

# Impact of Cropping Patterns on seed bank and emergence of *Orobanche* in infested field.



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

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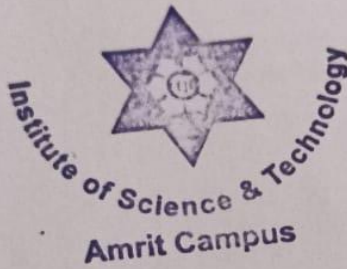
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DEPARTMENT OF BOTANY  
AMRIT CAMPUS  
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SEPTEMBER 2021

## DECLARATION

I, "Maya Karn", hereby declare that the work enclosed here is entirely my own, except where states otherwise by references or acknowledgements, 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

This is to recommend that the Master's thesis entitled "Impact of Cropping Patterns on seed bank and emergence of *Orobanche* in infested field at Lalbandi, Sarlahi" is carried out by "Maya Karn" 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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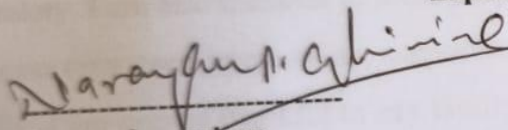
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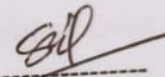
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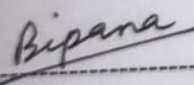


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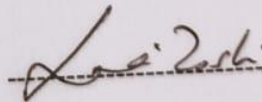
  
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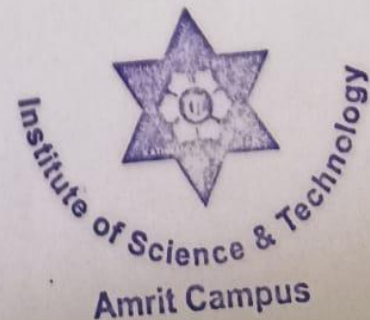
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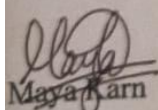
## ACKNOWLEDGMENTS

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

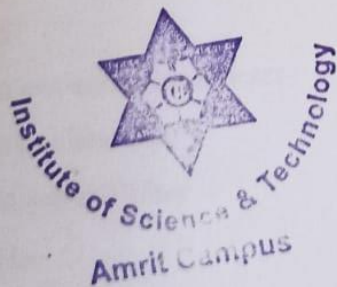
I am grateful to Dr. Shila Singh, Head of the Department, and Dr. Laxmi Joshi Shrestha, Coordinator, M.Sc Programme, Department of Botany, Amrit Campus, Tribhuvan University, for their support during the administrative work. I am also grateful to Prof. Dr. Kanta Poudyal, former Head, Department of Botany, Amrit Campus, Tribhuvan University, for her valuable suggestions, ever encouraging and motivating guidance.

My deep gratitude to Prof. Dr. Mukesh Kumar Chettri, former Coordinator, M.Sc Programme, Department of Botany, Amrit Campus, Tribhuvan University for his valuable suggestions, encouragements and guidance during field work and data analysis.

I would also like to thank all the teachers and staff, especially to Ms. Chandrika Singh former officer in-charge of Botany department, who helped me during the seed estimation in the laboratory. I am also thankful to local people/farmers of Tulsi Tole, Lalbandi-8, Sarlahi for their immense responses, interactions, supports and sharing their information about the field history. Finally, I am greatly thankful to my family member for their moral support, encouragement and financial support.

  
Maya Karn

Date: 26/08/19



## ACRONYMS AND ABBREVIATION

%	: Percentage
°C	: Degree Celsius
μ	: Micrometer
AHAS	: Acetohydroxy acid synthase
ALS	: Acetylcho line sterase
ANOVA	: Analysis of Variance
ATG	: Agricultural Technology Center
Avg	: Average
CaCl <sub>2</sub>	: Calcium chloride
cm	: Centimeter
DAP	: Diammonium phosphate
DHM	: Department of Hydrology and Meteorology
DMRT	: Duncan's Multiple Range Test
E	: East
EPSP	: Enzyme 5-enolpyruvyl shikimate-3-phosphate
FAO	: Food Agricultural Organization
Feb	: February
FGD	: Focus Group Discussion
Fig.	: Figure
g	: Gram
g/cm <sup>3</sup>	: Gram per cubic centimeter
g/hect	: Gram per hectare
g/ml	: Gram per milliliter
i.e.	: That is
kg	: Kilogram
kg/hect	: Kilogram per hectare
KII	: Key Informant Interview
m	: Meter
m <sup>2</sup>	: Meter square
MgSO <sub>4</sub>	: Magnesium Sulphate

mm	: Millimeter
N	: North
NARC	: Nepal Agricultural Research Council
No.	: Number
Oct	: October
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

*Orobanche* is a genus of more than 200 species of parasitic herbaceous plants in the family *Orobanchaceae* that cause damages to the important vegetables and field crops in many parts of the globe including Nepal. *Orobanche* is a problematic parasitic weed in tomato and brinjal fields of Lalbandi, a major tomato growing areas in Nepal. *Orobanche* emergence in the early sown six tomato and one brinjal fields, and in late sown six tomato and one brinjal fields were recorded along with the past history of each fields. The soil samples from these seven early sown and seven late sown fields were collected for the *Orobanche* seed bank study. The *Orobanche* seed bank study was conducted using the flotation technique of Ashworth (1976) with some modification (Acharya *et al.*, 2003). From this study on *Orobanche* emergence at early and late sown tomato and brinjal fields, it can be concluded that the severity of the parasitic weed in a field cannot be ascertain with the *Orobanche* shoot emergence alone.

The *Orobanche* seed bank study gave the real picture of parasitic weed infestation in the field. In the early sown tomato fields F7, *Orobanche* seeds/100g soil was significantly lowest, and this field had a history of only cultivation of cucumber for last 5 years with no winter crops and tomato was grown only after five years as winter crop. This clearly indicates that gapping of tomato cultivation reduces *Orobanche* seed bank in the fields. Similarly, reduction in *Orobanche* seed bank was also recorded in early sown fields like F1 and F4, where hand pulling of *Orobanche* shoot was in practice, as well as have the field history with the intervention of crop rotation with lentil and chickpea, respectively. Among the late sown fields of tomato and brinjal, *Orobanche* seeds/100g soil was found to be less than in early sown fields. The *Orobanche* seeds/100g soil in late sown fields was significantly ( $P=0,05$ ) low in F5 and F1 which had a history of growing only paddy for last 3 years and 2 years, respectively (with no winter crop), and then tomato was cultivated as winter crop after 3 and 2 years, respectively. This indicated that gapping the cultivation of host plants for some years also helps in reducing the seed bank of parasitic weeds. Beside this when *Orobanche* seed bank/100 g soil was compared between the paddy and maize fields of early and late sown tomato/brinjal fields, most of the paddy fields were recorded to have less *Orobanche* seed bank than at the maize fields. From the *Orobanche* seed bank study of early and late sown tomato and brinjal fields it can be concluded that late sowing helps in reducing the *Orobanche* seed bank in soil.

**Keywords:** *Orobanche*, cropping pattern, seed bank and quantitative estimation.

## Chapter-1 Introduction

### Background

About 4000 species of flowering plants have adapted to parasitize other plants (Rubiales, Fernandez-Aparicio, 2012). Parasitic plants fall into two classes, root or stem parasitic weeds, according to the host organs with which they are associated. Among the root parasitic plants, the root parasitic *Orobanchaceae* (*Striga spp.*) and broomrape (*Orobanche and Phelipanche spp.*) parasitize crops of economic importance, vegetables, and oil plants. It is the most destructive pest of agricultural weeds worldwide (Parker, 2009). The various broomrape (*Orobanchespp.*) are obligatory root parasites lacking chlorophyll, which parasitize on broadleaf plants and cause severe damage to vegetables and other field crops, both in Israel and abroad (Parker and Riches, 1993).

*Orobanche* lack chlorophyll and are completely dependent on other plants for nutrition, i.e. the holoparasite. Broomrape seeds remain dormant in the soil, often for many years, until they are stimulated to germinate by specific compounds produced by suitable host roots (Foy et al; 1989). Once the haustorium attaches to a host, it draws water and nutrients from its host through the haustorium. Some species are only capable of parasitizing a single plant species, such as the Ivy broomrape *Orobanche hederiae*, which is restricted to parasitizing ivy, and these species are often named after the plant they parasitize. Others can infect several genera, such as the lesser broomrape *O.minor*, which lives on clover and other related Fabaceae (Brown et al, 1952). Host crops of *Orobanche* species are different, for instance *O. aegyptica* parasitize crops like are cotton, crucifers, cucurbits, eggplant, potato, tobacco, and tomato; *O.cernua* parasitize crops sunflower, tobacco and tomato; *O.mutella* parasitize eggplant, tobacco and tomato; *O. ramosa* parasitize tomato; *O.brassicae* parasitize cabbage and tomato (Sauerborn, 1991).

### 1.2 Worldwide distribution and host range of *Orobanche spp.*

*Orobanche* is a genus of more than 200 species of parasitic herbaceous plants in the family *Orobancheaceae* that cause damages to the most important vegetables and field crops in the Mediterranean region of the Middle East (Pieterse, 1979; Saghir and Dastgheib, (1978). They are parasitic on a wide range of plant families (Asteraceae, Fabaceae, Solanaceae, Apiaceae an

Cucurbitaceae) (Linke et al., 1989; Suh et al., 2014). Yield losses range from 5 to 100% depending on the region and crop (Joel et al., 2007). It has been recorded among the five most serious types of weeds in at least 14 countries, which include Afghanistan, Cuba, Egypt, Hungary, India, Iran, Italy, Jordan, Lebanon, Nepal, Pakistan, Saudi Arabia, Turkey and Uganda mostly native to the temperate northern hemisphere (Holm et al., 1971). However, a few species are also found in other parts of the world. The countries, where heavy crop losses have been reported due to different species of *Orobanche* are Russia, Ukraine, Turkey, Spain, Eastern Europe and the Eastern Mediterranean (due to *Orobanche cumana*); Pakistan, India, Nepal, Arabia, and Egypt (due to *Orobanche cernua*); Nepal, Cuba, Chile, France and Australia (due to *Phelipancheramosa*) (Parker, 2009). It is a threat to about 16 million hectares of agricultural land which represents about 1.2% of the world's total arable land (Sauerborn, 1991b). Most *Orobanche* species have multiple hosts, and a single crop host can be infected with multiple *Orobanche* species. The single genus of *Orobanche* is separated into two genera *Phelipanche* and *Orobanche* by morphological tools and genetic evolution (Joel, 2009).

### **1.3 *Orobanche* species in Nepal**

In Nepal, eight species of *Orobanche* have been 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. (Press et al., 2000). There has been a limitation in the production of two important cash crops, namely tori (*Brassica campestris* var. toria) and tobacco (*Nicotiana tabaccum*) crops in Nepal (Rao et al. 1988, Jacobsohn et al. 1989). Tomato cultivation in Nepal is also affected by *Orobanche spp* namely *O.aegyptiaca*, *O.cernua* and *O. solmsii*. Tomato cultivation in Nepal is about 20,000 hectares, with an average production range of 15 tons per hectare in the fiscal year 2013/2014 (MoAD, 2014), and increased to 22,566 hectares of cultivated area with a productivity of 18 tons per hectare in the year 2018/19 (MoALD, 2020).

