Identification of Non-host Crops as Possible Trap Crops to Reduce *Orobanche* Seed Bank in Infested Tomato Field

A Dissertation submitted for the Partial Fulfilment of the Requirements

for the Master's Degree in Botany

To

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DECLARATION

I hereby declare that the work presented in this dissertation is a work done originally by me and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATION LETTER

This is to certify that dissertation work entitled "**Identification of non-host crops as possible trap crops to reduce *Orobanche* seed bank in infested tomato field"** submitted by "**Ms. Sanju Jyawali"** has been carried out under my supervision. The entire work is based on the results of her own work and has not been submitted for any other degree to the best of my knowledge. I therefore, recommend this dissertation work to be accepted for partial fulfillment of Master's degree in Botany from Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

This dissertation work entitled **"Identification of non-host crops as possible trap crops to reduce *Orobanche* seed bank in infested tomato field"** submitted by **"Ms. Sanju Jyawali"** has been accepted for the examination and submitted to the Botany Department, Amrit Campus, Tribhuvan University for partial fulfillment of the requirements for Master's degree in Botany (Ecology).

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ACRONYMS AND ABBREVIATIONS

°C: Degree Celsius

%: Percentage

Cm: Centimetre

Mm: Millimetre

M: Metre

Sq.m: Square Metre

N: North

E: East

Spp: Species

g/cm^3 Gram per cubic centimeter

W/w: Weight/ Weight

W/v: Weight/ Volume

G/ml: Gram/ millilitre

Kg/ hec: Kilogram per hectare

Gm/ hec: Gram per Hectare

Gm: Gram

Ml: Millilitre

Min: Minimum

Max: Maximum

Kg: Kilogram

$CaCl_2$: Calcium chloride

r.p.m: Revolutions per minute

No.of: Number of

SB: Sunflower broomrape

ANOVA: Analysis of Variance

DMRT: Duncan’s Multiple Range Test

NARC: National Agricultural Research Council

SPSS: Statistical Packages for Social Sciences

DAP Diammonium phosphate

ATC: Agricultural Technology Centre

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ABSTRACT

Orobanche is an obligate root parasitic weed which is epidemic all over the world. It completely depends upon the host plant to complete its life cycle. Trap crop is a non-host plant which stimulates the seeds of root parasite to germinate but cannot be infected itself and so *Orobanche* finally dies thus will reduces the seed bank of infested tomato field. This study was carried out at *Orobanche* infested tomato field at Lalbandi of Sarlahi District. A modified method (Acharya *et al*. 2003) of sieving and differential floatation technique of Ashworth (1976) has been used for standardization and estimation of pre sowing and post-harvest soil seed bank. The percentage of seed recovered was highest in sandy soil (81.8) followed by sandy loam soil (71.250), loam soil (63.5) and clay soil (57.25). The field and poly-bag experiments was done in randomized block design using 12 non-host crops locally available and fallow as a control, with three replications in the infested tomato field of Lalbandhi, Sarlahi. Data of pre-sowing and post-harvest were compared in order to assess the effect of test crops on *Orobanche* seed density. *Orobanche* seed density in soil samples collected from poly-bag and field before sowing and after harvest of each crop species was recorded. On the basis of degree of effects on the *Orobanche* seed bank, the investigated crop species could be classified in following three categories:

Highly potential trap crops: *Lens culinaris*, *Cicer arietinum* and Moderately potential trap crops: *Zea mays*, *Daucas carrota*, *Cuminum cyminum*, *Hordeum vulgare*, *Coriandrum sativum* and *Vigna unguiculata* Non-potential trap crop: *Triticum aestivum*, *Phaseolus vulgaris* and *Sesamum orientale*

Finally it could be concluded that any of the crops listed as potential trap crops (*Lens culinaris*, *Cicer arietinum* and *Fagopyrum esculentum*) in crop rotation will be effective to reduce *Orobanche* seed bank significantly in infested tomato field.

Keywords: *Orobanche*, trap crop, root parasite, seed bank, quantitative estimation

CHAPTER-I

INTRODUCTION

1.1 General Introduction

Orobanche commonly known as broomrape are obligate plant-parasitic weed which are devoid of chlorophyll and entirely depend upon the host plant for nutritional requirement and can only reproduce in response to chemicals produced by the host plant. It belongs to family Orobanchaceae and has more than 150 species (Musselman, 1980), found mostly in the temperate Northern Hemisphere. It is a threat to about 16 million hectares of arable land in the Mediterranean region and west Asia (Sauerborn, 1991).

Broomrapes germinate in response to specific chemicals released by the host plant. During germination, the seedlings attach to the host roots by the production of specialized feeding structures known as haustoria that form a functional bridge into their hosts. Broomrapes spend most of their life cycle underground, and complete the processes of germination, haustorial differentiation from the radicle, haustorial penetration of the host, formation of vascular connection with the host, acquisition of host nutrients, and storage of resources in a parasite organ called the tubercle or nodule (Fernandez-Aparicio *et al*., 2011).The particular characteristics (underground development, attachment to the host roots) of this parasitic weed is to destroy the development of effective control strategies. In addition, a single broomrape plant can release more than 500,000 seeds, which are known to remain viable for decades in the soil. This provides the parasite with a great genetic adaptability to environmental changes, including host resistance, agronomical practices and herbicide treatments (Joel *et al*., 2007).

Orobanche is a small plant which ranges from 10–60 cm tall depending on species. It has yellow- to straw-colored stems completely lacking chlorophyll. This species forms an underground stem in the form of tuber and establishes a connection to a host root system. The stem above the ground is upright, usually branched, yellowish, with chlorophyll traces, with little husks instead of leaves, 10-40 cm high bearing yellow, white, or blue snapdragon-like flowers. The flower shoots are scaly, with a dense terminal spike of between ten and twenty flowers in most species, although single in *O. uniflora*. The leaves are merely triangular scales. When they are not flowering, no part of the plants is visible above the surface of the soil. Flowers are collected into the inflorescence spikes-shaped or racemes. Flowers have flowery spathe with five parts. Calyx is bell-shaped with 4-5 teeth. Calyx teeth are in the form of triangle with points, shorter than tube. Crown is 10-15 mm long, about 2.5 times bigger than calyx, blue-violet, at the bottom yellow, with two lips. Crown slices are blunt. It flowers from June to October (Holm *et al*., 1979).

1.2 Life cycle of *Orobanche*

Life cycle of *Orobanche* plants is annual in most crop species (Borg, 1986) but the life cycle could be extended to longer period in perennial hosts (Kropac, 1973). Life cycle of *Orobanche* consists of two stages. The hypogeal stage lasts for 45-55 days during which the *Orobanche* grows slowly underground. During the epigeal stage seedling emerges out within 4-5 days and the seed ripe after another 20-25 days. Under adverse weather condition emergence of *Orobanche* may be delayed beyond 45-55 days (Lolas, 1986).

Orobanche disrupts physiological and metabolic processes in host plant resulting in changes in various organic acids, carbohydrate, protein content, certain enzyme activities, nicotine and total phosphorus (Tinchev, 1971). Seeds of *Orobanche* generally remain dormant and require a post-harvest ripening period for their germination in response to chemical stimulation (alectrol/orobanchol) from the host plant roots. The stability of the chemical stimulant is very short-lived in the soil. Before germination, seeds must undergo conditioning period under suitable temperature and moisture conditions (Van Hezewijk *et al*., 1993). These conditions ensure that only seeds with in the rhizosphere of an appropriate host root will germinate to contact a host root before exhausting its limited energy resources. Suitable temperatures of conditioning of *Orobanche* seeds are between 15-20 °C for at least 18 days for maximum germination. However, prolonged storage in these conditions causes the seeds to enter secondary dormancy (Van Hezewijk *et al*., 1994). Increasing storage temperatures increases the percentage of seeds going dormant, there is also some decrease in viability at higher temperatures, with viability reaching zero at 80 °C (Mauromicale *et al*., 2000).

Weed dispersal is mostly confined to contaminated crop seeds owing to poor quarantine services, however, animal grazing, unfermented contaminated manures, wool, fur and farm machinery could be the other sources of seed dissemination. The seeds can easily pass unharmed through animal’s alimentary tract and infest the host plants (King, 1966).

1.3 Worldwide distribution of Orobanche

The majority of *Orobanche* occur in northern hemisphere. The majority of species are found in the Mediterranean regions. It can be found in subtropical, semiarid and arid regions as well as in the temperate and humid regions. They occur in vegetation zones characterized by hardwood forest with winter rain or in natural savanna regions with roughly 250-500mm precipitation.

Table 1.1: Economically important *Orobanche* spp., their synonyms and distribution

Species	Synonyms	Geographical distribution
O. ramosa L.	*O. muteli* F.W Schultz	Central Europe, Former Soviet Union, Mediterranean basin. All countries between middle East, India and Eastern Africa
O. aegyptiaca Pers.	*O. ludoviciana* Nutt. *Phelypaea aegyptiaca* Pers. Races suspected	Introduced to Mexico and USA. *O.ramosa* will be prevalent in cooler regions and *O. aegyptiaca* in warmer regions (Bischof and Koch, 1973; Jacobsohn *et al*., 1980; Kasasian 1973a; Musselman, 1982 and 1986)
O. crenata Forsk.	*O. speciosa* D.C No races suspected	Mediterranean basin (Kasasian, 1973a; Mesa-Garcia and Garcia Torres, 1984 and 1986)
O. cernua Loefl.	*O. Cumana* Wallr. *O. cernua* var. *Dessertorum* Races reported.	Eastern Europe, Russia, From the Middle Eastern countries all the way to the Indian subcontinent (Beilin, 1968; Krenner, 1958 and Kumar, 1942)
O. Minor Smith	*O.picridis* F.W.Schultz Races reported.	Central and Southern Europe, Middle East. Sporadically in U.S.A, South Africa, East Africa, New Zealand (Evans, 1962; Frost and Musselman, 1980; James, 1976; Musselman, 1986)

(Source: Foy *et al*., 1989)

Center of origin of *Orobanche* spp. are located in Turkey, Italy, Spain and Morocco and a single species of these have achieved a wide distribution. The economically important species are *Orobanche aegyptiaca*, *O. cernua* (syn. name *O. solmsii*), *O. crenata, O. ramosa, O. mutelli* and *O. minor* (Sauerborn, 1991). The yield loss in solanaceaceae plants like potato, brinjal, tomato and tobacco by *O. ramosa is* reported to more than 75% in Algeria (Kamel, 2005).

