



**EVALUATION OF THE SYNERGISTIC EFFECT OF HOST PLANT
RESISTANCE, RHIZOBIAL INOCULANT AND TRICHODERMA ON
OROBANCHE INFESTATION OF FABA BEANS (*VICIA FABA L.*) IN
SOUTHERN TIGRAY, ETHIOPIA**

M.SC. THESIS

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HAWASSA UNIVERSITY, ETHIOPIA

NOVEMBER 2017

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L.) IN SOUTHERN TIGRAY, ETHIOPIA**

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A THESIS

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HAWASSA UNIVERSITY
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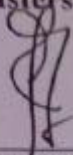
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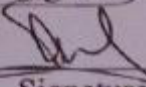
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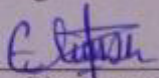
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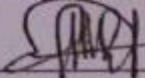
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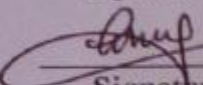
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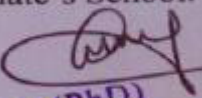
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DEDICATION

This work is dedicated to my mother and father who brought me up and gave me the opportunity to get educated, an opportunity they were unable to have.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bonafide work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for a M.Sc. degree at the Hawassa University and is deposited at the University Library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree.

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LIST OF ABBREVIATIONS AND ACRONYMS

AARC	Alamata Agricultural Research Center
ANOVA	Analysis of variance
APPRC	Ambo Plant Protection Research Center
BoARD	Bureau of Agriculture and Rural Development
CFU	Colony Forming Unit
CM	Centimeter
CSA	Central Statistical Agency
CV	Coefficients of variation
FA	Farmers Association
FAO	Food and Agriculture Organization
Ha	Hectare
ICARDA	International Center for Agricultural Research in Dry Areas
Kg	Kilogram
LSD	Least significant difference
MoA	Ministry of Agriculture
MSRC	Mekelle Soil Research Center
°C	Degree Celsius
PDA	Potato Dextrose Agar
RCBD	Randomized Complete Block Design
RCD	Randomized Complete Design
SNA	Slight Nutrient Poor Agar
TARI	Tigray Agricultural Research Institute

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THE SYNERGISTIC EFFECT OF HOST RESISTANCE, RHIZOBIAL INOCULANT AND *TRICHODERMA* ON OROBANCHE INFESTATION OF FABA BEANS (*VICIA FABA L.*) IN SOUTHERN TIGRAY, ETHIOPIA

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ABSTRACT

Broomrape (*Orobanche crenata* F.) has been seriously limiting faba bean production in northern Ethiopia. As a result there is a need to further enhance faba bean production through the development of management options that can be effectively used in combating the parasitic weed problem. This research was conducted with the objectives of quantify the infestation level of *O. crenata* and associated yield loss in faba bean fields of south Tigray, Ethiopia, evaluate the synergistic effect of host plant resistance and bio-inoculants on *O. crenata* naturally infested soils of faba bean and to assess pathogenic fungi associated with *O. crenata* and test their pathogenicity. A field survey was carried out to assess the distribution and infestation level of *O. crenata* in south Tigray. Field experiment was conducted by using four faba bean varieties and bio-inoculants of *R. leguminosarum* and *T. harzianum* that were applied singly or in combination. Un-inoculated plots with neither of the inoculants were included as control. The experiment was arranged in factorial RCBD with three replications. Results revealed the prevalence of *O. crenata* across the region. The highest prevalence (100%) of *O. crenata* was recorded in Ofra district while *O. crenata* incidence and severity were the highest in Adigolo Farmers Association (93 and 6.8 %, respectively). Estimated average faba bean yield loss as a result of *O. crenata* infestation ranged from 2 to 28 % at the district level while average yield loss was estimated to reach 99% at the field level. Various fungal pathogens were found associated with the roots and stems of the *O. crenata* plant. *F. oxysporum* was the dominant species with more than 56% incidence. Inoculation of faba bean with *R. leguminosarum* and *T. harzianum* singly or in combination significantly reduced *O. crenata* infestation as compared to untreated checks. Interactions between bio-inoculants and host variety had no significant effect at all. As compared to local variety, the varieties Hashenge, Walki and Moti reduced the incidence of *O. crenata* infestation by 74, 11 and 10 %, respectively. Similarly, the severity of *O. crenata* infestation was reduced by 79% on Hashenge variety, 30% on Walki and 16% on Moti variety. As compared to the negative check (no bio-control) *T. harzianum*, *T. harzianum* + *R. leguminosarum* and *R. leguminosarum* reduced *O. crenata* incidence by 11.5%, 8.4% and 7.6%, respectively. The same treatments reduced *O. crenata* severity by 39, 48, and 35%, respectively. Among the bio-inoculants, *T. harzianum* + *R. leguminosarum* treated plots had the highest grain yield (803.50 kg/ha), which was a 27% over the untreated control. The highest level of protection against the parasitic weed was achieved on Hashenge variety, which also meant that the same variety had the best agronomic performance and the highest yield. So based on current findings, we recommend that faba bean variety Hashenge be used in combination with *T. harzianum* + *R. leguminosarum* as integrated management option