Though there is no accurate data regarding the distribution of *Orobanche* in Nepal. According to Khatri, (1997) *Orobanche aegyptiaca* is found in all ecological regions of Nepal from tropical parts of Terai and inner Terai to temperate zone up to 3800 m altitude. *Orobanche* occurrence was high in the mustard and tobacco cultivated fields. It was also common in the Kathmandu valley in the Kalo Tori field (*B. campestris* var. *nigra*) near Badikhel, Godawari. *Orobanche* species have

the specificity of their host but some *Orobanche spp.* parasitizes more than one host. *Orobanche* attacks twenty-three species of plants belonging to seven families of flowering plants in Nepal (Table 1.1).

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

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

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

#### 1.4 Morphology and Life cycle

Broomrapes are usually small, 10-60 cm long, depending on the species. It is best recognized by its yellow to straw-colored stems that completely lack chlorophyll, and bears yellow, white or blue flowers resembling snapdragon. The flower buds are scaly, with a bushy terminal height of ten to twenty flowers in most species, although single in a single flower broomrape (*Orobanche uniflora*). Leaves are triangular scales and without chlorophyll. The seeds are small dust like (0.25-0.35 mm), tan to brown, and turn black with age. These plants generally bloom from late winter to late spring. No part of the plants can be seen when they are not flowering above the soil surface (Cubero and Moreno 1979).

Life cycle of *Orobanche* and *Phelipanche* spp. strongly related to that of its host. It consists of two phases- independent and parasitic life phase. In independent life phase in nature begins with seed conditioning, germination and last when it attach to the root of host. The seeds require preconditioning period of 2 to 14 days in a warm humid environment and subsequent exposure to a germination stimulator secreted by the roots of the host (Sato *et al.*, 2003). The parasitic phase start as soon as the haustorium develops. Broomrape seedlings develop root-like growths, the germ tube, which connects to the roots of neighboring hosts through the haustorium. It penetrates the living tissue of the host root and connects with the vascular tissues to form a physiological bridge for absorption of water and nutrients (Joel and Portnoy, 1998). At this stage the parasite are completely dependent on host for water and nutrients. After few days of underground development, the parasite emerges above the soil surface and develops flowering stems that produce seeds within a short period of time. In case of *Orobanche minor* life cycle is described in seven stages i.e (i) Seeds become responsive after a pre-incubation period at suitable moist temperatures. (ii) Seed germination is stimulated by host-derived stimulants including strigolactone. (iii) The growing roots attach to the rootstock of the host and develop the haustorium. (iv)The parasite penetrates the host epidermis and cortex tissues and connects to the vascular system. (v) The parasite absorbs water, minerals and photosynthetic structure from the host. (vi)The tubers of the parasite grow underground for several weeks or several months depending on the life cycle of host before the sprouts appear. (vii) The parasite produces a large number of seeds which remain viable for many years in the soil. (Bouwmeester *et al.* 2003).

## 1.5 Seed dispersal

There are various dispersal mechanisms by which *Orobanche* seeds can be easily transported from infested fields to non-infested fields. The dispersal of seeds takes place by 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 when it pass through the alimentary canal of live-stocks, so that it can easily infect the other fields through infected manure.

## 1.6 Seedbank

A single broomrape plant can produce more than 500,000 seeds and these seeds are known to persist for decades in the soil. This provides the parasite with a significant genetic capacity to adapt to environmental changes, including host resistance, agricultural practices, and herbicide treatments (Joel *et al.*, 2007). Most of the *Orobanche* seeds in the soil do not come in contact with the host root exudates (that stimulates seed germination), thus forming a seed bank for the next planting season. When the host crops are repeatedly planted, the seed bank in the soil increases exponentially resulting in the failure of the host crops.

There are several methods of seed bank study. One of the methods to study seed bank is of Ashworth, (1976), in which the seed bank can be studied by using sieving and differential floatation techniques using 40% (w/v) calcium chloride of specific gravity 1.3957. Khattri (1997) and Acharya *et al.*, (2003) slightly modified the technique of Ashworth (1976) to avoid the use of instruments likes centrifuge, millipore filter etc. for isolation of *Orobanche* seeds from heavier organic particles. Use of the equipment were compensated with floating the mixture containing *Orobanche* seeds and organic particles held on 100 $\mu$  sieve two times in CaCl<sub>2</sub> 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<sub>4</sub> 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.*, 1991a).

## **1.7 Different Control measures of *Orobanche***

Broomrape control is often difficult for various reasons and many control strategies have been tested over the years. The control methods used included changes in agricultural practices such as weeding, tillage, deep inversion, crop rotation, trap and catch crops, fertilization, and soil solarization, as well as chemical and biological control etc.

### **1.7.1 Cultural control**

#### **a. Weeding**

Hand weeding is one of the most common control methods in *Orobanche* as well as for weeds. Weeding begins only after two or three years when the infestation has already reached a high level. To prevent the spread of the parasite, it is important to start control measures at the beginning of the infestation. However, weeding reduces the production of new seeds, so the number of seeds can be reduced in the long run. In India, the number of emerging *Orobanche* shoots decreased from 366m<sup>2</sup> to 13m<sup>2</sup> in seven years (Vyas, 1966). Before flowering, weeding promotes the emergence of new *Orobanche* and *Striga* plants. ICARDA (1989) has also shown that the best time to remove weeds is after the *Orobanche* flower has fallen, but before the capsules and seeds have matured. Earlier weeding increases the number of emerging shoots and requires frequent weeding, which include high labour cost.

#### **b. Sowing date**

The degree of infection of the host plant with parasites is closely related to the date of cultivation of the crop. Farmers have long known that a late sowing date reduces infestation by *Orobanche* as well as *Striga*. According to Sauerborn (1991b), the late sowing crop have shown reduced attachment of *Orobanche* which is due to the environmental factors, mainly the temperature effects. The Kasasian (1973) reported that optimal germination temperature for *Orobanche* was between 18 and 23<sup>0</sup>C , only few seeds germinated at temperature below 8<sup>0</sup> C. In the past tomato

seeds were sown during August/September, but now a days they are sown up to September/October because of climate change. Similarly, all tomato phenology stages have been changed for 1 month because of climate change. In the past 30-40 years, tomato harvest has been between November/December to mid-February, while at present, it is during December/January to March (Bhandari *et al.*, 2021).

### **c. Trap and catch crop**

One of the best methods currently available for controlling agricultural root parasites is the use of trap and catch crops (Sauerborn, 1991a; Acharya, 2012, 2013, Acharya *et al.*, 2002, 2021). The trap crop is also known as the pseudohost, which stimulates the suicidal germination of parasite seeds but cannot be parasitized itself thus reducing the number of seeds in the soil. . Catch crops on the other hand are usual host which allow the development of the parasite. They are grown to stimulate a high percentage of parasite seeds to germinate, but are destroyed before the parasites bloom and develop seeds, so this also reduces the further addition of parasitic seed in the soil.

### **d. Intercropping**

Intercropping is a technique used for encouraging concurrent crop production and soil fertility building. There is a recharged interest in intercropping connected to the requirement for decreasing nitrogen cost and soil disintegration. Intercropping is generally utilized in locales of Africa as a minimal effort innovation of controlling the broomrapes (Oswald *et al.*, 2002). Recently 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. Maize and Snap bean were also complementing each other under inter-cropping and soil seed bank of *O. ramosa* and *O. cernua* were depleted by 72.5 per cent per season. Yield of tomato was significantly increased due to the reduction of *Orobanche* seed bank in the 3rd season (2004) (Abeb *et al.*, 2005). Field experiments observed that *O. crenata* contamination on *Vicia faba* and pea was reduced when these host crops were intercropped with oat. The number of *O. crenata* plants per host plant decreased as the proportion

of oats improved in the intercrop. Pot and rhizotron experiments confirmed the reduction of infection in *Vicia faba* intercropped with cereals. It was suggested allelochemicals released by cereal roots is the mechanism for reduction of *O. crenata* infection (Fernández-Apricio *et al.*, 2008).

#### **e. Solarization**

Soil Solarization based on covering the soil with transparent polyethylene sheets for several weeks under solar radiation, kills *Orobanche* seeds in the upper soil layers (Jacobson *et al.*, 1980). This is only effective when sunlight is sufficient and it is highly costly.

#### **f. Resistant varieties**

Resistant genotypes to host plant reproduction are among the most promising approaches to reducing losses from *Orobanche* invasion (Cubero, 1991). Resistant sunflower varieties were selected in Russia and Spain many years ago, and have been used successfully for decades. However, host plant resistance has been lost in many countries due to selection in *Orobanche* populations towards more aggressive biotypes adapted to newly introduced taxa (Antonova, 1994). This may also be the reason for the very limited practical success of introducing genetic resistance against *Orobanche* into other crops, such as beans, lentils and tomatoes. However, there are currently some resistance and some tolerant varieties of bean (Cubero, 1994; Monofi, 1994), sunflower (Cubero, 1994), and vetch (Cubero, 1991), which can be grown in *Orobanche* infested fields, but their preliminary investigation with local variety of parasites is needed.

### **1.7.2 Chemical control**

The chemical control of the broomrape has been widely used. It has been explored since the 1970s. However, this kind of control is complicated by a number of factors including: (i) Effective only as a prophylactic treatment, since in most cases we do not know the level of infestation; (ii) The parasite is directly related to the host; (iii) If the herbicide is applied to the parasite through the conductive tissue of its host, the host must be selective for herbicides without reducing their phytotoxicity; (iv) The parasite can germinate continuously throughout the season, new infections appear (Perez de Luc *et al.*, 2010).

Herbicides that are currently being used for broomrape are glyphosate and herbicides belonging to the imidazolinones (Eisenberg *et al.*, 2006a) or sulfonylureas. Glyphosate inactivates biosynthesis

of aromatic amino acids inhibiting key enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP). Imidazolinone and sulfonylurea herbicides inhibits the synthesis of acetylcholinesterase (ALS), also called Acetohydroxy acid synthase (AHAS), a key enzyme in biosynthesis of branched amino acids Isoleucine, leucine and valine. All of them are regular, the herbicides are absorbed through the leaves and roots of plants with a quick jump to the attached parasite serves as a strong trough (Colquhoun et al., 2006).

### **1.7.3 Biological control**

Biological control is particularly attractive in suppressing root parasitic weeds in annual crops because the intimate physiological relationship with host plants makes it difficult to apply conventional weed control measures. Both insects and fungi that attack parasitic angiosperms have been isolated. Muller-Struve *et al.*, (2009) reported that future biological control may lead to a reduction of *Orobanche*, with particular attention to research on fungi of the genus *Fusarium*, which breaks the haustorial connection between host and parasite.

Most of the insects reported to be present on *Orobanche* species are without any host specificity and thus damage to these parasitic weeds is limited (Klein and Kroschel, 2002). For biological control, oligotrophic and monophagous herbivorous insects are of interest. The *Phytomyza Orobanchia* fly (Diptera: Agromyzidae) has been reported to be host-specific and attack only *Orobanche* species. Its distribution is associated with the natural appearance of *Orobanche spp.* and *Smicronyx spp.* (Coleoptera: Curculionidae). These insects inhibit seed production by developing larvae inside the seed capsules of their target hosts and thus contribute to reducing their ability to reproduce and spread. However, research on both insects has revealed that their efficacy in preventing seed set is limited and would not be sufficient to significantly reduce the soil seed bank (Klein and Kroschel, 2002).