1.4. Geography of Nepal

Nepal is a south Asian country which lies in between India and China bordering with India in the south, east and west and China in the north. It covers an area of 1,47,181 sq.km and stretches

145 to 241 kilometers north to south and 850 kilometers west to east. It extends from 26˚22' to 32 ˚27' N latitude and 80˚4' to 88˚12'E longitude.

1.5 Distribution and problems of Orobanche in Nepal

There is no accurate documentation regarding the distribution of *Orobanche* in Nepal. According to Khattri (1997) *O. aegyptiaca* is found in all ecological regions of Nepal from tropical parts of terai and inner terai to sub-tropical in mid-hills to temperate region in temperate up to an elevation of 3800m. The occurrence of it was high in terai and inner terai where there is extensive cultivation of tori (*B. campestris* var. toria) and tobacco (*Nicotiana tabaccum*). It was also common in Kathmandu valley in kalo tori (*B. campestris* var. *nigra*) field at and 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., *O. coerulescens* Steph., *O.ramosa* L. and *O. solmsii* C.B. Clarke ex Hook.f. (Press *et al*., 2000). In Nepal, two species *Orobanche aegyptiaca* and *O. cernua* are causing threat to wide range of important vegetables and other field crops, particularly tori (*Brassica campestris* var. toria), Sarson (*Brassica campestris* var. sarson), rayo (*B. juncea*), tomato *(Lycoperiscon esculentum*) and tobacco (*Nicotiana tabaccum*) grown in terai and dun valleys (Khattri *et al*., 1991). *O. aegyptiaca* attacks all above mentioned crops while *O. cernua* attacks solanaceous crops only (Khattri *et al*., 1991). Yield loss due to *Orobanche* is estimated upto 49% in tori (Khattri, 1997). This problem is severe in tomato growing areas in terai, where it is estimated to be 30-40% yield loss in field of Lalbandhi, Sarlahi district (Personal communication with NARC officials and farmers).

1.6 Host range

Orobanche species have their own host specificity. *Orobanche* attacks twenty-three plants species belonging to seven families of flowering plants in Nepal (**Table 1.2**). Only five species are regarded as either widespread or acute agricultural problems- *O. crenata* Forsk. (=*O. speciosa* D.C); *O. cernua* Loefl. (=O. solmsii C.D Clark); *O. ramosa* L. (=*O. nana* Noe and *O. mutelli* Schultz); *O. aegyptiaca* Pers. and *O. Minor* Sm. (Parker, 1986).

Table 1.2: 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	*Nicotiana tabacum* L.	Solanaceae	+++	+++
14	*Lycopersicon esculentum* Mill	”	++	+++
15	*Solanum melongena* L.	”		++
16	*S.nigrum* L.	”		+
17	*Vicia faba* L.	Fabaceae	+	
18	*Cicer arietinum* L.	”	+	
19	*Lens esculentum* Medic.	”	+	
20	*Foeniculum vulgare* Mill	Umbelliferae	+	
21	*Chenopodium album* L.	Chenopodiacea	+	
22	*Argemone Mexicana* L.	Papaveraceae		+
23	*Hordeum vulgare* L.	Gramineae	+	

(Source: Khattri, 1997)

(+= rare, ++= common, +++= vary common)

A

B

Figure 1.1: Photograph showing *O. cernua* in (A) Tomato *(Solanum lycopersicum*) (B) Brinjal (*Solanum melongena)*

1.7 Control Measures used Worldwide

Currently there is no consistent and sustainable method to control the weed all over the world (Parker and Riches, 1993). However some study showed that use of trap crops has sustainable and useful potential to control *Orobanche* species. Several available control methods (chemical, physical, biological and cultural etc.) have been tried around the world for control of this weed but none of the single method is found to be effective in term of economic returns (Perez-de-luque *et al*., 2010; Goldwasser and Kleifield, 2002).

1.7.1 Preventive method

The strength of broomrape lies in its ability to form seed bank in the soil. The main aim of the management or eradication program is reducing this seed bank, while minimizing the production of new seeds and their dispersal to new sites. Quarantine is therefore an essential element in control or eradication programs. Preventive measures could be found more effective if the initial specific infestation is abundant and timely precautionary measures are being adopted to counter long distance seed dispersal.

1.7.2 Sanitary method

The main aim of phytosanitation is preventing the spread of viable seeds by minimizing the movement of infested soil by farm machinery and vehicles, preventing grazing on infested plant material, treating manure (e.g. composting) and avoiding the use of hay made of *Orobanche*-infested plants (Jacobsohn, 1984). One should also avoid the use of *Orobanche*-infested crop seeds.

1.7.3 Cultural method

1.7.3.1 Avoidance

Delaying the sowing of winter crops may decrease *O. crenata* infestation and reduces the damage caused by it and early sowing of sunflower reduces infestation caused by *O. cumana* (Garcia Tores, 1994).The other way to reduce infestation is to grow non-host crops in infested field. It is necessary to know the host of the particular *Orobanche* in the field and one should remembered that these seeds remain viable in soil upto 15 to 20 years (Garcia and Tores, 1994)

1.7.3.2. Crop rotation using trap and catch crops

A crop rotation system includes *Orobanche* host crops, trap crops and catch crops as non-host crops (Parker and Riches, 1993). Crop rotation of mustard with non-host crops like wheat, barley, chickpea etc. is the most effective and commonly used management strategy for reducing the weed seed bank in heavily infested areas. The major restriction in adopting crop rotation in long-run is the longer viability of its seeds. Thus, heavy infestations may remain in a field despite absence of host crops for several years. Weed seeds buried in the soil beneath the crop root zone can be brought up to surface soil as a result of subsequent ploughing, germinate and provide competition to the host crop in later years. Frequent planting of susceptible crops on the same field should be avoided and as far as possible grow mustard in alternate years with diverse growing habit genotypes (Braun *et al*., 1984)

Kleifeld *et al.* (1994) justified the importance of using 'trap crops yielding suicidal parasite germination' as a management option for reducing *Orobanche* seed bank in the infested fields. These crops exude stimulants that induce *Orobanche* seed germination but no viable attachment to the host plant roots is established and the weed seedlings withers away and die up and ultimately their seed bank in the soil gets reduced. Acharya *et al*. (2002) noticed that by using a local cultivar of *Brassica campestris* as a catch crop in Nepal, can reduce *O. aegyptiaca* seed bank by around 33.35 per cent. Experimental results in Tehran indicated that using trap crops namely sesame, brown indian-hemp, and common flax and black-eyed pea decreased broomrape biomass by 86, 85.3, 75.2, and 74.4 per cent, respectively. It was found that these plants have a great potential to reduce broomrape damage and they can be used in rotation in broomrape infested fields (Sirwan *et al*., 2010).

Krisnamurthy and Rao (1976); Sauerborn *et al*. (1986) and Kleifeld *et al.* (1994) listed some trap crops which are found to be effective and help to reduce seed bank of different *Orobanche* 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 (lucerne) (*Medicago sativa*), soybean (*Glycine max*), vetches (*Vicia* spp.) and chickpea (*Cicer arietinum*). An additional cultural means for reducing *Orobanche* seed bank in the soil is the use of catch crops i.e., planting an *Orobanche* host crop for inducing parasite seed germination and attachment and that will be destroyed later on by means of light tillage practices or residual soil herbicides. But the use of trap and catch crops to manage this weed is somewhat limited due to (a) enormous amount of *Orobanche* seeds dispersed in the soil and only a small proportion may be exposed to germination stimulants in the rhizosphere (b) feasibility and economics of growing these crops in the existing situations.

Abu Irmaileh (1994) studied that increasing of nitrogen concentration has a result in linear decreasing of seed germination and length of *Orobanche* rootlet in flax, lentil, pepper, tomato, common wheat. Seed germination of parasite was considerably lower on common wheat than on other crops, the lowest being in the lack of crops.

1.7.3.3 Sowing date

Germination of *Orobanche* is found to be very much reduced below 8℃ and further development is reduced low temperatures. Delaying the planting date affects *Orobanche* more than its hosts; the delay should be two weeks only from the date optimal for sowing in an un infested field. However, this method must be adapted for different regions and for different hosts. Early planting dates are beneficial in certain instances. Late planting of mustard (last week of October-first fortnight of November) is observed to be helpful in reducing the parasitism of *Orobanche* a result of specific weed and host plant differential response to low temperatures (Yadav *et al*., 2005)

1.7.4 Biological control

Bioagents include insects such as *Phytomyza orobanchia* and fungi such as *Fusarium oxysporium* fsp *orthoceras.* Only *Phytomyza orobanchia* (Diptera, Agromyzidae) is the best for the biological control of *Orobanche*. Seed production in *Orobanche* was found to be reduced significantly in many countries (Kroschel and Klein, 2003). Natural enemies can reduce the fly population considerably. The only way to make use of *Phytomyza orobanchia* is to collect *Orobanche* infested with the fly's larvae, and to distribute these shoots in the field at the time when *Orobanche* is expected to emerge. These insects prevent seed production through the development of larvae inside the seed capsule of their target hosts and contribute to reduce their reproductive capacity and spread it. Several fungi have been reported to be virulent on *Orobanche*. Current investigations concentrate mainly on *Fusarium oxysporum* f. sp. *orthoceras*, a pathogen with high potential to control *Orobanche cumana* by disconnecting the host parasite connection (Abuelgasim and Kroschel, 2003).