against *O. crenata*. Further evaluations involving additional faba bean varieties and bio-inoculants need to be undertaken under different agro-ecological settings.

Key words: faba bean, *Orobanche crenata*, synergistic effect, *Rhizobium leguminosarum*, *Trichoderma harzianum*

1. INTRODUCTION

Faba bean (*Vicia faba* L.), also known as broad bean, horse bean, fava bean and field bean, is one of the earliest domesticated food legumes in the world (Metayer, 2004). In Ethiopia, faba bean is grown in the highlands (1800-3000 m.a.s.l), where the need for cold temperature is met (Yohannes, 2000). It is believed that the crop was introduced to Ethiopia from the Middle East via Egypt around 5000 B.C., immediately after domestication (Asfaw *et al.*, 1994). Ethiopia is now considered as one of the centers of secondary diversity for faba bean (Asfaw *et al.*, 1994; Yohannes, 2000; Torres *et al.*, 2006). The crop takes the largest share of the area under pulse production and occupies close to 509,373.92 ha of land with annual production of about 8,557,565.54 quintals (CSA, 2015).

The food value of faba bean is high and it has been considered as a meat extender or substitute due to its high protein content (20-41 %) (Crepona *et al.*, 2010). Along with lentil, field pea and chickpea, faba bean is one of the major sources of protein in almost every rural Ethiopian household. The straw is an important livestock feed. Ethiopian farmers recognize the role faba bean in improving soil health through atmospheric nitrogen fixation (Sahile *et al.*, 2008) and its importance as a rotation crop. Particularly in the cooler highlands where other pulse crop types are least cultivated, it is the major and sometimes only rotation crop planted following cereals every two to three years. Even though Ethiopia is said to be the world's second largest producer of faba bean (Chopra *et al.*, 1989), yet the productivity of the crop has remained far below its potential. The average yield of faba bean under smallholder farmers is not more than 1.6 t/ha (MoA, 2011; CSA, 2015). This is due to a number of factors amongst which are the inherently low grain yielding potential of indigenous cultivars and their susceptibility to biotic and abiotic stresses (Mussa *et al.*, 2008; Sahile *et al.*, 2008).

From among the biotic stresses, parasitic plant Broomrape (*Orobanche crenata*) and fungal diseases such as chocolate spot (*Botrytis fabae*), rust (*Uromyces vicia fabae*), foot rot (*Fusarium avenaceum*) and black root rot (*F. solani*) highly contribute to the low productivity of the crop (Teklay *et al.*, 2013). The parasitic weed *O. crenata*, though known to have been a problematic weed in parts of North Wello and neighboring areas in the Amahara region for quite some period, has become a major pest of faba bean and field pea in the highlands of the southern zone of Tigray in the last two decades (Rezene and Gerba, 2003; Teklay *et al.*, 2013). The weed reduces the ability of the host plant to absorb water from the soil under moisture stress conditions, and causes drought stress and wilting resulting in heavy yield loss. The effect is more pronounced in northern Ethiopia where moisture availability is limited. Yield losses due to this weed may reach as high as 75-100 % in certain parts of the Sudan and Ethiopia (ICARDA, 2006) and it is not different in Tigray, where farmers have reported complete crop loss due to orobanche infestation. Measures that have been taken so far, including chemical herbicide sprayings and manual weeding of *O. crenata* plants, were not effective enough to reduce the parasitic weed population and curb its further distribution. As a result it has become a major threat to faba bean production as it is colonizing previously un-infested neighboring districts (Kiros and Mulubrhan, 2005).