### **1.8 Justification**

*Orobanche*, the parasitic weeds absorb water and minerals from the host plant causing damage and obvious symptoms such as wilting, reduced plant size, reduced yield, and reduced quality. It is a serious problem globally and now it is creating a huge problem in Nepal. Lalbandi in Sarlahi district is highly infested with *Orobanche* species in cash crops such as tomato and brinjal, which may reduce crop yields up to 30-40 % (personal communication with NARC officials and local

farmers). Terai farmers are mainly affected by this problem, according to them up to 120-130 *Orobanche* shoots were found/per 1sq.m in severely infested tomato fields. Tomato plants in Lalbandi are experiencing drastic changes in climatic conditions and several diseases have been observed for a few years and are on an accelerating trend. In Lalbandi, the parasitic plant *Orobanche* is a big problem for farmers because they are economically dependent on tomato and/or brinjal cultivation.

*Orobanche* control method through change in crop rotation and shift in tomato plantation date has not been investigated to reduce the *Orobanche* seed bank. Therefore in the present study, an attempt has been made to investigate the severity of infection of *Orobanche* in early and late sown tomato fields and also with different cropping pattern. The results of this research will be helpful to farmers to find out the appropriate sowing date and cropping pattern in *Orobanche* infested tomato/brinjal fields to reduce its seed bank.

### **1.9 Research questions**

- a. Which sowing date of tomato is appropriate to reduce *Orobanche* emergence?
- b. What is the effect of cropping pattern on the seed bank of *Orobanche* in infested fields?

### **1.10 Objectives**

### **1.10.1 General objective**

- To evaluate the intensity of *Orobanche* emergence and seed bank in tomato/briinjal fields having different cropping pattern at Lalbandi.

### **1.10.2 Specific objectives**

- To estimate *Orobanche* emergence in the infested field with different cropping patterns.
- To evaluate the *Orobanche* emergence at different sowing dates of tomato/brinjal.
- To estimate *Orobanche* seed banks in different infested field at Lalbandi area.

## **Chapter 2: Literature review**

## Host range

More than 200 *Orobanche* species have been identified in the world (Linke et al., 1989), but only a few parasitize economically important herbs in crop systems. It is parasitic in a wide range of plant families (*Asteraceae*, *Fabaceae*, *Solanaceae*, *Apiaceae*, *Cucurbitaceae*). Muromical *et al.* (2008) showed that *Orobanche* plants not only reduce the above-ground mass of the tomato plant, but also have the effect of reducing the mass of chlorophyll in the leaf and decreasing the photosynthetic activity.

The problem of parasitic weeds has been well described by several authors during the past three decades. Root parasites *Striga* (witchweed) and *Orobanche* (broomrape) are predatory pests in both tropical and subtropical regions (Joel, 2000). Globally, parasitic weeds represent one of the most devastating and intractable problems for agricultural production in both developed and developing countries (Ali, 2007).

Generally the cultivated plants or crops can be categorized into three groups in relation to *Orobanche* seed germination. The first group is the cultivated plants that stimulate *Orobanche* seed germination and establish contact with a host plant afterwards, the second group is pseudohosts that stimulate germination without establishing contact with the host plant, and the third group is plants that do not stimulate the germination of *Orobanche* seeds. *Orobanche* causes severe direct damage to crop diversity as they absorb the water and nutrients of host through the haustorium. The extent of damage depends on the specificity of host/parasite relationships, and on the extent of the infestation. *Orobanche* is an obligatory root parasite that attacks the roots of both young and mature host plants. The parasite can only be seen on the roots of the host, and its flowering stem from the soil near the host plants. On the other hand, damage can be seen in any host organs (Lins *et al.*, 2006).

Some species of *Orobanche* that are particularly destructive in the Mediterranean and in Western Asia and parasitize the important cash and food crops. *Orobanche aegyptiaca* and *O. ramosa* attacks mainly legumes, eggplant, tobacco and tomato (Sauerborn, 1991). The weed species in Central Europe is the most widespread of the family *Orobancheae* causing great damage to many crops, especially tobacco, hemp and tomato.

A survey was conducted by FAO Project in 1993, indicating that broomrapes (*Orobanche spp.*) are aggressive parasitic weeds that attack strategic food crops, such as legumes and vegetables, and threaten the livelihoods of many nations. *Orobanche* problem often force farmers to abandon the cultivation of more profitable host crops. Eight species of *Orobanche*(*Orobanche cumana* Wallr., *O. ramosa* L., *O. nana* Noe, *O. muteli* Sch., *O. minor* Sutt., *O. lutea* Baumg., *O. aegyptiaca* Pers. and *O. crenata* Forsk.). *Orobanche cumana* Wallr. (*O. cernua* Loefl.) were reported as a parasite in sunflowers and also frequently were found growing on the rootstock of tomatoes, tobacco and some weed plants (Kojic et al., 2001, Masirevic and Kojic, 2002). The range of crops affected by *Orobanche cernua* is very narrow, and includes Solanaceous crops such as tobacco, tomatoes, eggplant and potatoes. Tobacco is seriously affected by *Orobanche cernua* in India and Pakistan and locally in Jordan, Ethiopia, Saudi Arabia and no doubt a number of other countries in Western Asia. Tomatoes are known to be seriously affected by *Orobanche cernua*, currently found in Israel, Jordan, India and Ethiopia. Eggplant is moderately affected by *Orobanche cernua* in Israel, India and Ethiopia and potatoes are known to be affected locally in Jordan(Parker, 1994). In Nepal, *Orobanche cernua* on tobacco was reported by Sahu and Sinha (1983).

Lentils can be parasitized mainly by two different species, namely *Crenate broomrape* (*Orobanche crenata* Forsk.) and the *Egyptian broomrape*(*Phelipanche aegyptiaca* (Pers.) Pomel). Due to broomrapes infestation, up to 95% yield loss of lentil have been reported depending on the severity of infestation and planting history (Rubiales et al. 2009). Fields of red lentils located in southeastern Anatolia region of Turkey are highly infested with *O. crenata* and *P. aegyptiaca*/*P. ramosa*, and have been identified as mixed in 65% of the area. More than 10% of the fields are severely endemic and 25% are severely endemic(Sauerborn 1991; and Rubiales et al., 2009a). 50% and 80% average crop loss for fields were reported in highly infested fields. Economic losses of approximately 60 million Euro per year was estimated in Turkey (Uludag and Demirci, 2005). In the Middle East, annual losses from this parasitic weed was estimated to be 1.3-2.6 billion dollars (Ali, 2007).

## **Control measures**

According to Gold, et al., (1976), all species produce a very large number of small seeds that remain viable for many years, germinating only in the presence of a suitable host. Reducing the *Orobanche* soil seed bank by using natural hosts, for example beans, peas, lentils, which act as false hosts stimulate the parasite seeds to germinate, but are destroyed before the attachment. Flax (*Linum usitatissimum L.*), basil (*Ocymumbasilicum L.*) and coriander (*Coriandrum sativumL.*) also stimulate seed germination of *Orobanche*, but do not allow attachment or development of parasites (Menoufi, 1991; Khalaf 1992; Park A. W. Tharawat, 1993; Schnell et al., 1994). Cultivation of host species (beans, peas, chickpeas, lentils, etc.) with lupine (*Lupinus termis Forsk.*), turnip (*Brassica rapa L.*), fenugreek (*Trigonella foenum-graecum, L.*), oats (*Avena sativa L.*) etc. have been suggested, as root secretions of these non-host crops reduce the germination of *O.crenata* seeds by allelopathic mechanisms and thus reduce their attacks (Al-Menoufi et al., 1996; Bakheit et al., 2002; Rubiales et al., 2004; Fernàndez-Aparicio et al., 2006; Fernàndez-Aparicio et al., 2007a; Fernàndez-Aparicio et al., 2007b; Evidente et al., 2007).

The results of investigation on the various aspects of broomrape control clearly indicate different degrees of success in controlling the parasite. This provides scope for parasite management by incorporating several methods. Among them, tool control using 'spear' and 'mini-spear + leaf pusher', immersion, deep summer ploughing, crop rotation using trap (non-host crops) and catch crops (host crops) are the most promising crops in India. From these methods it was evident that total control of *Orobanche* species is very difficult, but reduction of this weed has been attempted through the use of trap crops (Krishnamurti et al., 1977).

Studies were conducted by Acharya et al., (2002) to assess the effects of non-host crops on *Orobanche* seed bank in infested tori field in Nawalparasi district, Nepal and reported that the native cultivar of *Brassica campestris*, could reduce the seed bank by 33%. Further, 21 different types of non-host crops were tested for reduction of *Orobanche* seed bank and the results categorized the tested crops into 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 (Acharya, 2012).

Several researchers indirectly examined the effect of temperature on *Orobanche* parasitism by changing the planting dates of the host, which were legumes in most of these studies. It was found that under a Mediterranean climate, delaying the sowing date of beans or lentils from autumn to winter reduced the infestation of *O. crenata* and *O. aegyptiaca*, possibly due to the delay in parasite development at the lower temperatures prevailing in winter. In most of these studies, temperature data were not presented (Linke et al., 1991; van Hzewijk, 1994; MesaGarcia and Garcia Torres, 1986; Kukula and Masri, 1984; Kukula et al., 1985; ter Borg, 1986). Germination of *Orobanche* seeds occurs after a period of preconditioning (humid temperatures suitable for several days) and exposure to germination stimulants secreted by the roots of the host (Sato et al., 2003). The seed was pre-conditioned, for example by exposure to a moist environment at about 18°C for 11 days, in order to be stimulated by the host plant to germinate (Kasasian, 1973, Bista 2015).

Tomatoes can grow under a wide range of climatic conditions. However, it is highly sensitive to hot and cold growing conditions, especially to the prevailing summer weather as in Bangladesh (Ahmed, 2002). Field experiments and a controlled environment have shown that tomatoes are resistant to *O. crenata* only at high temperatures (spring and summer) and are sensitive under low temperatures (Eizenberg *et al.*, 1998).

The use of trap and catch crops is one of the best methods currently available to control agricultural root parasites (Sauerborn 1991). The effect of trap crops on the management in tomato cultivation was studied in pot experiments using eight crops: Egyptian clover (*Trifolium alexandrinum*), sesame, mungbean (*Vigna radiata*), flax, brown Indian hemp (*Hibiscus cannabinus*), cotton (*Gossypium hirsutum*), pepper (*Capsicum annuum*) and black-eyed pea (*Vigna unguiculata*). In particular, sesame and brown hemp reduced *P.aegyptiaca* biomass and increased tomato yield and were found to be the best trap crops (Babaei S, Alizadeh H, Jahansouz MR, *et al.*, 2010).

To reduce *Orobanche* infestation in tomato fields of Lalbandi, Sarlahi, Acharya *et al.* (2021) investigated 22 non-host plants to identify potential trap crops in two fields and poly bags. On the basis of *Orobanche* seed bank reduction, the investigated crop species like *Capsicum frutescens*, *Cicer arietinum*, *Lens culinaris* and *Vicia faba* were classified as highly potential trap crops and reduced seed bank from 52 to 70%. Crops like *Cuminum cyminum*, *Daucus carota*, *Foeniculum vulgare*, *Phaseolus vulgaris*, *Sessamum orientale* and *Trigonella foenum-graecum* were classified

as moderately potential trap crops and reduced seed bank from 41 to 52 %. Among all the tested crops *Capsicum frutescens*, *Cicer arietinum*, *Lens culinaris* and *Vicia faba* were identified as the potential trap crop to reduce *Orobanche* seed bank in infested tomato fields.