1.7.5 Chemical control

Chemical control of *Orobanche* is very difficult and very often non effective. Soil fumigation with methyl bromide has been effectively used in the past, especially in light soils (Jacobsohn, 1984), but it is no longer permitted for this purpose. Alternatives, including metan-sodium and dazomet, may provide good control, but methods of use are critical and best results are normally achieved with soil coverage by plastic (Parker and Riches, 1993). Recommended doses of these compounds are usually very high and costly, but much lower doses were reported by Chalakov (1998) to be effective in Bulgaria, perhaps resulting from a germination-stimulatory effect and death by suicidal germination.

1.7.6 Integrated Management

A single control method is not sufficient to control parasitic weeds due to high production of seeds in one cropping season. Therefore, applications of different control method have been suggested to reduce the infestation on a tolerable level. Integrated management strategies need to combine low cost control methods that enhance crop tolerance to the parasite through improvement of soil fertility, particularly nitrogen status, utilize the most tolerant cultivars that are available, in addition to potential preventive means. Cultural methods include manipulating seeding rate, planting date, proper phytosanitary measures, help in reducing infestations. Herbicides application could be carried out by trained farmers. The aim of integrated management is to constantly reduce the parasite population leading to the reduction of soil seed bank. For the planning of proper weed management strategy the amount of weed problem in infested field should be known correctly. In case of *Orobanche*, it is the seed density in the soil which gives a correct picture of infestation than the visual inspection. The infestation of *Orobanche* in a field is not necessarily limited to areas where infected plants are observed in a particular year (Nash and Wilhem, 1960). Sauerborn *et al.* (1991) developed technique for soil sampling to estimate the seed bank of *Orobanche.* Intensive seed bank studies can be useful in estimation and prediction of *Orobanche* infestation in field and can be performed any season of the year. Seed bank study help to plan for integrated management of weed (Auld *et al*., 1979)

1.8 Justification of the study

Orobanche spp. is causing serious problem over the world and causes major losses to crops in Nepal especially in terai region . It causes major infestation in tomato field of Lalbandhi, Sarlahi reducing crop yield from 5 to 100%. Due to this they are facing high economic loss. The Orobanche infestation appears when tomato plants are in vegetative to flowering stage. At

Lalbandhi field it is found that about 120-130 *Orobanche* shoots were recorded in one square meter in tomato field (Personal Communication of NARC people and farmers). Hand pulling is a general weeding practice but this process is laborious and also causes uprooting of the host as the parasite is closely connected to the roots of the host. Herbicide spraying using glyphosate was tried by farmers but it also killed the host. Hence to minimize loss from *Orobanche spp.* without damaging the host the present study has been conducted 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. So this research work was conducted to evaluate potential trap crops that can reduce the *Orobanche* seed bank in infested tomato fields of Lalbandhi. The outcome of this research will help tomato growing farmers of the study area for the reduction of *Orobanche* problems in a growing season.

1.9 Research Question

In this research, an attempt is made to answer:

Which non-host crops have potential to reduce *Orobanche* seed bank as trap crops in infested tomato field?

1.10 Objectives

1.10.1 Broad Objectives

The main objective is to evaluate the potential trap crops in *Orobanche* infested tomato fields.

1.10.2 Specific Objectives

- To adopt and standardize the available method of *Orobanche* seed bank estimation
- To estimates pre sowing soil seed bank in infested fields and pots
- To estimate post-harvest soil seed bank estimation
- Identification of effective non host crops as potential trap crop

CHAPTER-II

LITERATURE REVIEW

Orobanche are obligate plant-parasitic weed which are devoid of chlorophyll and entirely depending on the host for nutritional requirements and can only reproduce in response to chemicals produced by host plant as trap crop and catch crops. Following germination, the seedlings attach to the host roots by the production of specialized feeding structures, described as haustoria that form a functional bridge into their hosts. Broomrapes spend most of their life cycle underground, where they undergo processes of germination, haustorial differentiation from the radicle, haustorial penetration of the host, formation of vascular connection with the host, acquisition of host nutrients, and storage of resources in a parasite organ called the tubercle or nodule (Fernandez-Aparicio *et al*., 2011). Haustoria penetrate the host tissues until they reach the vascular system for uptake of water, nutrients, assimilate, and grow at the expense of the host plant's resources (Joel *et al*., 2007).

According to Musselman (1980) it is a genus of over 150 species of parasitic herbaceous plants in the family Orobanchaceae, mostly native to the temperate Northern Hemisphere. It is a threat to about 16 million hectares of arable land in the Mediterranean region and west Asia Sauerborn (1991).The most common species which occurred on cultivated crops are *O. cumana, O. ramosa, O. minor* and *O. aegyptiaca.* They are obligatory parasites that attack hundreds of varieties of dicotyledonous plants in warm and dry areas all over the world. All these species have got completely atrophied root, thus they live on the roots of other plants. They are particularly parasites on sunflower, maize, tobacco, tomato, peas, hop, hemp, and broad-bean crops (Karacic *et al*., 2010).

Habimana *et al.* (2014) found that *Orobanche* species cause considerable yield losses (5-100 %) in the crops, especially in the drier and warmer areas of Europe, Africa and Asia where it is reported to mainly parasitize species of leguminous, oilseeds, solanaceous, cruciferous and medicinal plants. It is a serious root parasite threatening the livelihood of the farmers with its devastating effect on the some of the fore mentioned crop. The long-term impact of the broomrapes is even more serious: their seeds may easily spread to other fields, and can persist in soil up to 20 years, leading to an accelerated increase in the infested areas in which susceptible crops are under danger.

Dhanapal *et al.* (1996) from their studies stated that depending upon the degree of infestation, environmental factors, soil fertility, and the crops' response damage from *Orobanche.* It may cause complete crop failure. This parasitic weed has capacity to proliferate well in coarse textured soils with high pH, low in nitrogen status having poor water holding capacity where the crop cultivation is either rain fed or dependent on sprinkler systems for irrigation.

The yield loss in solanaceaceae plant like potato, brinjal, tomato and tobacco by *O. ramasa is* reported to more than 75% in Algeria, (Kamel, 2005).

Khatri *et al.* (1991) reported that *O. aegyptiaca, O. cernua* and *O. solmsii* cause significant damage to mustard and tobacco crops in Terai (plains), Inner Terai and central and W. Midhill regions of this country. Cruciferous plants, especially toria (*Brassica campestris* var. *toria*), appear to be the most susceptible host to *O. aegyptiaca*, while some Solanaceous crops such as tobacco, aubergines and tomato (*Lycopersicon esculentum*) are most susceptible to *O. solmsii.* Miscellaneous hosts of *O. aegyptiaca* are chickpeas (*Cicer arietinum*,broad beans (*Vicia faba*), *Chenopodium album* and Taraxacum sp. Barley is also suspected to be a host of *Orobanche.* Temperature variation seems a major factor for occurrence of *Orobanche* in different seasons at different altitude zones of Nepal. Phenology shows that there is no specific preference of *O. aegyptiaca* to the growth stages of *B. nigra.*

According to Fernandez *et al.* (2016) *O. crenata* attacks legumes complicating their inclusion in cropping systems along the Mediterranean area and west Asia. The effect of broomrape parasitism in crop yield loss can reach upto 100% depending on infection severity and broomrape crop association. This work provides field data of the consequences of *O. crenata* infection severity in three legume crops, i.e. faba bean, field pea, and grass pea in field. Regression functions modeled productivity losses and revealed trends in dry matter allocation in relation to infection severity. Reductions in host biomass occurred in both vegetative and reproductive organs, the latter resulting more affected. The increase of resources allocated within the parasite was concomitant to reduction of host seed yield indicating that parasite growth and host reproduction compete directly for resources within a host plant. However, the parasitic sink activity does not fully explain the total host biomass reduction because combined biomass of host–parasite complex was lower than the biomass of uninfected plants. In grass pea, the seed yield was negligible at severities higher than four parasites per plant. In contrast, faba bean and field pea sustained low but significant seed production at the highest infection severity. Data on seed yield and seed number indicated that the sensitivity of field pea to *O. crenata* limited the production of grain yield by reducing seed number but maintaining seed size. In contrast, the size of individual parasites was not genetically determined but dependent on the host species and resource availability as a consequence of competition between parasites at increasing infection severities.

Mauromicale *et al.* (2008) studied the influence of the holoparasite branched broomrape on the vegetative growth, leaf chlorophyll content, photosynthetic rate, and chlorophyll fluorescence of tomato was studied over two growing seasons on plants grown in a commercial greenhouse. They found that presence of the parasite strongly reduced the aerial biomass by acting as a competing sink for assimilate, but more importantly, by compromising the efficiency of carbon assimilation via a reduction in leaf chlorophyll content and photosynthetic rate. The degree of damage to the host was not dependent on either the number or the biomass of parasitic plants per host plant. The study suggest that the ability to maintain a high photosynthetic rate, leaf chlorophyll content, or both and the ability to minimize photo inhibition can be developed as indirect assays for improved tolerance to branched broomrape.