In some locations, *O. crenata* has created severe pressure and is forcing farmers to abandon cultivating faba bean particularly in the highlands where other pulse crops, other than faba bean are rarely grown. The absence of a legume crop in the crop rotation is affecting the traditional organic soil fertility management system and is forcing farmers to depend on chemical fertilizers. Besides, this will severely affect poorer households nutritionally as this may be the only or major source of household protein rich nutrition (Crepona *et al.*, 2010; Haas *et al.*, 2010) and impact livestock production as faba bean straw is a good feed

supplement. There were some attempts to separately alleviate the problems of *O. crenata* and foliar diseases in Ethiopia in general and South Tigray in particular. Few lines with some level of resistance to chocolate spot disease and with certain level of tolerance to *O. crenata* were identified (Teklay *et al.*, 2013). However, an integrated approach need to be devised so as to alleviate the problem that is challenging faba bean production and household food, income and nutrition security. Therefore, the present study was conducted with the following objectives:

General objective

- ✓ To contribute toward improved faba bean production through management of *O. crenata*

Specific objectives

1. To quantify the prevalence, incidence, and severity of *O. crenata* and associated yield loss in faba bean in south Tigray
2. To isolate and identify pathogenic fungi associated with *O. crenata* and test their pathogenicity
3. To evaluate the combined effect of host plant resistance, rhizobial inoculant (*R. leguminosarum*) and Trichoderma (*T. harzianum*) on *O. crenata* infestation and yield of faba bean

2. LITERATURE REVIEW

2.1. Parasitic Weeds

About 4,000 flowering plant species have adapted to parasitize other plants (Fernandez-Aparicio *et al.*, 2010). Parasitic plants are flowering plant that directly attached to another plant via a haustorium, a modified root that forms a morphological and physiological link between the parasite and host (Kuijt, 1969; Agrios, 2005). , The most economically damaging parasitic flowering plants on agricultural crops are the broomrapes (*Orobanche* spp.), witchweeds (*Striga* spp.) and dodders (*Cuscuta* spp.) (Joel *et al.*, 2007; Parker, 2009). These species have become severe constraints to major crops of cereals and legumes (Rubiales and Heide-Jorgensen, 2011).

Crenate broomrape (*Orobanche crenata*) is occasionally known by the synonym *Orobanche speciosa*. This out-crossing species shows genetic diversity, but no clearly defined local races (Paran *et al.*, 1997). The parasite belongs to the class Dicotyledonae order Scrophulariales, and family Orobanchaceae.

2.2. Distribution of Crenate Broomrape

Orobanche is commonest in the Mediterranean countries, the Middle East and East Africa (Ethiopia), while other species have a wider distribution (Perez-de-Luque *et al.*, 2010). *O. crenata* is important in Ethiopia where it infests many legume crops, particularly faba bean, field pea, chickpea, lentil and dekokko (*Pisum sativum* var. *abyssinicum*) (Rezene and Gerba, 2003; Rubiales *et al.*, 2006; Teklay *et al.*, 2013). The parasite causes up to 100% yield loss in faba bean and field pea (Rezene and Gerba, 2003). The distribution of the weed in Tigray region is increasing at alarming speed from some localized areas to almost throughout the whole southern zone of the region (Teklay *et al.*, 2013). In highly infested areas, farmers are avoiding growing food legume crops, resulting in substantial reductions to both the extent of cultivated areas and to food legume production (Besufikad *et al.*,

1999). The spread and occurrence of this parasitic weed in Ethiopia is an alarming development in a country where faba bean is a major crop.

2.3. Host Range

Broomrapes (*Orobanche* spp.), the phytoparasitic weeds, are obligate parasites which grow on roots of a broad range of broadleaf plants. Some species attack food and ornamental crops, causing considerable yield losses (Parker and Wilson, 1986). The host range of *O. crenata* is somewhat narrower than that of *O. ramosa*, with the main host crops restricted to the family Fabaceae and Umbelliferae (Apiaceae) and relatively sporadic occurrence on crops in a few other families such as Compositae (Asteraceae) and Cucurbitaceae. Wild hosts are mainly in these same families.

2.4. Crenate Broomrape Biology and Physiology

O. crenata is holoparasite plant, needing to establish a connection to a host root within a few days of germination. *Orobanche* spp. depends totally on their hosts for all nutrition, and draws most of their water from the host root. Effects on the host are directly proportional to the biomass of the parasite, such that the mass of the parasite is reflected in a very similar loss in mass of the host crop (Manschadi *et al.*, 1996).