According to Grenz et al. (2005) changing the planting date of agricultural crops also influences germination of *Orobanche* species. Delaying the ideal date of sowing up to two weeks decreases the germination of parasitic plant. In *Vicia faba* delaying the planting date from October to November, December or January decreased the emergence of both *O. crenata* and *O. foetida* as well as their dry weight. Garcia Torres, (1994) had also reported that, sowing sunflowers in early date reduced the infestation by *O. cumana*.

Different practices of soil management can severely affect seed banks, including broomrape seeds. Ploughing a trench 45-50 cm deep with a die plate plow reduces *O. ramosa* by 80-90 percent in Eastern European tobacco fields by burying seeds at depths where they are not likely to germinate. This method will have limited utility here due to the risk of erosion in light soils and the need to follow up with minimal tillage for several years. Minimal tillage can contribute to broomrape control by reducing the amount of viable seeds incorporated into the soil (Habimana et al. 2013).

Chemical control of *Orobanche* is very difficult and often ineffective. Soil fumigation with methyl bromide has been used effectively in the past, especially on light soils, but is no longer permitted for this purpose (Jacobsohn, 1984).

Current approaches to parasitic weed control focus on reducing the soil seed bank, preventing seed collection and inhibiting the movement of seeds from infected to uninfected areas (Fernandez Aparicio et al., 2008; Rubiales et al., 2009). The use of catch crops (Acharya et al., 2002) and trap crops (Acharya, 2012, 2021; Sauerborn, 1991b) have been suggested as tools to reduce broomrape in infected soils. Currently, there is no established and sustainable way to control the *Orobanche* anywhere else in the world (Parker and Riches, 1993).

### **Seed bank estimation methods**

The seed bank can be studied by using differential flotation and sieving techniques using 40% (w/v) calcium chloride with a specific gravity of 1.3957. Acharya et al (2003); Khattri, (1997) modified the method of Ashworth (1996) by avoiding the use of tools such as centrifugation,

Millipore filter, etc. to isolate *Orobanche* seeds from heavy organic particles. In another method, which was used by Visser and Wentzel, (1980), and Kachelriess (1987) 4 g of soil were mixed with 20 ml of a MgSO<sub>4</sub> solution with a specific gravity of 1.16 g/ml. This mixture was shaken for 90 seconds and then centrifuged at 2500 rpm. for 5 minutes. This allowed soil particles to precipitate while the seeds remained floated.

### **Research gap**

Tomato in Nepal is cultivated in both paddy fields (which remains inundated condition for some months) and maize fields (i.e bari -which have no irrigation facilities). *Orobanche* problem exists in both tomato growing fields. Because of prevailing climate change, the farmers in Lalbandhi have practices of growing tomato in early season (September) as well as late season (November –December) in terai of Nepal. From the review of the literature it is evident that *Orobanche* emergence and its seed bank study comparing early sowing and late sowing dates have not been conducted in tomato fields. Besides this, if the different cropping rotation in paddy and maize fields in terai has any impact on *Orobanche* soil seed bank has also not been studied. Hence these research gap of (a) studying *Orobanche* emergence in early and late sown tomato fields and (b) *Orobanche* seed bank in fields with different cropping patterns, have been identified for the present study.

## **Chapter-3 MATERIAL AND METHOD**

### **3.1 Description of the study area**

The study was conducted in and around Tulsitole, ward no 8, Lalbandi. Lalbandi is a Municipality in Sarlahi District in the Province 2, in South-eastern Nepal (Fig. 3.1 and 3.2). It is located at

27.0583° N, 85.6333° E. with an area of 64.9 square kilometers (Fig,1). About 45.5% of the total land area in the study area is agricultural fields. The soil in the study area is mostly sandy loam and loamy with cobbles and pebbles. It is stretched from Northern Pattharkot VDC in Chure Hills to Southern Jabdi VDC central Terai. The area is famous for tomato cultivation and supplies it to the whole country.

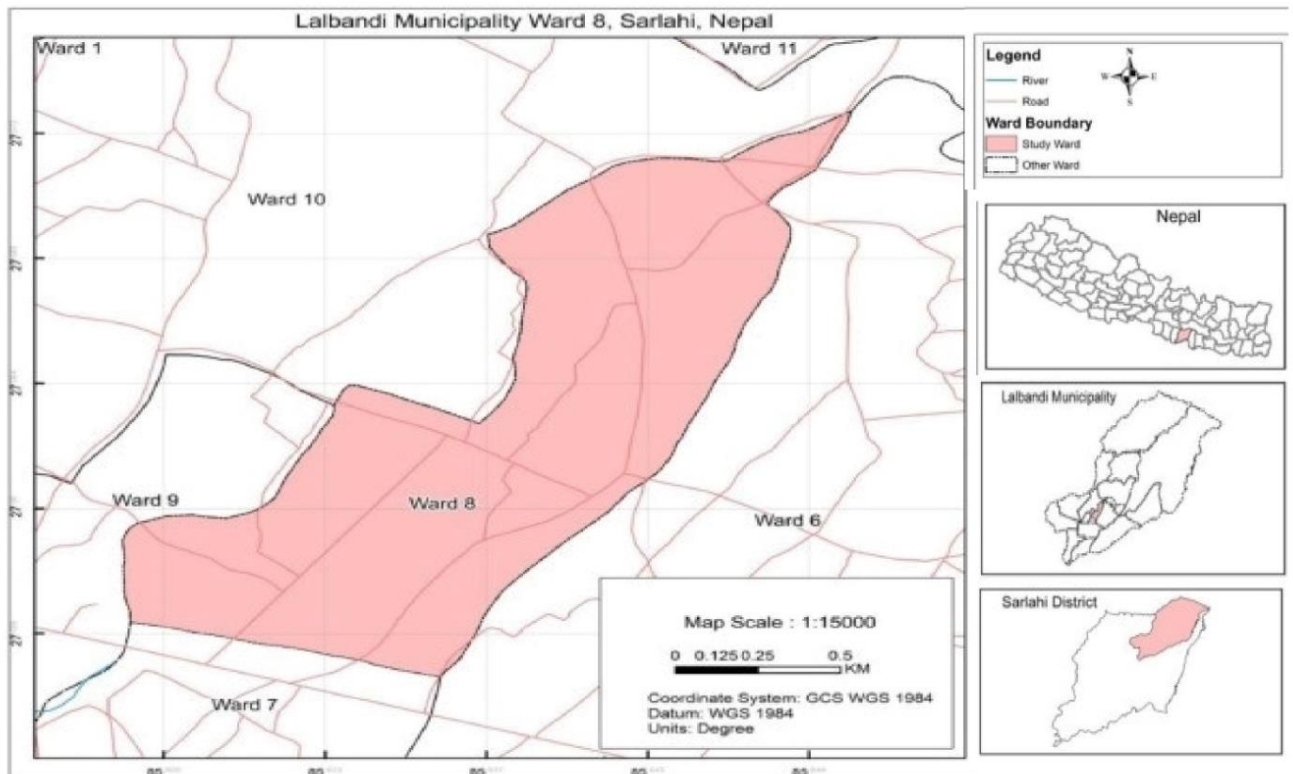


Fig 3.1:- Location map of the study area(Lalbandi).

## Lalbandi field map

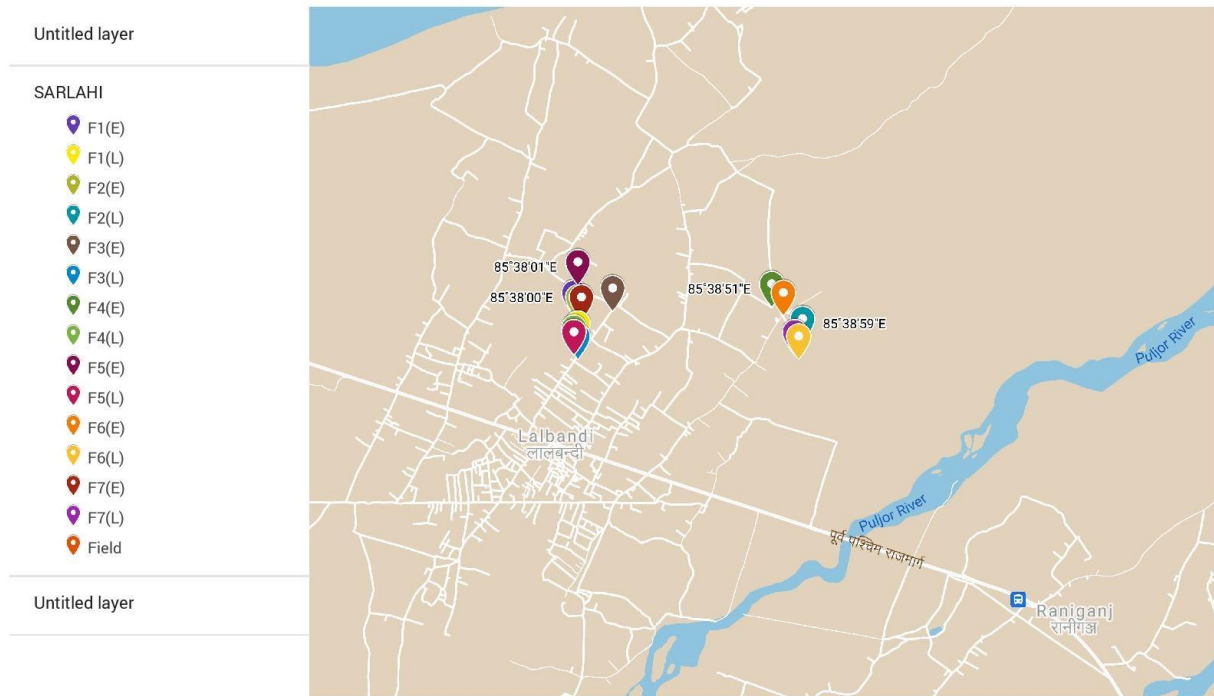
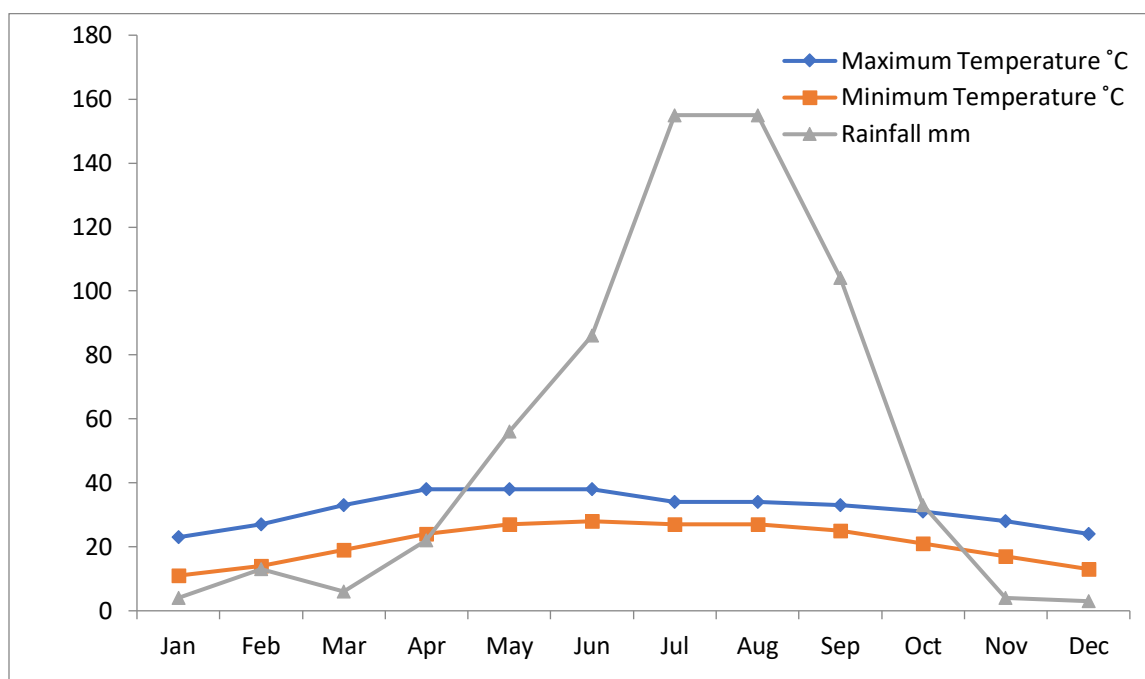


Figure 3.2: Field location of the study area.