Hershenhorn *et al*. (2009) studied the *Phelipanche aegyptiaca* management in tomato. *Phelipanche* and *Orobanche* spp. are widespread in Mediterranean areas, in Asia and in Southern and Eastern Europe, attacking dicotyledonous crops and depending entirely on their hosts for all nutritional requirements. *Phelipanche aegyptiaca, Phelipanche ramosa* and *Orobanche cernua* are extremely troublesome weeds on tomatoes and exert their greatest damage mainly to their shoot emergence and flowering. This study summarizes the four main control measures for the weedy root parasites *Phelipanche* and *Orobanche* in tomato, namely chemical and biological control, resistant varieties and sanitation. Some of these methods are commercially widely used by farmers in Israel (chemical control), some are in the final stages of development towards commercialization (resistant varieties and sanitation), and some still require further development and improvement before commercial implementation (biological control). The study presents an up-to-date summary of the available knowledge on their use for broomrape management in processing tomatoes.

Ashworth (1976) described the method of seed bank study by using sieving and differential flotation techniques using 40% (w/w) calcium chloride of specific gravity1.3957. Acharya *et al*. (2003) modified this method by avoiding the use of instruments like 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 100 sieve two times in $CaCl_2$ solution.

Visser and Wentzel (1980) and Kachelriess (1987) studied seed bank by mixing 4g of soil with 20ml of magnesium sulphate solution with a specific gravity of 1.16g/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 seed remained floating. The supernatant was retained in meshed filter paper and the seeds were then counted under the binocular microscope.

Several available control methods (chemical, physical, biological and cultural etc) have been tried around the world for control of this weed but none of the single method is found to be effective in term of economic returns (Perez-de-luque *et al*., 2010, Goldwasser and Kleifeld, 2002).According to Parker and Riches (1993) and flooding of rice cropping throughout the growing season destroys *Orobanche* seed.

Krisnamurthy and Rao (1976); Krishnamurthy *et al.* (1977); Abu-Irmaileh (1984); Sauerborn and Saxena (1986) and Kleifeld *et al*. (1994) listed some trap crops which are found very effective and help to reduce seed bank of *Orobanche* 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 (lucerne) (*Medicago sativa*), soybean (*Glycine max*), vetches (*Vicia* spp.) and chickpea (*Cicer arietinum*).

Bista (2015) investigated effect of soil moisture on *Orobanche* seed germination. *Orobanche* seed were pre-conditioned in three different soil moisture conditions. 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. 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 study of effect of light showed that exposure of seeds to continuous light during pre and post conditioning period inhibited seed germination. The inhibition was more effective when seeds were exposed to light during post conditioning phase rather than during pre- conditioning phase.

Kleifeld *et al.* (1994) justified the importance of using 'trap crops yielding suicidal parasite germination' as a management option for reducing *Orobanche* seed bank in the infested fields

Acharya (2013) studied the seed bank of *Orobanche aegyptiaca* in infested mustard field of Nawalparasi found that reduction by using certain trap crop. But all the seed bank reduction is not due to trap crop only; some reduction is also contributed by edaphic and/or pathogenic factors. There are some reports that the crops rotation with certain non- host crop can reduce *Orobanche* seed bank in the infested field (Parker, 1991; Sauerborn, 1991; Acharya, 2013).

Acharya (2012) 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,

Fernandez *et al*. (2007) studied the intercropping with cereals and found that it reduces infection by *Orobanche crenata* in legumes. *Orobanche crenata* is a root parasitic weed that causes huge damage to legume crops. Control strategies have centered on agronomic practices and the use of herbicides, although success has been marginal. Their field experiments show that *O. crenata* infection on faba bean 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 intercrop. Pot and field experiments confirmed the reduction of infection in faba bean 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.

Daniel *et al*. (2006) studied parasitic weed *Orobanche minor* that attaches to the roots of red clover (*Trifolium pratense*) and a number of other broad-leaved plant species in

Pacific Northwest USA. Wheat (*Triticum aestivum*), was found to be a false-host of *O. minor*; therefore, growth chamber, glasshouse and field soil experiments were conducted to evaluate the effect of six soft white winter wheats (*T. aestivum*), one durum wheat (*Triticum turgidum*), and one triticale (*Triticale hexaploide*) on *O. minor* germination. In growth chamber experiments, wheat and triticale reduced germination of *O. minor* from 20–70%. In glasshouse studies, *O. minor* attachment was minimal on red clover plants grown in pots previously planted to wheat or triticale. In pots red clover plants averaged 4.2 *O. minor* attachments per plant. Red clover plants also had fewer *O. minor* attachments when grown in field soil taken from the plots where wheat or triticale were grown compared with plants grown in soil where no wheat or triticale were previously grown. The study demonstrated that wheat may have the potential to be effectively integrated into an *O. minor* management system.

Babaei *et al.* (2010) study was conducted to evaluate the effect of some trap crop on reducing *Ph. aegyptiaca* damage in tomato. Trap crops consisting of Egyptian clover, sesame, mungbean, common flax, brown Indian hemp, cotton, pepper and black-eyed pea were studied during 2008 and 2009. Through the first year, trap crops were cultivated in pots and in the next year, tomato was transplanted into those pots. 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. Meanwhile, sesame, brown Indian hemp, Egyptian clover and mungbean increased total biomass of tomato by 71.4, 67.5, 65.5, and 62.5 %, respectively. It was found that these plants have a great potential to reduce broomrape damage and they can be used in rotation in broomrape infested fields.

Abebe *et al.* (2005) performed experiment with selected crop on a naturally *Orobanche* infested soil to test the potential of selected crop in *Orobanche* seed bank exhaustion preceding tomato field at Melkassa Agricultural Research Center, Merti Upper Awash Agro-Industrial Enterprise and Ziway Horticulture Development Enterprise in 2002 and 2003. They planted all plots with tomato, host plant in the third season to see the cumulative effect of trap crops to reduce soil seed bank of *O. ramosa* and *O. cernua*. 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 increased due to the reduction of *Orobanche* seed band in the third season (2004).

Use of trap crop is one of the best methods currently available to control agricultural root parasites. Trap crops also called false host are plants which stimulate the germination of the parasite seed but cannot be infected and thus reduce the seed population in the soil.

Seed bank study and use of potential traps crops in infested field is an important aspect of integrated management of parasitic weed *Orobanche*.

CHAPTER-III

MATERIALS AND METHODS

3.1 Standardization of *Orobanche* seed bank estimation

A method of sieving and differential floatation technique of Ashworth (1976) with some modification (Acharya *et al*., 2003) used for the *Orobanche* seed bank estimation. For the standardization of seed has been done by using four different types of soil namely: sandy, sandy loam, loam and clayey soil. This sieving method has been used for the seed estimation of soil sample from poly-bags and field experiments as shown in flow chart below (**Fig.3.1**).

The seeds were identified from other soil particles due to its ornamented seed coat, shape and size. There were three replication for each soil types. The seeds not recovered were most probably lost in the soil during floatation in the present method. The total number of the seeds in the soil sample was calculated with a correction factor based on the percentage of seeds recovered (Lopez Grandos and Garcia Tores, 1993).

100 *Orobanche* seed in 100 gram soil in 250ml beaker

↓

Added tap water filling 3/4th of the beaker

↓

Left undisturbed for 24 hours

↓

Sample was stirred and poured over the stack of two sieves (upper 279 micron, lower 120 micron).

↓

Soil washed down with the help of shower

↓

Debris contents of lower sieve transferred to beaker (100 ml) with 40% $CaCl_2$, left undisturbed to settle heavier particles

↓

Floating materials was collected on 100 micron sieve

↓

Contents of sieve washed several times with water and treated with 2% sodium hypochlorite solution

↓

Washed with water and transferred to lined muslin cloth

↓

Debris spread evenly and seeds present in each line were counted under stereomicroscope

Figure 3.1: Work flow chart

3.2 Description of study area

Lalbandi lies in the central development region of Nepal. It is located on 27.0583° N, 85.6333° E. Average elevation is 154 meters above sea level. Temperature is moderate over here ranging from minimum 15^{0}C in winter season to maximum 35^{0}C in summer season. Lalbandi is stretched from Norther Pattharkot VDC (Chure Hill) to Southern Jabdi VDC central Terai. Now the lands are being register under Lalbandi municipality of Province 2. Lalbandi is famous for supplying tomato demand of whole country. (**Fig 3.2)**

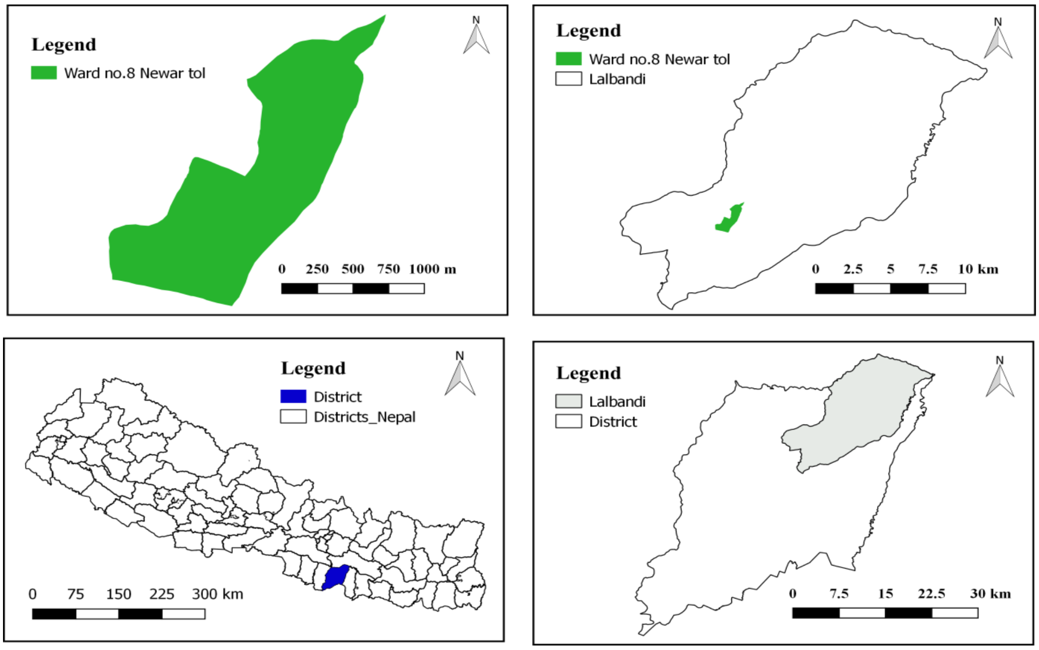

Figure 3.2: Map showing study area of Lalbandhi, Sarlahi

3.3 Climate of study area

The climate of the study area is typically of monsoon type with high rainfall in June to October. Minimum temperature ranges from 8˚C in January to 25 ˚C in July and August and maximum temperature ranges from 16 ˚C in January to 37 ˚C in June and July. The maximum precipitation was in the month of August (355.8 mm) and minimum was in November, December and January (0 mm).