The *O. crenata* seed is approximately 0.2 x 0.4 mm in size, from which only the radicle emerges and this can grow only a few mm long. A chemical stimulus is needed to initiate *Orobanche* germination. This stimulus normally comes from host roots. However, a moist environment is required (for several days), together with suitable temperatures, before the mature seed is responsive to germination stimulants. This preparatory period is known as conditioning or preconditioning. Conditioned seeds remain responsive to germination stimulants for several months. . This combination of characteristics ensures that the seeds have a characteristic cycle of germinability through the year and tend to germinate freely

over a relatively short period in early to mid-winter. On contact with the host root, a swelling, the haustorium, is formed and intrusive cells penetrate through the cortex to the vascular bundle to establish connection with the host xylem and phloem (Dorr and Kollmann, 1995).

The ability to respond to germination stimuli fades gradually when the seeds dry and they then remain dormant until re-conditioned (Timko *et al.*, 1989; Joel *et al.*, 1995). The parasite develops into a tubercle on the surface of the root, developing to a diameter of 5-20 mm. Secondary roots may develop on the tubercle and make separate contacts with the host root system. After several weeks, the tubercle develops a flowering shoot which emerges above the soil. Seeds are produced in very large numbers and may remain viable in soil for many years. Lopez-Granados and Garcia-Torres (1999) showed that seed in the field persisted for 6-9 years.

2.5. Management of Crenate Broomrape

The management of parasitic weeds is more difficult than the control of other weeds. This is because they produce more than 500,000 of very small seeds from a single plant, which can remain viable for decades in the soil until conditions become suitable for germination. This provides the parasite with a great genetic adaptability to environmental changes, including host resistance, agronomical practices and herbicide treatments (Joel *et al.*, 2007). Due to this, different control measures against broomrapes have not proven as effective, economical and applicable as predicted (Goldwasser *et al.*, 2008; Perez-de-Luque *et al.*, 2010).

Although several potential control measures were developed over the past few decades for some crops, any control method applied alone is often only partially effective and the results were sometimes inconsistent due to variable environmental conditions. The only

effective way to control weedy root parasite like *O. crenata* is through recent advances that have been made with existing technologies and which have the potential to make significant impact on the control of parasitic weeds in the short to medium term. These include bio-control, intercropping with best trap crop, use of wild relatives as sources of resistance, the use of new technologies and molecular genetics approaches which involve the use of mutants like herbicide resistant pulses (Joel *et al.*, 2007).

2.5.1. Preventive Measures

The durability of broomrape lies in its ability to form a bank of seeds in the soil. A management or eradication program must aim at reducing this seed bank, while reducing the production of new seeds and their dispersal to new sites. Quarantine is therefore an essential element in control or eradication programs. The best option for winning against broomrapes is avoiding the fight. It is impossible when the fields are already infested with the seeds, but preventive measures must be taken into consideration to avoid distributing the infestation into neighboring fields (FAO, 2008).

The dispersal of broomrape seeds are through machinery and tools, and together with the host seeds; thus proper phytosanitary measures in and around the field are necessary to minimize the spread of *O. cernata*. Farm equipment and machinery should be cleaned prior to their use in un-infested fields. Special care must be applied to disinfestations and cleaning of field machinery and harvesters, and avoid trucks going from infested to non-infested fields. Containment is necessary to avoid spreading of the infestation and eradication programs should be considered. *O. crenata* shoots should be removed prior to flower opening. The collected shoots should be burnt or disposed of properly. Good extension agents could easily convince the farmers to execute such task, especially when they made aware of the tremendous production of *Orobanche* seeds per plant. One important spreading agent of various weeds, including *Orobanche*, is the uncontrolled

movement of grazing animals. Grazing animals should be forbidden to enter un-infested fields after grazing infested areas (Panetta and Roger, 2005). Furthermore, farmers should use certified seed in order to ensure themselves it is clean of parasite seeds.

Strict quarantine measures, at various levels, national and international, help in preventing the introduction of the parasite into parasite-free areas. Technical inspection of imported agricultural materials should be carried out by a subject matter specialist (FAO, 2008).