### 3.2 Climate

It has tropical to sub-tropical types of climate. There is a lot of rainfall in the summer, and in the winter it is quite dry again. The average annual temperature for Lalbandi is 21° degrees and there is about 1377 mm of rain in a year. It is dry for 152 days a year with an average humidity of 61%. On average, it is maximum of about 38°C in summer months April, May, and June in Lalbandi and at least around 11°C degrees in winter month January as shown in figure 3.3.



**Fig. 3.3.** Average monthly Temperature and rainfall of Lalbandi past 30 year (Meterological station, Janakpur).

### 3.3 Data collections

The data were collected directly from field observation, a tomato and brinjal field survey were conducted twice in the month of September/October for early sowing and in the month of November-December for late sowing. Data of different seven fields, i.e number of hosts and number of *Orobanche* emergence per square meter were collected. For this 5 to 10 quadrats of 1mX1m were laid in each field, depending on the size of fields. The number of hosts and *Orobanche* emergence were counted in each quadrat.

### 3.4 Effect of cropping pattern on emergence of *Orobanche*

To assess the effects of different crops on the emergence of the *Orobanche*, field history of various fields containing different crops in Tulsitole, ward no. 8 of Lalbandi were recorded through an interview with the respective farmers having different crops. Field history of the past ten years was noted. Besides, the method of farmers to control the emergence of *Orobanche* was also observed. The emergence of *Orobanche* in the field was recorded along with the crops in 1m × 1m quadrat. Five to 10quadrats were laid in each field.

### 3.5 Soil sample collection for seed bank estimation

The soil samples up to 15 cm depth were collected from four corners and center of a quadrat i.e by composite sampling method. A representative sample of about half kg was prepared from each quadrat. The collected soil samples were well labelled and carried to the laboratory of Amrit Campus for the *Orobanche* seed bank estimation.

Bulk density of soil in different fields were also measured by using a steel rod (10cm in height and 7cm in diameter), an undisturbed flat horizontal surface in the soil with a spade at the depth of the taken quadrat were pushed or gently hammered the steel ring into the soil. Soil around the ring were removed and then the ring with soil was carefully removed with the soil intact. (McKenzie *et al.*, 2004). The collected soil samples for bulk density measurements were well labelled and carried to the laboratory of Amrit Campus.

The bulk density were measured by using the formula of Cresswell and Hamilton (2002).

$$\text{Bulk density (g/cm}^3\text{)} = \text{Dry soil weight (g)} / \text{Soil volume (cm}^3\text{)}$$

#### 3.5.1 Standarization of seed bank estimation method

The seed bank estimation was done by the differential floatation technique according to Ashworth (1976) with some modification (Acharya *et. al* 2003). To standardize the method, about ½ kg of four different types of soil(i.e sandy, loam, silt, and clay)were taken. Three replication were taken for each types of soil. 100g soil of each type were weighed,100 seeds of *Orobanche* were counted with the help of a microscope. The counted seeds and 100g of soil were mixed in 250ml of a beaker with water(¾). The mixture was left overnight. The mixture of soil and seeds was poured on the stack of two sieves (upper-sieve 297µ and lower-sieve 125µ). The mixture inside the sieve was washed properly with shower spray so that all content was collected on the lower sieve. The surface of lower sieve was wiped with tissue paper and content of the lower sieve was transferred to 100ml of a beaker with the help of a scalpel (Calcium chloride solution (40 %) was poured on the beaker with the content and left for 10 minutes. The upper floated part with seed was poured into the small sieve (100µ) prepared with nylon mesh and placed in a petri-dish containing 2% sodium hypochloride for 1-2 minutes and then washed with water several times. The seed and debris content in the petri- dish was again refloated in the calcium chloride solution in a beaker (100 ml). The upper floated material was transferred in a sieve(100µ) washed the content several times

and the content was spread evenly on the lined muslin cloth(1-25) over a glass plate. The content was examined under a stereomicroscope and counted seeds of each line and total number of seeds/100g were calculated. *Orobanche* seeds were recognized from other debris due to ornamental seed coats.

The mean value of seed recovered from different types of soil, above mentioned were calculated to find out the standardization index.

### 3.5.2 Seed bank estimation of soil sample

Three replicants 100gof each soil sample collected from different fields of Lalbandi area were done by following the above method.

#### *Orobanche* seed estimation

The actual numbers of *Orobanche* seeds recovered were calculated by the following formula (Lopez-Granados and Garcia-Torres,1993).

$$\text{Actual no of } Orobanche \text{ seeds} = \frac{\text{No. of recovered } Orobanche \text{ seeds/100g soil}}{\text{Orobanche seeds standardization index}^*} \times 100$$

\*Mean value of *Orobanche* seed recovered in 4 different types of soil

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

$$\text{No. of } Orobanche \text{ seed /m}^2 = (\text{no. of } Orobanche \text{ seeds /100 g}) \times \text{Soil depth (cm)} \times \text{soil density} \times 100$$

### 3.6 Data analysis

Data obtained were analyzed statistically for *Orobanche* seed bank estimation by SPSS version 21 using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at P=0.05 level of significance. Some of the data were also analyzed by excel 2007.

## Chapter -4 Result

#### 4.1 Field history and *Orobanche* emergence in early sown tomato and brinjal fields

Altogether seven fields for *Orobanche* emergence was considered for the early sowing study and one field of which was a field with brinjal cultivation (F6) and six fields were with tomato cultivation. The field history of different early sowing crops had different stories and summarized in Table 4.1. Field 1 (F1) had a practice of sowing paddy and tomato every year, but lentil was grown last year instead of tomato, just to observe of crop change will reduce *Orobanche* infestations or not. Field 2, 3 and 5 (F2, F3 and F5) are highland (bari in Nepali) and hence regularly there was maize as summer crop and tomato as winter crop. In Field 4 chickpea was grown for last two years in winter and maize as summer crop, but this year tomato was grown instead of chickpea. In field 7 (F7) only cucumber was cultivated in summer and no crop was cultivated in winter for 5 years and this year tomato was grown as winter crop after 5 years interval. Number of host/m<sup>2</sup> i.e number of tomato plant/m<sup>2</sup> was highest in F7 (i.e 5.8±1.166) and lowest in F4 (4.1±0.831).

The field 6 (F6) was the only one early brinjal sowing field studied for knowing the emergence of *Orobanche* and was found to have host number 4.6±0.490 individuals /m<sup>2</sup>. Field 6 (F6) had a field history of Brinjal as winter crop and paddy as summer crop.

**Table 4.1.** Number of host plant/meter square in early sowing tomato and brinjal fields.

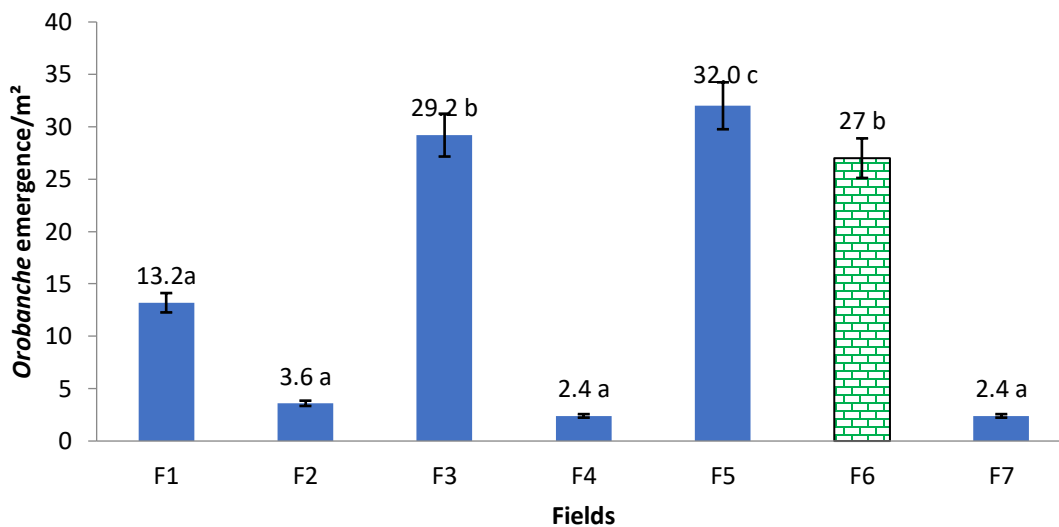
Field Code	Field History of Early Sowing Fields	<i>Orobanche</i> Control Measures	No. of Host Plant/m <sup>2</sup> (Mean±Sd)
F1	Paddy field with crop rotation of paddy in summer season and tomato in winter season, but just last 1 year lentils was cultivated instead of tomato, just to observe if it will control the infestation of <i>Orobanche</i> . <b>(Paddy – Tomato)</b>	Hand pulling	4.8±1.166

F2	Maize field with crop rotation of maize in summer and tomato in winter for many years. <b>(Maize –Tomato)</b>	Hand pulling, pesticides used	5.6±2.059
F3	Maize field with crop rotation of maize in summer and tomato in winter for many years. <b>(Maize –Tomato)</b>	Not used	4.8±0.748
F4	Maize field with crop rotation of maize in summer and tomato in winter season this year, but for last two year chickpea were cultivated instead of tomato to reduce the infestation of <i>Orobanche</i> . <b>(Maize-Tomato)</b>	Hand pulling	4.1±0.831
F5	Maize -Tomato crop rotation. <b>(Maize –Tomato)</b>	Not used	4.1±0.833
F6	Paddy field with crop rotation of paddy in summer and brinjal in winter. <b>(Paddy- Brinjal)</b>	Not used	4.6±0.490
F7	Cucumber was cultivated in summer and tomato cultivated in winter season. For last five year no winter crops were cultivated instead of tomato to reduce the infestation of <i>Orobanche</i> , and after five years again tomato was cultivated. <b>(Cucumber-Tomato)</b>	Hand Pulling	5.8±1.166

#### 4.1.1 *Orobanche* emergence in early sown fields

*Orobanche* shoot emergence was highest in field 5 ( $32.0 \pm 13.45/m^2$ ) in which hand pulling or other control measures were not adopted (Figure 4.1). Similarly, in fields F3 and F6, not any control

measures were used, hence had significantly ( $P=0.05$ ) high *Orobanche* emergence. In fields F1, F2, F4 and F7, practice of removing *Orobanche* shoot by hand pulling was common as a result of which its emergence recorded in these fields were significantly ( $P=0.05$ ) low.