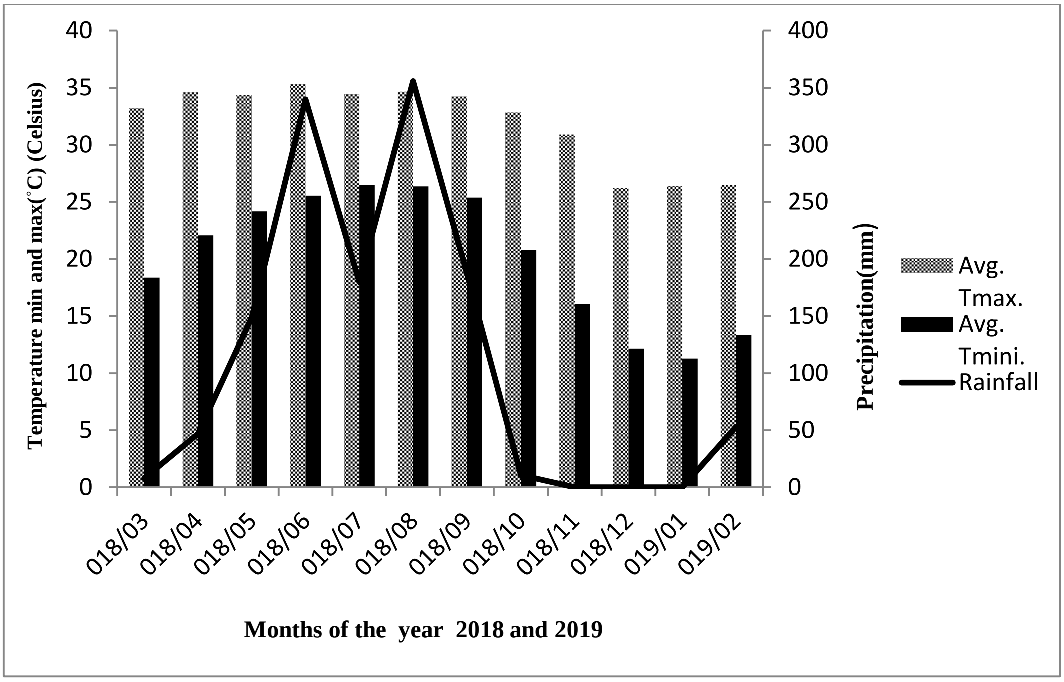

Figure 3.3: Ombothermic graph showing temperature min/max and rainfall of Karmaiya, Sarlahi

(Source: Department of Hydrology and Metrology)

3.4 Poly-bag experiments

Altogether, twelve winter crops have been tested for poly-bag experiments. The poly-bag mixture included soil collected from naturally infested field by *Orobanche* seeds. The poly-bags were first filled with soil mixture and then moisten with water. About 3/4th portions of bags have been buried into the soil to avoid rapid fluctuation of soil temperature and moisture. Seeds or seedling of test crops has been collected from the local market. Crop seeds has sown 3-4 cm deep in the soil. To avoid dehydration of the germinating seeds, regular watering was done. There were three replications for 2each treatment, including fallow as control. Soil samples (500gm) for quantitative estimation of *Orobanche* seeds was collected from each bag at the time of crop sowing and after harvest.

Table 3.1: List of crop plants, their number sown/poly-bag and number maintained/poly-bag

Crop tested (Botanical name)	Common name	Seed sown/ poly-bag	Plant maintained/ Poly-bag
Cicer arietinum L.	Chickpea	5	3
Coriandrum sativum L.	Coriander	10	5
Cumin cyminum L.	Cumin	10	5
Daucas carrota L.	Carrot	10	3
Fagopyrum esculentum Moench	Buckwheat	5	2
Hordeum vulgare L.	Barley	7	3
Lens culinaris Medic.	Lentil	7	3
Phaseolus vulgaris L.	French bean	3	1
Sesamum orientale L.	Sesame	10	3
Triticum aestivum L.	Wheat	7	3
Vigna unguiculata L.	Cowpea	5	3
Zea mays L.	Maize	3	1

3.5 Field Experiments

3.5.1 Field history

The experimental field was rain fed with maize in rainy season and tomato in winter season. The field had homogenous nutrient and moisture regimes. The soil type was loam with 47.8% sand, 43.4% silt and 8.8% clay. Soil nitrogen was 0.09%, phosphorus 35.02 kg/ha, potassium 455.6 kg/ha, organic content 1.02% and soil pH 7.5 (Soil analysis was done in Agricultural Training 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).

3.5.2 Field preparation and field design

Orobanche infested tomato field at Lalbandhi, Sarlahi has been selected for present study. Twelve locally grown non host crops has been selected for trap crops experiment and fallow as control treatment. Trap crops experiment has been conducted in poly-bags and fields. Poly-bag and field experiment has been conducted in randomized complete block design with three replication and fallow as control. The total plot area was 15 x 12.10 m^2 and each plot is 2m X 1m.

Figure 3.4: Photograph showing preparation of field and poly-bag for experimental work

4 **12**	**5** **4**	**12** **8**	**16** **5**	**17** **12**	**24** **3**	**28** **10**	**29** **3**	**36** **7**	**→Plot Number** **→Treatment Number**
3 1	**6** **11**	**11** **7**	**15** **8**	**18** **F**	**23** **7**	**27** **1**	**30** **2**	**35** **5**	
2 **6**	**7** **F**	**10** **10**	**14** **2**	**19** **10**	**22** **F**	**26** **9**	**31** **4**	**34** **12**	
1 5	**8** **9**	**9** **3**	**13** **6**	**20** **11**	**21** **1**	**25** **11**	**32** **6**	**33** **8**	
37 **2**	**38** **4**	**39** **9**							

Figure 3.5: Field lay out: RCB; **Area 15 x 12.10 m2 / Plot size 2m x1m**

Trt. no. Scientific name

1. ***Sesamum orientale* L.**
2. ***Lens culinaris* Medic.**
3. ***Hordeum vulgare* L.**
4. ***Cuminum cyminum* L.**
5. ***Daucas carrota* L.**
6. ***Coriandrum sativum* L.**
7. ***Cicer arietinum* L.**
8. ***Fagopyrum esculentum* Moench**
9. ***Zea mays* L.**
10. ***Triticum aestivum* L.**
11. ***Vigna unguiculata* L.**
12. ***Phaseolus vulgaris* L.**
13. **Fallow**

3.6 Plantation of test crops

The seeds of twelve test crops obtained from local market of Kathmandu and Lalbandhi were planted after the final preparation of the field in the first week of October. Plots were irrigated a day before sowing. Later irrigation was done as required. The distance maintained between rows and between plants was given in **table 3.2**.

Table 3.2: Name of crops planted, distance maintained between the rows and between the plants during field experiment

Botanical name	Common name	Distance maintained	
		Rows(cm)	Plants (cm)
Cicer arietinum L	Chickpea	30	5
Coriandrum sativum L.	Coriander	25	5
Cuminum cyminum L.	Cumin	18	2
Daucas carrota L.	Carrot	30	10
Fagopyrum esculentum Moench	Buckwheat	30	5
Hordeum vulgare L.	Barley	18	2
Lens culinaris Medic.	Lentil	30	10
Phaseolus vulgaris L.	French bean	30	5
Sesamum orientale L.	Sesame	30	10
Triticum aestivum L.	Wheat	18	2
Vigna unguiculata L.	Cowpea	25	10
Zea mays L.	Maize	40	15

Soil was sampled (composite sampling method) two times from each plot: first before sowing and the second after harvest. Finally 1kg of soil was collected in a poly-bag, labeled and transported to lab for seed estimation.

3.7 Seed bank estimation

Orobanche seed bank estimation in soil was done by the method of Ashworth (1976) with some modification (Acharya *et al*., 2003). Seed recovery was calculated using the following formula (Lopez Granados and Garcia Tores, 1993):

Actual number of *Orobanche* seeds $= \frac{No.of\ recovered\ orobanche\ seeds\ per\ 100\ g\ soil}{Orobanche\ seed\ standarization\ index*}$ x 100

*mean value of *Orobanche* seed recovered in 4 different types of soil

Soil samples from the poly-bag before sowing and after harvest of non-host crops was collected from up to 15cm depth by composite sampling method. Collected soil samples was placed in a labeled poly-bag and carried to laboratory for seed estimation. All the seed bank study from each soil sample was conducted in triplicate.

The number of *Orobanche* seeds per meter square will be determined by using the formula of Sauerborn *et.al* (1991)

Number of *Orobanche* seeds/m^2 = number of *Orobanche* seed/100 gm × soil depth (cm) × soil density × 100

3.8. Statistical Analysis

All the data obtained from different experiments conducted were analyzed statistically by SPSS var 7 using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at P= 0.05 level of significance.

CHAPTER: IV

RESULTS

4.1 Quantitative estimation of *Orobanche* seeds in infested soil

4.1.1 Standardization of *Orobanche* seed bank estimation

The result showed that recovery percentage varies with soil types. The recovery percentage was highest in sandy soil (81.8±3.11, followed by sandy loam (71.25±3.59), loam soil (63.5±5.19) and clay soil (57.25±5.12)

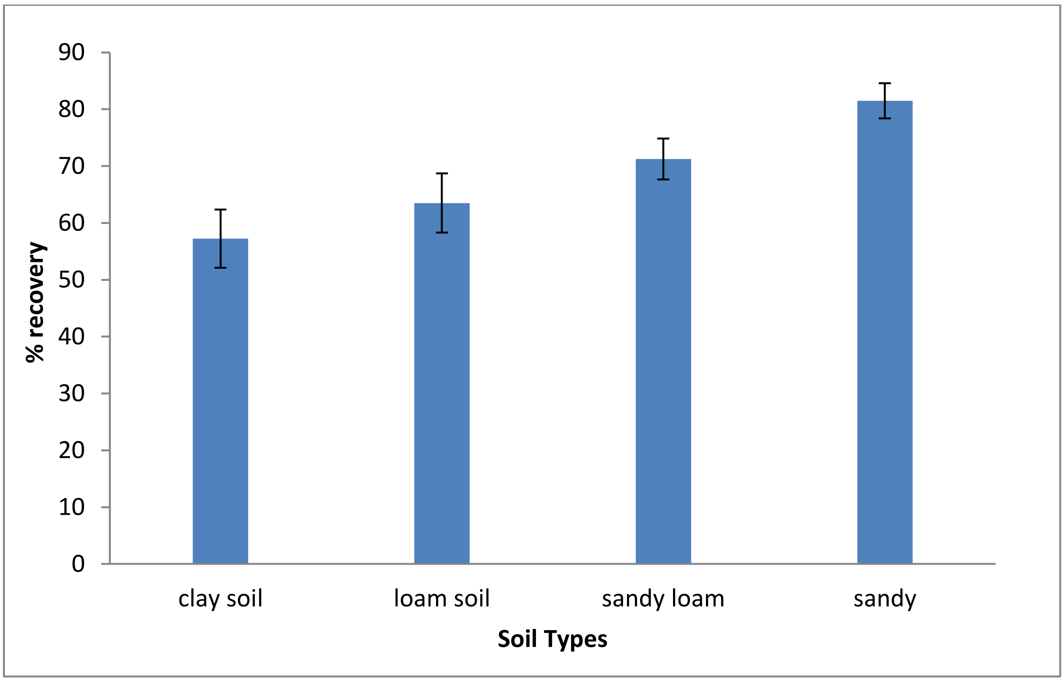

Figure 4.1: Recovery of *Orobanche* seeds from different types of soil (n=9)

4.2. Pre-sowing *Orobanche* seed bank in infested soil of poly-bags and fields experiment

The *Orobanche* seeds in soil for poly-bag experiment ranged from 18.33 to 25.3 seeds per 100 gm soil. The highest number *Orobanche* seed in poly-bag was found to be in *C. arietinum* i.e 25 and lowest in *T. aestivum* i.e 18 per 100 gram soil.

The *Orobanche* seed bank in the soil for field experiment ranged from 19.66 to 31.33 seeds per 100 gm soil, which is estimated to be 46905 to 74730 seeds per m^2. The highest number *Orobanche* seed in field was found to be in *C. arietinum* i.e 31 and lowest in *C. cyminum* i.e 20 per 100 gram soil.

Table 4.1: Number of pre-sowing *Orobanche* seed bank in 100 gm soil of poly-bags and seed in 100gm soil and seed per sq.m of fields experiment.

Non-host crops	**Poly-bag experiments**	**Field experiments**	
	No of pre-sowing seeds in 100gm soil	**No of pre-sowing seeds in 100gm soil**	**Seed per sq.m**
C. arietinum L.	25.33±4.189	31.33±6.599	74730±15740.2
C. sativum L.	18.67±1.247	26.67±4.189	62805±9202.79
C. cyminum L.	20.67±2.494	20.33±2.624	48495±6259.83
D. carrota L.	19.66±1.247	22.33±2.624	53265±6259.83
F.esculentum Moench	20.66±1.699	26.66±2.867	63600±6838.84
H. vulgare L.	22±2.160	22.33±2.624	53265±6259.83
L.culinaris Medic	22.33±3.299	29.76±6.798	70755±16214.88
P. vulgaris L.	19.67±1.699	22.66±1.885	54060±4497.19
S. orientale L.	20±1.632	20.66±1.247	49290±2974.618
T. aestivum L.	18.33±1.699	21.33±2.624	50880±6259.83
V.unguiculata L.	22.33±5.436	22±2.160	52470±5152.18
Z. mays L.	19.33±2.054	23.66±3.091	56445±7372.52
Fallow	21±3.559	19.66±1.699	46905±4053.721

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

Comparison of seed bank in soil before sowing and after harvesting of different test crops showed that the number of *Orobanche* seeds reduced in all cases and in fallow poly-bag and plots too.

4.3.1 Impacts of test crops on *Orobanche* seed bank in poly-bags

The number of *Orobanche* seeds/100g soil in pre and post harvesting of different test crops including fallow poly-bag is presented in **fig 4.2**. This shows that number of *Orobanche* seeds get reduced in most of the test crops.

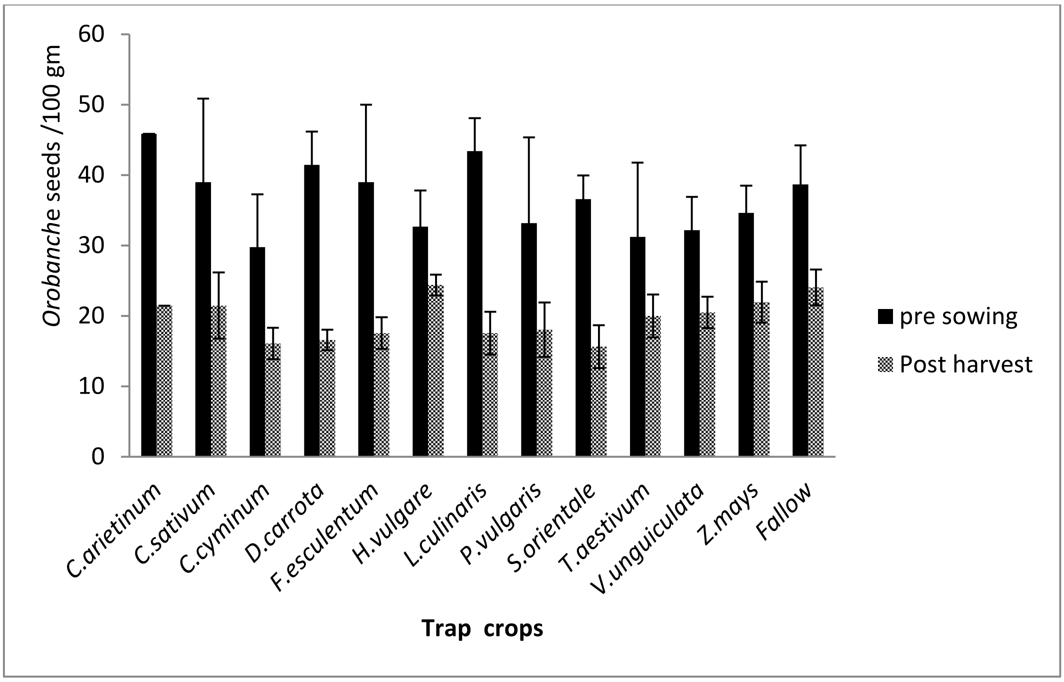

Figure 4.2: Number of *Orobanche* seeds per 100 g soil of pre-sowing and post-harvest of test crops in poly-bag experiment (n=9)

Lowest percentage of reduction of *Orobanche* seed bank was in *H. vulgare* (31.33± 4.163 %) and highest percentage of reduction was *C. arietinum* (56.33± 4.041). Out of 12 crops investigated, seed bank was reduced significantly (P= 0.05) in *L. culinaris, C. arietinum, V. unguiculata* and *F. esculentum*

Test crops like coriander (*C. sativum*), wheat (*Triticum aestivum*), carrot (*Daucas carrota*), cumin (*Cuminum cyminum*) and maize (*Zea mays*), (**Table 4.2)** showed reduction in *Orobanche* seed density but insignificantly at P=0.05 when compared with control plots. The percentage reduction of *Orobanche* seeds in crops like*, H. vulgare, S. orientale* and P. *vulgaris* were more or less same as in control plots, hence showed same grouping in Duncan's multiple range tests followed after ANOVA.

Table 4.2: Reduction (%) of *Orobanche* seed density in different test crops grown in poly-bags (n=9).