**Figure 4.1** *Orobanche* emergence/ m<sup>2</sup> of early sown tomato fields ( F1, F2, F3. F4, F5 and F7) and brinjal field (F6).

#### 4.2 Field history and *Orobanche* emergence in late sown tomato and brinjal fields

Altogether six tomato fields and one brinjal field, which were cultivated lately, were considered for investigation of *Orobanche* emergence. The field history of these seven fields are different and is mentioned in the Table 4.2. In field 1 and 5 (F1 and F5) only paddy was cultivated for 2 and 3 years, respectively, and no winter crop was grown, but after two and three years, respectively, tomato was cultivated as winter crop. In fields (F3, F4 and F6) maize was cultivated as summer crop and tomato as winter crop. In field 7 (F7) paddy and faba bean were grown for two years, but after two years paddy was shown as summer crop and tomato were sown as winter crop.

Physical weed control practice by hand pulling of emergent *Orobanche* was common among all the studied fields. The number of tomato host/m<sup>2</sup> ranged from 4.7 to 7.6 individuals/m<sup>2</sup> among the studied tomato fields. In Field 2 (F2) brinjals were sown lately. In this field Paddy was grown as summer crop and brinjal as winter crop and the number of brinjal/m<sup>2</sup> was 7.6 (Table 4.2).

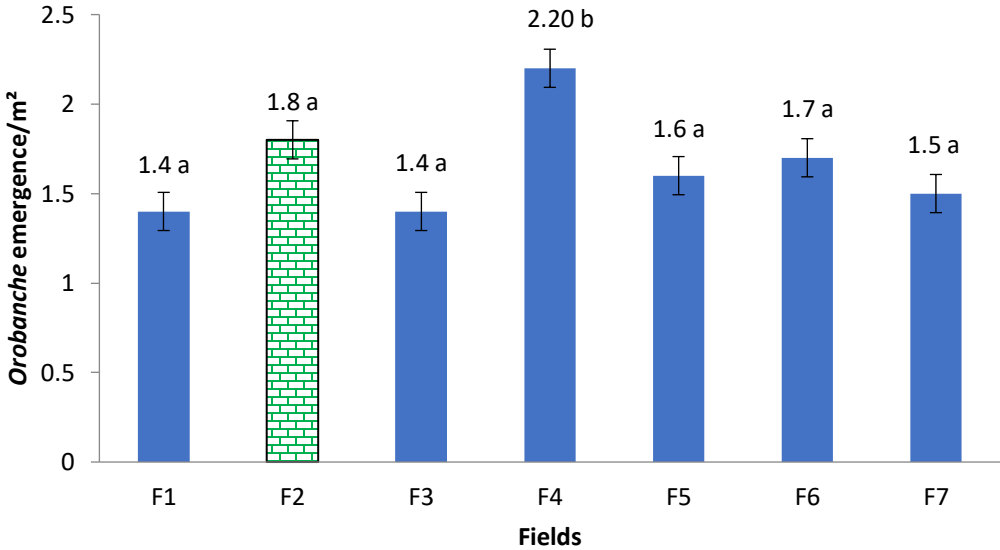
**Table 4.2** Number of host plant/meter square in late sowing tomato fields.

<b>Fields</b>	<b>Field History of Late Sowing Fields</b>	<b><i>Orobanche</i> Control Measures</b>	<b>No. of Host Plant/ m<sup>2</sup> (Mean±Sd)</b>
F1	Paddy field- paddy was cultivated in summer season and tomato cultivated in winter season this year, but for last two year only paddy was cultivated not any winter crops were cultivated to reduce the infestation of <i>Orobanche</i> . <b>Paddy-Tomato</b>	Hand Pulling	7.6±1.02
F2	Paddy field- with paddy cultivated in summer season and brinjal cultivated in winter season. <b>Paddy-Brinjal</b>	Hand Pulling,	7.6±1.28
F3	Maize field- with maize in summer and tomato in winter. <b>Maize –Tomato</b>	Hand Pulling	6.3±1.10
F4	Maize field- with maize in summer and tomato in winter. <b>Maize –Tomato</b>	Hand Pulling	5.2±0.60
F5	Paddy field- paddy cultivated in summer season and tomato in winter season this year, but for last three years only paddy was cultivated not any winter crops were cultivated to reduce <i>Orobanche</i> emergence in the field. <b>Paddy- Tomato</b>	Hand Pulling	4.7±1.00
F6	Maize field- with maize as summer crop and tomato as winter crop. <b>Maize –Tomato</b>	Hand Pulling,	6.3±0.78
F7	Paddy field- paddy was cultivated in summer season and tomato was cultivated in winter season	Hand Pulling,	5.8±0.75

	<p>this year, but for last two years faba bean was cultivated instead of tomato. Emergence of less <i>Orobanche</i> shoot was observed in such tomato cultivated field.</p> <p><b>Paddy- Tomato</b></p>		
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**4.2.1 Orobanche emergence in lately sown fields**

The *Orobanche* emergence in all the studied fields were controlled by physical practice of simply hand pulling of emergent shoot. *Orobanche* emergence in late sowing of tomato and brinjal fields is given in Figure 4.2. *Orobanche* shoot emergence was found to be significantly higher ( $2.20 \pm 1.03/m^2$ ) in field 4 i.e with tomato and maizecrop rotation than that in most of other fields. *Orobanche* shoots in other fields were statistically insignificant.



**Figure 4.2** *Orobanche* emergence/ m<sup>2</sup> of late sown tomato fields (F1, F3, F4, F5, F6 and F7) and brinjal field (F2).

While comparing the results of early and late sown fields with *Orobanche* control by hand pulling, *Orobanche* emergence ranged from 2.4 to 13.2 shoot/m<sup>2</sup> in early sown fields, and it ranged from 1.4 to 2.20 shoot/m<sup>2</sup> in the late sown fields (Figure 4.1 and 4.2). The *Orobanche* emergence were

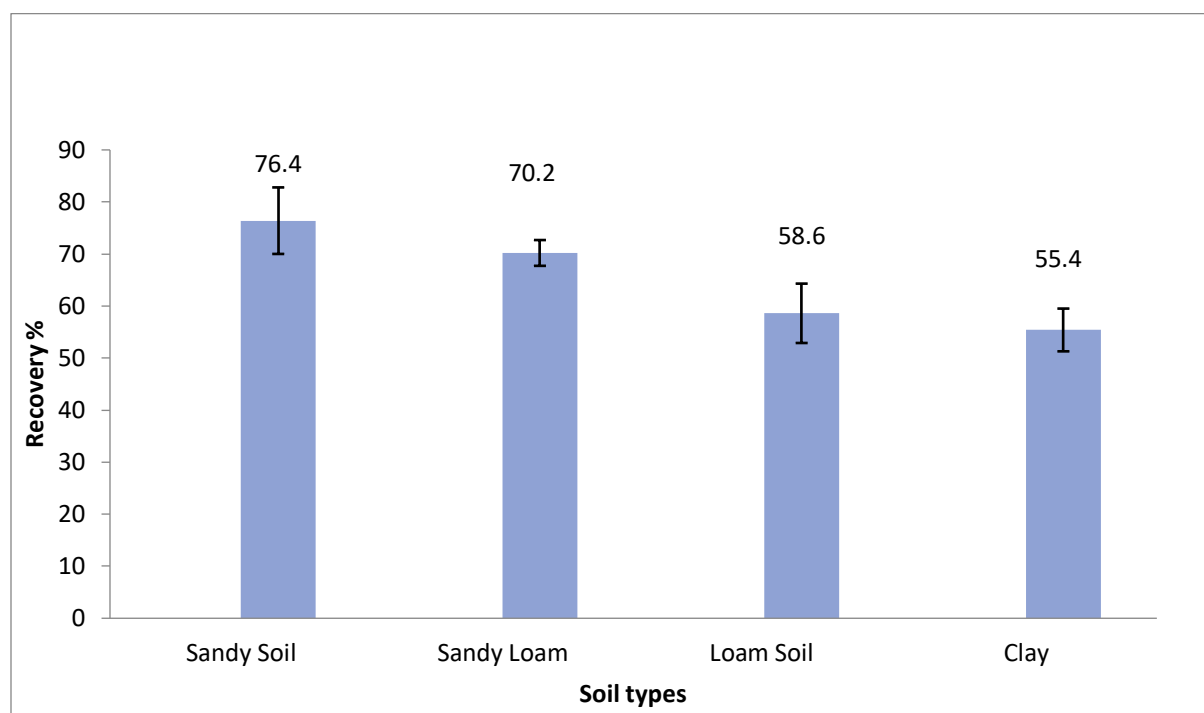
higher in early sown fields than in late sown fields. Similarly, when *Orobanche* emergence in Paddy and Maize fields among the early and late sown fields were compared, high emergence were observed among maize fields (Figure 4.1 and 4.2).

### **4.3 *Orobanche* seed bank in soil**

Though the *Orobanche* emergence result is related with physical control by hand pulling, but the emergence of *Orobanche* shoot in the field does not reflect the severity of the parasitic weed infestations, hence *Orobanche* seed bank in the soil was investigated in this study. To study seed bank in soil, first of all the standardization of the method was conducted using known number of *Orobanche* seeds amended in different types of soil.

#### **4.3.1 Standardization of *Orobanche* seed bank estimation method**

The recovery percentage of *Orobanche* seeds from different types of soil is shown in Figure 4.3. The highest percentage of *Orobanche* seeds recovery was obtained in sandy soils ( $76.4 \pm 6.39$ ) followed by sandy loam ( $70.2 \pm 2.47$ ), loam ( $58.6 \pm 5.7038$ ), and clay soil ( $55.4 \pm 4.11$ ) respectively. From this it was quite evident that the *Orobanche* seed recovery is dependent on the soil texture as a result highest recovery was obtained in sandy soil and lowest in clay soil. The mean recovery % value obtained from this estimation method was 65.15, which was further used as standardization index for the estimation of *Orobanche* seed bank in other field samples.



**Figure 4.3** Recovery of *Orobanche* seeds (%) from the different type's soil (n=12)

#### 4.4 *Orobanche* seed banking early sown tomato and brinjal fields

*Orobanche* seed bank/100g soil was estimated for all seven fields, where the tomato or brinjal were sown earlier and the result obtained is given in Table 4.3. *Orobanche* seeds/100gsoil was significantly ( $P=0.05$ ) high in tomato field F3 ( $18.43\pm 1.16$ ) i.e a field which was situated along the roadside and had not applied any *Orobanche* controlling measures. While the *Orobanche* seeds/100g soil was significantly ( $P=0.05$ ) lowest in field 7 ( $3.27\pm 0.41$ ), which had a history of only cultivation of cucumber for last 5 years with no winter crops and tomato was grown only after five years as winter crop. This showed that significant reduction in number of *Orobanche* seeds in soil can be achieved when cucumber is cultivated as summer crop in tomato fields. Generally paddy fields have less *Orobanche* seed bank than the maize fields, but the paddy fields with brinjal as early winter crops had comparatively more *Orobanche* seed bank than in other tomato growing maize fields (Table 4.3). Reduction in *Orobanche* seed bank was recorded in fields like F1, F4 and F7, where hand pulling of *Orobanche* shoot was in practice, as well as have the field history with the intervention in normal cropping pattern. For instance in Field F1 lentil was grown (for 1 year instead of tomato), in field 4 chickpea (for 2 years instead of tomato) and in F7 no winter crops

was grown in winter. Reduction in *Orobanche* seeds in F2 was not achieved with hand pulling practice.

**Table 4.3** *Orobanche* seed bank in early sown tomato and brinjal fields.

Field	Cropping Pattern (History)	<i>Orobanche</i> Control Measures	<i>Orobanche</i> seeds/100g (Mean±Sd)
F1	Paddy -Tomato (last 1 year Lentils - Paddy)	Hand pulling	5.32±0.41b
F2	Maize-Tomato	Hand pulling, spreading pesticides	14.34±1.52e
F3	Maize-Tomato (Road side field)	Not used	18.43±1.16f
F4	Maize -Tomato (last 2 years chickpea - Maize)	Hand pulling	8.81±0.40c
F5	Tomato- Maize	Not used	12.70±0.60d
F6	Paddy -Brinjal	Not used	11.98±0.61d
F7	Tomato- Cucumber (Tomato cultivated after 5 yrs)	Hand Pulling	3.27±0.41a

#### 4.5 *Orobanche* seed bank in late sown tomato and brinjal fields.

*Orobanche* seed bank/100 g soil was estimated for all seven late sown tomato and brinjal fields and the results are given in table 4.4. *Orobanche* seeds/100g soil was highest in F3 (9.831±0.752531d) among the studied fields. In all the late sowing fields, *Orobanche* control by hand pulling of emergent shoot was common. *Orobanche* seeds/100gsoil was significantly (P=0,05) low in F5 and F1 which had a history of growing only paddy for last 3 years and 2 years, respectively (with no winter crop), and then tomato was cultivated as winter crop after 3 and 2 years, respectively. In paddy field F7, where faba bean was cultivated as winter crop for two years

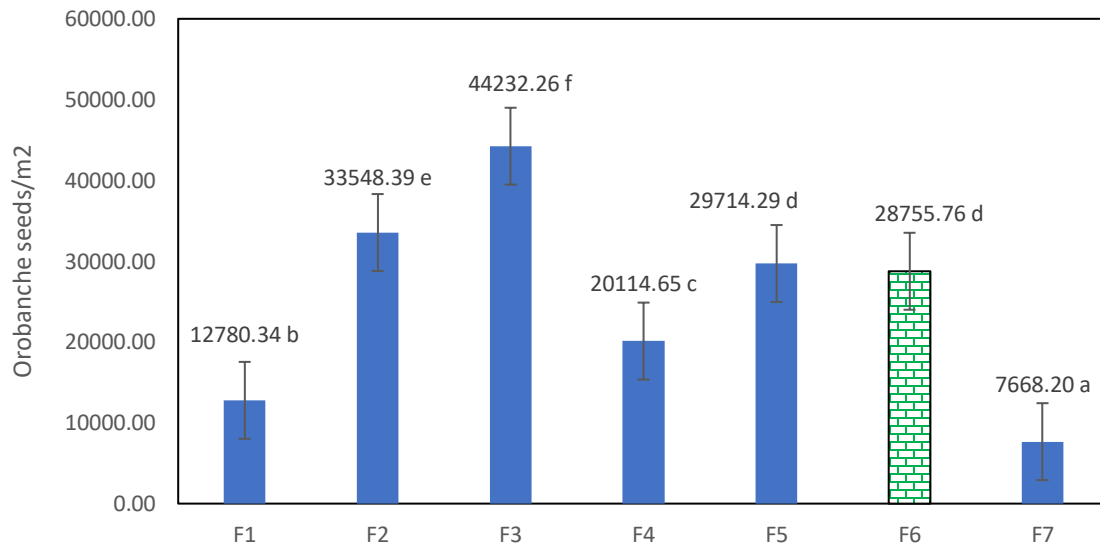
and then tomato, had the seed bank of  $6.144 \pm 0.971514$  seeds/100g soil. The *Orobanche* seeds/100gsoil of paddy field F2 was  $6.144 \pm 1.37$ , where the brinjal was cultivated as winter crop. The *Orobanche* seed bank in most of the paddy fields were recorded to be less than in the maize fields. Similarly the *Orobanche* seed bank in the early sowing fields were mostly higher than in the late sowing fields.

**Table 4.4** *Orobanche* seed bank in late sown tomato and brinjal fields

Field	Cropping Pattern (History)	<i>Orobanche</i> Control Measures	<i>Orobanche</i> seeds/100g (Mean±Sd)
F1	<b>Paddy-Tomato</b> (Tomato Cultivated after 2 yrs)	Hand Pulling,	$5.837 \pm 1.15ab$
F2	<b>Paddy – Brinjal</b>	Hand Pulling,	$6.144 \pm 1.373928ab$
F3	<b>Maize –Tomato</b>	Hand Pulling, pesticides used	$9.831 \pm 0.752531d$
F4	<b>Maize- Tomato</b>	Hand Pulling	$7.373 \pm 1.149511b$
F5	<b>Paddy-Tomato</b> (Tomato cultivated after 3 yrs)	Hand Pulling	$5.530 \pm 0.752531a$
F6	<b>Maize –Tomato</b>	Hand Pulling,	$7.987 \pm 1.149511bc$
F7	<b>Paddy- Tomato</b> (Faba bean for 2yrs and then Tomato cultivated after 2 yrs)	Hand Pulling,	$6.144 \pm 0.971514ab$

#### 4.6 Estimation of *Orobanche* seed bank/m<sup>2</sup>in early sown tomato and brinjal fields

*Orobanche* seed bank estimation/m<sup>2</sup> in the early tomato and brinjal sowing fields are given in figure 4.4. *Orobanche* seed bank/m<sup>2</sup> was highest in F3 i.e. the maize fields with tomato as winter crop and the farmers had not used any type of controlling measure. *Orobanche* seed bank/m<sup>2</sup> was recorded lowest in F7 i.e. the fields where only Cucumber was cultivated and no winter crops were grown for last 5 years and after 5 years tomato was cultivated as winter crop.

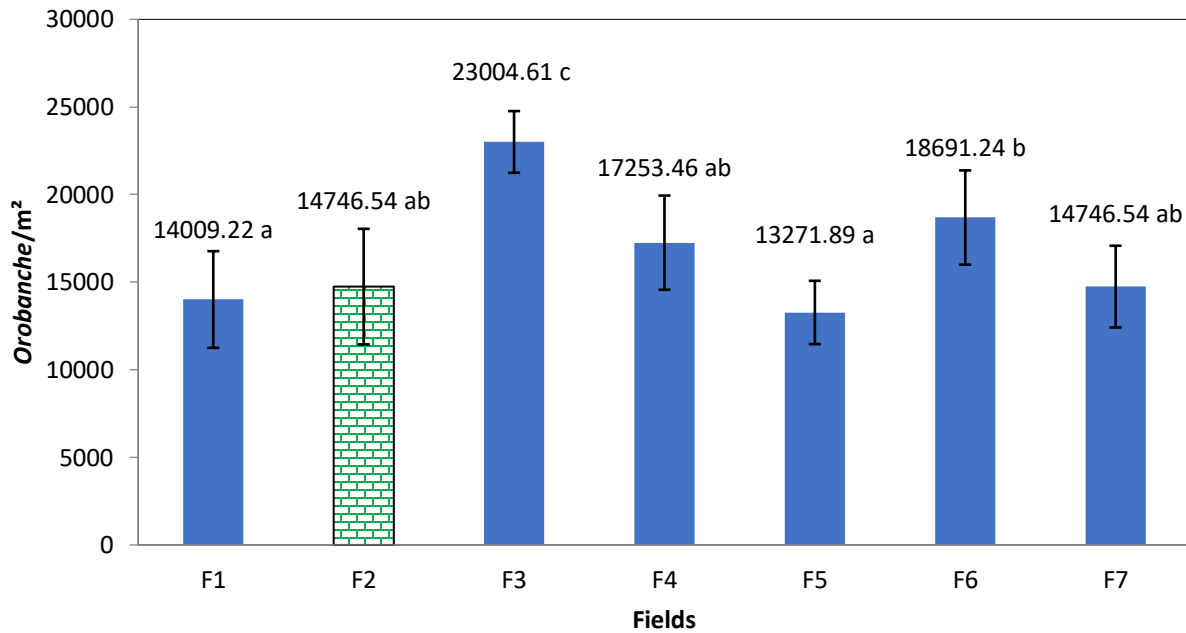


**Figure 4.4** *Orobanche* seed/m<sup>2</sup> of early sown tomato and brinjal fields.

#### 4.7 Estimation of *Orobanche* seed bank/m<sup>2</sup> in late sown tomato and brinjal fields

*Orobanche* seed bank/m<sup>2</sup> was highest in F3 (23004 seed/m<sup>2</sup>) i.e. field with maize as summer crop and tomato as a winter crop, and had only physical measures like hand pulling for *Orobanche* control. The low value of *Orobanche* seed bank/m<sup>2</sup> was observed in F1 and F5, where the only paddy was grown for 2 and 3 years, respectively and no winter crops grown, and after 2 and 3 years of gap tomato was cultivated. Similarly, significantly less *Orobanche* seed bank was also observed in F7 i.e. the paddy field with crop rotation by growing Faba beans instead of tomato for two years and then after 2 years tomato was cultivated. This clearly indicates that gapping of tomato cultivation reduces *Orobanche* seed bank in the fields.

Paddy fields with brinjal grown lately in F2 also had nearly 50% less *Orobanche* seed bank(14746 seeds/m<sup>2</sup>)than the early sown brinjal fields F6(28755 seeds/m<sup>2</sup>) (figure4.4 and4.5). Similarly most of the late sown tomato field were having less *Orobanche* seed bank than the early sown tomato fields, which indicated that *Orobanche* seed bank can be reduced to some extent by sowing the tomato lately.



**Figure 4.5** *Orobanche* seed/m<sup>2</sup> of late sown tomato and brinjal fields.

## Chapter-5 Discussion

### 5.1 *Orobanche* emergence

Because of climate change, the phenology stages of tomato have changed for 1 month. In the past tomato seeds used to be sown during August/September, but now a days they are sown up to September/October. In the past 30-40 years, tomato harvest used to be between November/December to mid-February, while at present, it is during December/January to March Bhandari *et al.*, (2021). To cope up with this situation, the farmers of the Lalbandi area cultivate tomatoes for two periods which were names as early tomato sowing and late tomato sowing, especially to overcome the infestation of *Orobanche*. Farmers used to cultivate tomatoes in Sep/Oct (early sown) because they found seedling of tomato at a low price and the selling price of tomatoes after harvesting is high, but in case of late sowing the seedling of tomato was found at a high price and the harvested tomato price after November is low. Late sowing has been recommended by various researchers (Moreno-Mairquez, 1947; Mesa-Garcia & Garcia-Torres, 1991; Zaitoun & Ibrahim, 1998) in order to prevent severe attacks of the parasite.