Test crops	Common name	% Reduction in seed density Mean ± sd
C. arietinum L.	Chickpea	56.33± 4.041C
C. sativum L.	Coriander	40.33± 10.59 ABC
C. cyminum L.	Cumin	47.67± 5.508 BC
D. carrota L.	Carrot	43.33± 9.609 ABC
F. esculentum Moench	Buckwheat	52.33±10.116 C
H. vulgare L.	Barley	31.33± 4.163 A
L. culinaris Medic.	Lentil	54.33 ± 7.024 C
P. vulgaris L.	French bean	32.67± 15.30 A
S. orientale L.	Sesame	32.00± 17.09 A
T. aestivum L.	Wheat	43.00 ± 7.937 ABC
V. unguiculata L.	Cow pea	50.01± 4.583 C
Z. mays L.	Maize	49.67± 2.517 BC
Fallow	Control	33.00± 3.00 A

(Same letter followed after the mean ± standard deviation do not differ significantly at P= 0.05 according to Duncan's Multiple range tests followed after ANOVA)

4.3.2 Impact of tests crops on *Orobanche* seed bank in fields

Number of *Orobanche* seeds per meter square recorded before and after harvest of different test crops in field is shown in **fig 4.3**. Comparing seed bank data of before and after harvest showed that *Orobanche* seed density was found to be reduced in all test crops including fallow.

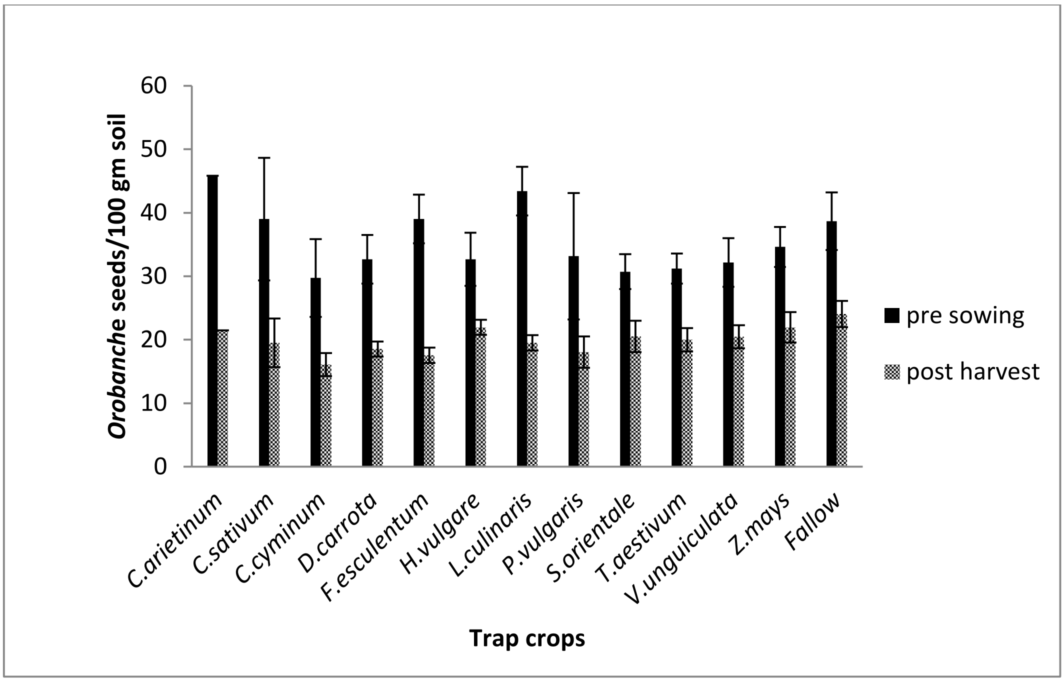

Figure 4.3: Number of *Orobanche* seeds per 100 g soil of pre-sowing and post-harvest of test crops in field (n=9).

Lowest percentage of reduction of *Orobanche* seed bank was in *H. vulgare* (25.16±4.55%) and highest percentage of reduction was in *L. culinaris* (57.59±13.7). Out of 12 crops investigated, seed bank was reduced significantly (P= 0.05) in *L. culinary, C. arietinum* and *F. esculentum*

Plants like *C. cyminum, D. carrota, C. sativum* and *P. vulgaris* (**Table 4.3)** showed reduction in *Orobanche* seed density but insignificantly at P=0.05 when compared with control plots. The percentage reduction of *Orobanche* seeds in crops like *H. vulgare, Z. mays, T. aestivum, S. orientale* and *V. unguiculata* were more or less same as in control plots, hence showed same grouping in Duncan's Multiple range tests followed after ANOVA.

Table 4.3: Reduction Percentage of *Orobanche* seed density in different test crops grown in fields (n=9).

Test crops	Common name	% Reduction in seed density Mean ± sd
C. arietinum L.	Chickpea	52.78± 5.99 CD
C. sativum L.	Coriander	44.30± 5.47 BC
C. cyminum L.	Cumin	45.52± 4.06 BCD
D. carrota L.	Carrot	45.95± 5.94 BCD
F. esculentum Moench	Buckwheat	54.58±5.99CD
H. vulgare L.	Barley	25.16± 4.55 A
L. culinaris Medic.	Lentil	57.59 ± 13.7CD
P. vulgaris L.	French bean	45.83± 4.17 BCD
S. orientale L.	Sesame	36.40± 3.99 AB
T. aestivum L.	Wheat	35.61 ± 5.11 AB
V. unguiculata L.	Cow pea	34.61± 5.22 AB
Z. mays L.	Maize	36.08± 7.99 AB
Fallow	Control	38.16± 8.37 AB

(Same letter followed after the mean ± standard deviation do not differ significantly at P= 0.05 according to Duncan's Multiple range tests followed after ANOVA)

CHAPTER-V

DISCUSSION

5.1 *Orobanche* seed bank estimation

The importance of seed bank study 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/w) $CaCl_2$ solution of specific gravity 1.3957 for quantitative detection of *Orobanche* seeds in infested soil.

The technique used in the present study principally follows the sieving and flotation technique of Ashworth (1976) with some modification (Khattri, 1997; Acharya *et al*., 2003) to isolate the *Orobanche* seeds from heavier organic particles. The data obtained in present study indicated that recovery percentage of *Orobanche* seeds from soil depend on texture of the soil. Larger soil particles showed highest percentage as compared to smaller particles. This result was similar to the findings of Acharya (2002) which showed that the recovery was high in sandy and low in clay soil. The statistical analysis ANOVA followed by Duncan's Multiple Range Test showed that among four types of soil tested for seed standardization process, the percentage recovery was highest significantly in sandy soil than in other three types of soil like sandy loam, loam and clay. 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 with nearly similar size to *Orobanche* seeds, which not only creates error in the seed recovery but also made seed counting difficult.

5.2 Investigation of trap crops

In present study altogether 12 different plant species were studied in poly-bag and field experiments, with fallow as control. 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. *Orobanche* seed bank in soil was counted before sowing and after harvest of different crop in poly-bag and fields. In most of the cases the seed bank reduction were found to be higher in poly-bag experiment than in field experiments. The findings of *Orobanche* seed bank reduction in soil after sowing of different crops have been discussed below in each crops investigated:

5.2.1 Control poly-bag/plots (Fallow)

In the present study, it was interesting to note that there was some reduction in *Orobanche* seed bank in fallow poly-bag and plots also. This might be due to 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 (i), some empty (without embryo) and some broken possibly by soil insects.

5.2.2 *Cicer arietinum* L. (Chickpea)

Chickpea is a common host of *O. aegyptiaca, O. ramosa* and *O.cernua* (Parker, 1986). The crop was tested in both polybag and field in which significant reduction was observed than that of control. Although this crop has been reported as host in other countries, but not a single crop plant was found parasitized by the *Orobanche* in the present study, indicating that the *Orobanche* species present in Nepal is physiologically different from other species found elsewhere. Data of seed bank clearly indicate that chickpea can be used as a trap crop in crop rotation in order to reduce *Orobanche* in infested field. Its cultivation also increases soil nitrogen content which may reduce *Orobanche* emergence as suggested by Abu Irmaileh (1994) in *O. ramosa.*

5.2.3 *Coriandrum sativum* L. (Coriander)

There are some reports of using this crop as a trap crop for the different species of *Orobanche* like *O. cernua, O. mutely, O. crenata and O. minor* (Foy *et al*., 1989; Sauerborn, 1991). Schnell *et al*. (1994) reported that *Coriandrum sativum* can reduce 19.0% seed bank of *O. crenata* in pot experiment.

In the present study, the reduction of *Orobanche* seed bank with cultivation of coriander were found to be reduced insignificantly than in control plots or poly-bag. Thus from our experimental data, it is clear that coriander cannot be used as potential trap crop.

5.2.4 *Cuminum cyminum* L. (Cumin)

The reduction of seed bank of *O. crenata* in pot experiment with cultivation of cumin was found to be 21.7% (Schnell *et al*., 1994) but there was no record of testing the crop plants with *O. aegyptiaca*. Cumin when tested in pot in infested tori field in Vedabari and Beldiha of Nawalparasi showed significant reduction of seed bank than that of control and is capable of inducing *O. aegyptiaca* (Acharya, 2002). But in the present study, the reduction of *Orobanche* seed bank was found to be insignificant. It might be due to presence of *O. cernua* abundantly in infested tomato field of Lalbandhi which were not stimulated by root exudation of cumin. So it cannot be considered as possible trap crop.

5.2.5 *Daucas carrota* L. (Carrot)

Carrot has been described as potential trap crop to reduce *O. aegyptiaca* (Foy *et al*., 1989). The area with carrot cultivation is found to be infested with *O. ramosa* and *O. crenata* and causes yield losses up to 75% (Kamel, 2005). In present study, the potentiality of this crop was tested in poly-bag and field condition. As it was regarded as host of *Orobanche* but in present study plant was found neither parasitized nor caused suicidal germination of *Orobanche* seeds indicating that the *Orobanche* species present in Nepal is physiologically different from the species found elsewhere which show insignificant reduction in seed density. The experimental data clearly shows that carrot cannot be used as a trap crop in crop rotation in order to reduce *Orobanche* seeds in infested field of tomato.