In the present study, under the physical control of *Orobanche* by hand pulling in early sown fields, *Orobanche* emergence ranged from 2.4 to 13.2 shoot/m<sup>2</sup>, but in the late sown fields all the studied fields had used *Orobanche* control by hand pulling methods and ranged from 1.4 to 2.20 shoot/m<sup>2</sup>. This clearly indicates that the early sown fields mostly had higher number of *Orobanche* emergence than the late sown fields. Sauerborn (1991) examined the degree of infestations of the Faba bean and Lentil by *Orobanche* and found that it is closely correlated to the sowing date of the crop. In faba bean and lentil the number of *Orobanche* attached plants was reduced by 90-95% through later sowing in the month of December. Reduced attachment in late sown crops can be attributed to environmental factors, particularly temperature effects. Kasasian (1973) reported that the optimal germination temperature for *Orobanche* is between 18 and 23<sup>0</sup>C, while only a few germinated below 18<sup>0</sup>C. The minimum temperature in Lalbandhi during September and October is 25 and 21<sup>0</sup>C, which might have favored the *Orobanche* seed germination, but in November and December it is 17 and 13<sup>0</sup>C, which are below its favorable temperature range. Hence temperature is speculated to be the major environmental factor to reduce *Orobanche* germination and ultimately its emergence and infestations. This findings also support the recommendations made above.

*Orobanche* emergence in Maize fields were higher than in Paddy field, which might be due to inundated condition of paddy field for 2-3 months during paddy cultivation. Germination of *Orobanche* seeds occurs after a period of preconditioning (humid temperatures suitable for several days) and exposure to germination stimulants secreted by the roots of the host (Sato et al., 2003). But in inundated conditions the stimulant secreted by host might be very dilute and/or might induce suicidal germination to reduce seed bank. Hence, Cicerone and Piglionica 1979 and Cubero (1983) have also suggested inundated condition of fields to be a control measures for reduction of *Orobanche* infestation.

## **5.2 *Orobanche* seed bank estimation**

Different workers have used various methods to estimate *Orobanche* seeds in infested soils. In the method developed by the working team at ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria), MgSO<sub>4</sub> specific gravity solution of 1.16 g/cm<sup>3</sup> was used to suspend *Orobanche* seeds (Kachelriess, 1987). Ashworth (1976) used a 40% (w/v) CaCl<sub>2</sub> solution for the quantitative detection of *Orobanche* seeds in infected soil. In the present study mainly the Ashworth's(1976) flotation and sieving technique was used with some modifications (Acharya et al., 2003) to isolate *Orobanche* seeds from heavy organic particles. The *Orobanche* seed recovery in this study were 76.6, 70.2, 58.6 and 55.4% for sandy soil, sandy loam, loam and clay soil, respectively, which were more or less similar to that of Acharya (2002). The high recovery in sandy soil is mainly due to presence of large soil particles (0.02 to 2 mm in diameter) and less organic matter. Since the size of soil particles in sandy soils is large, most of them were kept in an upper sieve (279 µm) which makes seed counting easier. In sandy, loam and clay soils, there was more organic matter and soil debris of roughly the same size as *Orobanche* seeds, which not only caused seed recovery error but also made seed counting difficult. Seeds that were not recovered might have lost during floatation and sieving in this method.

## **5.3 Seed bank estimation of early sown fields**

The importance of seed bank in infested soils has been emphasized by several workers for proper management of weeds (Sauerborn et al., 1991, Auld et al., 1979). The soil seed bank study is found to be a more reliable method to monitor *Orobanche* infestation than other visual field surveys (Sauerborn et al., 1991), which is also evident from the present study. For instance in early sown fields F1, F2, F4 and F7 *Orobanche* emergence was significantly ( $p=0.05$ ) less because of physical control by hand pulling, but the *Orobanche* seed bank was significantly high in F1 and F2. Hence, to understand the severity of *Orobanche* infestation, it is important to examine the soil seed bank along with the past history of the field.

In the study area there was either paddy fields or maize fields. Paddy fields are mostly low lying areas as plenty of water is needed for growing paddy in summer, whereas maize fields are mostly upper plain highland areas where maize are grown in summer. In the early tomato sown paddy field F1, the *Orobanche* seed bank is insignificantly less than in maize field F5 but significantly less than in Maize field F2, and F3. This indicated that Paddy fields have less *Orobanche* seed bank than the maize fields. The inundated condition of paddy fields have been reported to reduce *Orobanche* seeds (Bista, 2016). The inundated conditions become favorable for preconditioning of *Orobanche* seed germination but unable to get attached with the host and thus causes suicidal germination of *Orobanche* seeds. This might be the reasons for less *Orobanche* seed bank in Paddy fields.

Besides the inundated condition in F1, the farmers had also practiced change in the crop rotation by growing lentil last one year instead of tomato. Lentil being leguminous plant increases nitrogen content in soil and when the nitrogen content is high, *Orobanche* infestation is less (Abu-Irmaileh, 1994). Lentil has also been reported to be one of the suitable potential trap crops by Acharya et al (2021) as it has reduced 57.59% *Orobanche seeds* in the field experiments. This might be the reason for less seed bank in paddy field F1 than in F6. The seed bank in paddy field F6 where brinjal was sown early (and was without any intervention of crop rotation) had quite high *Orobanche* seed bank than in paddy field F1.

In the early sown maize field F4, the *Orobanche* seed bank was significantly less than in other maize field F2 and F3. The field history indicated that chickpea was grown as winter crop for last

2 years instead of tomato. This finding also supports the findings of Acharya et al 2021, who have also identified Chickpea as one of the potential trap crops and had reported the reduction of *Orobanche* seed bank nearly by 52%. *Orobanche* seed bank was highly reduced in F7 i.e field with cucumber cultivation in summer and tomato in winter (after five years). The main reason behind this reduction could be due to lack of host crop for last five years in winter season. *Orobanche* are holoparasite, they need host to complete their life cycle. In absence of host, on one hand, the parasitic plants could not grow and new *Orobanche* seeds were not been added, and on the other hand, there is decay and decomposition of seeds which leads to reduction of *Orobanche* seed bank (Acharya et al 2021). This could be the reason for highly reduction of *Orobanche* seed bank in F7.

#### **5.4 Seed bank estimation of late sown fields**

*Orobanche* seed bank in late sown fields were comparatively less than in early sown fields. The possible reason for this is the climatic factor, especially temperature as suggested by the Sauerborn (1997 b). According to Kasasian (1973) the optimal germination temperature for *Orobanche* is between 18 and 23<sup>0</sup>C and a only few seed could germinate below 18<sup>0</sup>C. The minimum temperature ranged from 25 to 21<sup>0</sup>C at the time of early sowing in Lalbandi during September and October and are within the optimal temperature range for *Orobanche* seed germination. The minimum temperature during the late sowing period (November and December) ranged from 13 and 17<sup>0</sup>C, which are below the favorable temperature range, and hence only a few *Orobanche* seed could germinate and infest the host plants. When the infestation is low, the seed bank expected is also low. This might be the reason for less *Orobanche* seed bank in late sown fields.

Seed bank in paddy field F1, F2, F5 and F7 is comparatively less than in maize fields F3, F4 and F6, which might be due to inundated condition of paddy fields. Bista (2016) conducted *Orobanche* seed germination in the laboratory also reported significantly low germination of *Orobanche* seeds in flooding soil condition and speculated it to be due to direct effect on the dilution of endogenous chemicals responsible for the receptiveness of stimulant produced during pre-conditioning period. Germination of *Orobanche* seeds occurs after a period of preconditioning (humid temperatures suitable for several days) and exposure to germination stimulants secreted by the roots of the host

(Sato et al., 2003). But in the flooding or inundated conditions the stimulant secreted by host might be very dilute and this possibly induce suicidal germination of *Orobanche* seeds and undergo decaying process. This might be the reason for less *Orobanche* seed bank in paddy fields.

Besides this in paddy field F1 and F5, tomato was cultivated after 2 and 3 years, respectively, this interruption of tomato cultivation for two or three years reduced the addition of *Orobanche* seed in soil, as the parasite *Orobanche* cannot grow in the absence of host. This might have reduced the *Orobanche* seed bank than in other fields.

In paddy field F7 faba bean (*Vicia faba*) were grown for two years instead of tomato. Faba bean was reported as a rare host of *O.aegyptiaca* in Nepal (Khatti, 1997). Faba bean has also been reported as a potential trap crop to reduce *Orobanche* seeds by Acharya et al (2021) as the reduction of *Orobanche* seed bank was about 55.56% in paddy fields. Schnell et al (1994) demonstrated that faba bean was most effective for the *O. crenata* which reduced the seed bank by 47.3%. The hormone bioassay of *Vicia faba* root exudates revealed clear gibberellin activity and suggested it to be responsible for seed germination of *O. crenata* (Lopez-Granados and Garcia-Torres, 1991). From the above literature it can be said that in field F7, faba bean were grown for two years instead of tomato acted as trap crop, i.e it induces suicidal germination of *Orobanche* seeds because of their root exudates, but the germinated seeds could not parasitized the faba bean plant as it is not the actual host. This phenomenon most probably reduced the seed bank in the soil of F7 fields.

In paddy field F2, where brinjal was grown as late winter crop had seed bank more or less similar to F1 and F7, but were less than in early sown paddy fields with brinjal (F6 of early sown). This further indicated that late sowing date can reduce the *Orobanche* seed bank in soil.

## Chapter-6 Conclusion and Recommendation

### 6.1 Conclusion

From the *Orobancha* emergence study of early and late sown tomato and brinjal fields, it can be concluded that the severity of the parasitic weed in a field cannot be ascertain with the *Orobancha* emergence alone. From the *Orobancha* seed bank study of early and late sown tomato and brinjal fields it can be concluded that late sowing helps in reducing the *Orobancha* seed bank in soil. Besides this when paddy fields and maize fields are compared for *Orobancha* seed bank, paddy fields were found to have less *Orobancha* seed bank than the maize fields and from this it can be speculated that the inundated condition of soil may help in reducing the *Orobancha* seed bank. Crop rotation by substituting tomato by lentil for one year in paddy field (F1 of early sown fields) and substituting tomato by chickpea for two years in maize field (F4 of early sown fields), substituting tomato by faba beans for two years in paddy fields (F7 of late sown fields) showed significant less *Orobancha* seed bank. Similarly, only cucumber cultivation in summer with fallow in winter for 5 years (F7 of early sown fields), only paddy cultivation in summer and fallow in winter for 2 years and 3 years (i.e F1 and F5 of late sown fields)also showed significant less *Orobancha* seed bank. Hence it can be concluded that crop rotation using leguminous crop like lentil, chickpea, faba bean instead of tomato as winter crop can reduce *Orobancha* seed bank and ultimately its infestation. Similarly, keeping the tomato fields without any winter crop cultivation for some years is also an option to reduced *Orobancha* seed bank in infested fields.

### 6.2 Recommendations

Followings are the recommendations made to the farmers from this study.

1. The physical control of *Orobancha* by hand pulling only is not enough, as the fleshy shoots helps in the maturation of seed and it further adds seed bank in the fields. For this proper management of removed *Orobancha* shoots by burning them in a pit is important to reduce *Orobancha* seed bank.

2. Crop rotation with leguminous crops like lentil, chickpea and faba bean (rather than keeping the field fallow) is highly recommended, as they will add nitrogen content in soil on one hand and also reduce *Orobanche* seed bank in tomato or brinjal growing fields.
3. Crop rotation with leguminous crops like lentil, chickpea and faba bean (rather than keeping the field fallow) is highly recommended, as they will add nitrogen content in soil on one hand and also reduce *Orobanche* seed bank in tomato or brinjal growing fields.

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## Annexes

### Photoplates



**Photoplate A: *Orobanchae* shoots at tomato field.**



**Photoplate B: *Orobanchae* shoots at brinjal field.**