5.2.6. *Fagopyrum esculentum* Moench. (Buckwheat)

This crop plant is commonly known as pseudo-cereals. It was tested in pot and field condition in the area having *O. aegyptiaca* problem in infested tori field of Nawalparasi but showed insignificant reduction in seed bank (Acharya, 2002). In the present study, when studied in both poly-bag and field it shows significant reduction (P=0.05) compared to control plots in *Orobanche* seed bank. The possible reason might be it is capable in inducing suicidal germination of *Orobanche cernua* seeds in Lalbandhi. This plant can be used as vegetable also. Thus, buckwheat can be used as possible trap crops in *Orobanche* infested tomato fields.

5.2.7 *Hordeum vulgare* L. (Barley)

Barley is referred as an occasional host of *O. aegyptiaca* (Khattri *et al*., 1991).Barley showed significant reduction in seed density of *Orobanche* in infested tori field in Vedabari and Beldiha of Nawalparasi (Acharya, 2002). In present study, the percentage reduction in *Orobanche* seeds in soil after cultivation of barley was insignificantly different than reduction in control. The possible reason for this might be due to difference in species of *Orobanche* i.e in the infested tomato field *O. cernua* is prevalent. So, there is no possibility of using barley in rotation as a trap crop for infested tomato field.

5.2.8 *Lens culinaris* Medic. (Lentil)

Lentil is referred as an usual host of *Orobanche* which was mainly attacked by *O. aegyptiaca*, *O. muteli* and *O. crenata* (Foy *et al*., 1989; Parker, 1986). Due to broomrape infestation, lentil yield losses up to 95% have been reported depending upon the severity of infestation and the planting date (Rubiales *et al*., 2009).

According to Khattri (1997) lentil is a rare host of *O. aegyptiaca* in Nepal. According to him parasitization in the lentil was limited to subsoil stage only. This study supports the finding of many workers like Acharya (2002); Sauerborn (1991); Kleifeld *et al.* (1994) and Schnell *et al.* (1994). Lentil as a catch crop reduces number of shoots of *O. crenata* by 50% is very important for controlling this species in the Mediterranean region of Turkey. The growing of lentil two month before the sowing of lentil as a crop can be a main element in integrated management which causes to reduce the soil seed bank of *crenata* broomrape (Aksoy *et al*., 2016). In the present study crops was not found to be parasitized by *Orobanche* but was found useful in reducing *Orobanche* seed bank significantly. It was grown as a pure and mixed crop together with tori in infested areas of Nawalparasi. As it is a leguminous plant which stores the fertility of soil by fixing atmospheric nitrogen can be used for crop rotation. So it can be used as trap crops in *Orobanche* infested tomato field also.

5.2.9 *Phaseolus vulgaris* L. (French bean)

French bean is regarded as potential trap crop for *O. aegyptiaca* and *O. crenata* which was able to germinate 30% seeds of them (Sauerborn, 1991). In the present study it was tested in both polybag and field experiment. The results obtained in poly-bag and field experiment did not support the outcomes of Sauerborn (1991) as there was insignificant reduction of *Orobanche* seed density. So it cannot be used as possible trap crop in crop rotation in studied area.

5.2.10 *Sesamum orientale* L. (Sesame)

Sesame is considered as a potential trap crop for *O. aegyptiaca* which can be able to stimulate broomrape biomass by 86% in broomrape infested field (Sirwan *et al*., 2010). In the present study there was no significant reduction in *Orobanche* seed bank both in poly-bag and field after sesame cultivation. The above finding showed that sesame cannot be used as trap crop in infested tomato field of Nepal.

5.2.11 *Triticum aestivum* L. (Wheat)

Wheat is considered as false host of *O. minor* and has the potential to be implemented into an integrated *O. minor* management system (Ross *et al*., 2004).

Although it is reported as host of *Orobanche* species, but in present study not a single plant was found neither parasitized nor caused significant reduction in *Orobanche* seed bank. So there are no possibilities of using wheat in rotation as a trap crop in infested tomato field.

5.2.12. *Vigna unguiculata* L. (Cow pea)

Cow pea is considered as usual host of root parasitic plant like *Striga* spp. It is found that cow pea has been infested by *O. aegyptiaca* and *O. crenata* (Schnell, 1989). In present study, it was tested in both poly-bag and field. *Orobanche* seed bank was found to be reduced significantly in polybag after cow pea cultivation but insignificantly in fields. Higher moisture content associated with concentrated root density could be possible reason for the greater reduction of the parasitic weed seed reduction in poly-bag than in fields. From the present study it was found that if cow pea is grown densely than the normal, it may act as an effective trap crop for reducing seed bank and being a leguminous plant also increases nitrogen contents of field.

5.2.13 *Zea mays* L. (Maize)

Monocots are generally regarded as non-host plant of *Orobanche* species. Girma *et al.* (2005) reported maize as potential trap crop which showed better performance in stimulating germination of *Orobanche* seed and depleted soil seed bank of *O. cernua* and *O. ramose* by 72.5%. In present study maize was able to reduce *Orobanche* seed bank compared to fallow but insignificantly when tested in both poly-bag and field experiment. So, it cannot be considered as potential trap crop in infested tomato field.

CHAPTER-VI

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Quantitative estimation of *Orobanche* seeds from the infested soil has been one of the major problems in *Orobanche*. In the present study a method given by Ashworth (1976) with some modification given by Khattri (1997) and Acharya *et al*. (2003) was used to make it less expensive. This method was found to be fairly simple, easier and quickly reliable. The present seed estimation technique showed that recovery of seed depended on soil texture and amount of organic matter present in soil.

From the investigations for trap crops in poly-bag and field clearly showed that crops like lentil (*Lens culinaris*), chickpea (*Cicer arientinum*) and buckwheat (*Fagopyrum esculentum*) ranked highest among the crops which reduce *Orobanche cernua* seed bank significantly.

On the basis of results obtained from *Orobanche* seed bank study in both poly-bag and field, the crops investigated could be listed in one of the following categories:

Highly potential trap crops: *Lens culinaris, Cicer arientinum* and *Fagopyrum esculentum*

Moderately potential trap crops: *Zea mays*, *Daucas carrota*, *Cuminum cyminum, Hordeum vulgare*, *Coriandrum sativum* and *Vigna unguiculata*

Non-potential trap crop: *Triticum aestivum*, *Phaseolus vulgaris* and *Sesamum orientale*

6.2. Recommendations

Further extensive repeated field trails with these potential trap crops are recommended before suggesting to the farmers.

From the present studies it can be recommended to farmers that any of the crops listed in the category of highly potential trap crops (lentil, chickpea and buckwheat) could be used in crop rotation and which help to reduce *Orobanche* seed bank in infested tomato field of Lalbandhi, Sarlahi.

It is suggested to collect and burn the shoots of *Orobanche* after harvesting tomato.

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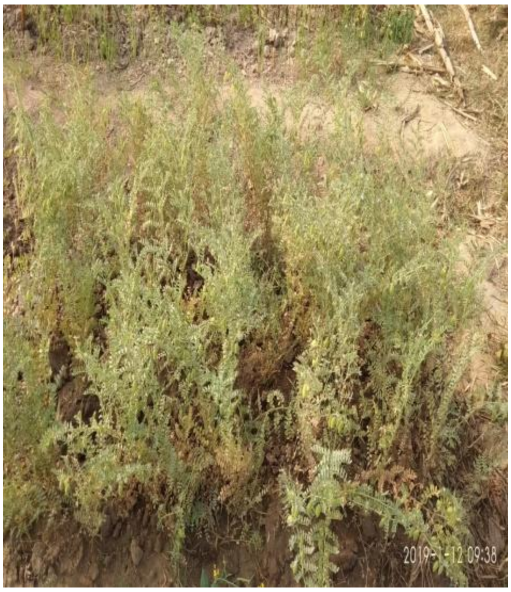
ANNEXES

Number of post-harvest *Orobanche* seed bank in 100 gm soil of poly-bag and seed in 100 gm soil and seed per sq.m of field experiments.

Non-host crops	**Poly-bag experiments**	**Field experiments**	
	No of post-harvest seeds in 100gm soil	**No of post-harvest seeds in 100gm soil**	**Seed per sq.m**
C. arietinum L.	11±2.449	14.67±2.624	34980±6259.83
C. sativum L.	11±0.816	13.33±1.247	31800±2974.61
C. cyminum L.	10.67±0.471	11±0.816	26235±1947.34
D. carrota L.	11±0.816	12±0.816	28620±1947.34
F.esculentum Moench	9±0.816	12±0.816	28620±1947.34
H. vulgare L.	15±0.816	15±0.816	35775±1947.34
L.culinaris Medic	10±1.632	13.33±1.697	31800±4053.72
P. vulgaris L.	13±1.632	12.33±1.699	29415±4053.72
S. orientale L.	13.33±1.697	13.33±1.247	31800±2974.61
T. aestivum L.	10.33±1.247	13.67±1.247	32595±2974.61
V. unguiculata L.	10.67±1.699	14±1.632	33390±3894.68
Z. mays L.	9.67±1.247	15±1.414	35775±3372.89
Fallow	14±2.828	12.67±2.054	30210±4900.70

PHOTO PLATES

A **B**



C **D**

Photo plates: (A) Showing different test crops in sapling stage (B) Showing *Zea* at Matured stage in poly-bag (C) Irrigating test crops (D) *Cicer* at fruiting stage

E F

G H

Photo plates: (E) *Triticum* at fruiting stage (F) *Fagophyrum* at flowering stage (G) Flowering Cumin at poly-bag (H) Hand wedding test crops