2.5.2. Cultural Methods

Germination of *O. crenata* tends to be very much reduced below 8°C and further development is greatly reduced at low temperatures (Kroschel, 2001). Delaying the planting date affects *Orobancha* more than its hosts; the delay should be only two weeks from the date optimal for sowing in an un-infested field. However, this method must be adapted for different regions and for different hosts. Earlier planting dates are beneficial in certain instances. The change of the sowing date seems not to be very promising due to uncertainty of the environmental conditions, specifically temperature and rainfall situation. Nitrogen compound application and manure fertilization also appear to be candidates for putative control of broomrapes: some studies have shown that nitrogen in ammonium form negatively affects broomrape germination and/or elongation of the seedling radical. In addition, manure fertilization augments the killing effect of solarization on *O. crenata* seeds (Haidar and Sidahmed, 2000).

Weeding of *O. crenata* is mainly accomplished after the parasitic damage has already been done. It is not likely to show any yield increase in the short term. Weeding is laborious and time-consuming, and not very promising in highly infested areas. However, in combination with other methods, it can reduce the seed bank very efficiently (FAO, 2008).

Intercropping is already used in regions of Africa as a low-cost technology of controlling parasitic plants (Oswald *et al.*, 2002). It has been demonstrated that intercrops with cereals or with fenugreek or berseem clover can reduce *O. crenata* infection on faba bean and pea due to allelopathic interactions (Fernandez-Aparicio *et al.*, 2010). 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).

The use of trap crops or rotation with non-host offers the advantage of preferentially stimulating broomrape suicidal germination. Flax, fenugreek and Egyptian clover are successful trap crops for *O. crenata* (Sauerborn and Saxena, 1986).

2.5.3. Physical Methods

Soil solarisation has been proven as the most effective method in controlling broomrape in open crops fields (Haidar and Sidahmad, 2000). Soil solarisation is the heating of soil by sunlight trapped under a mulch of black, or more usually clear, polyethylene film. The temperatures of 48-57 °C kill *Orobanchae* seeds that are in the imbibed state; therefore, soil must be wet at the time of treatment. The temperature increase achieved is primarily the result of the elimination of evaporation, but is also partially because of the greenhouse effect created. This technique has been used successfully on croplands in many countries around the world like Middle East with an endemic *Orobanchae* problem, as a pre-planting treatment for tomato, carrot, eggplant, faba beans and lentils. As global environmental quality considerations grow in importance, along with an increasing human population, evolving concepts, such as soil solarization and other uses of solar energy in agriculture, will become more important (Ashrafi *et al.*, 2009).

2.5.4. Chemical Methods

The use of chemicals with the ability to stimulate broomrape seed germination in the absence of a suitable host may lead to a reduction in the *Orobanchae* seed bank. This is "suicidal" germination because; once germinated, the *Orobanchae* seeds cannot return to dormancy and cannot survive for longer than a few days without nutritional supply from a host. This is an ideal control strategy, but its utility is limited in real-world applications. Initial attempts to deplete broomrape seed banks using synthetic Strigolactone were made by using the synthetic Strigolactone analogue GR7. However, field application of these GR type Strigolactones provided only partial control of *Orobanchae* to the instability of the compound, particularly at pH >7.5 (Fernandez-Aparicio *et al.*, 2011). New Strigolactone analogues are continuously being produced (Mwakaboko and Zwanenburg, 2011), and these could serve as candidates for the suicidal germination approach, as long as they can be properly formulated for field application. Although trans-22-dehydrocampesterol stimulates *O. aegyptiaca*, *O. crenata*, *O. foetida*, and *O. minor*, Soyasapogenol B was very specific, stimulating the germination of *O. minor* seeds only (Evidente *et al.*, 2011).

The herbicides that are currently in use for broomrape control are glyphosate, and herbicides belonging to the imidazolinones (Eizenberg *et al.*, 2006) or sulfonylureas. Glyphosate disrupts the biosynthesis of aromatic amino acids inhibiting the key enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP). Imidazolinones and sulfonylurea herbicides inhibit acetolactate synthesis, also called acetohydroxyacid synthesis, a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine and valine. All of them are systemic herbicides absorbed through foliage and roots of plants with rapid translocation to the attached parasite, which acts as a strong sink (Colquhoun *et al.*, 2006).

Foliar herbicide application for broomrape control normally requires lower herbicide rates. Sequential foliar application of low rates of glyphosate could be effective for broomrape

control only on a few hosts in the families' *Apiaceae*, *Fabaceae* and *Brassicaceae*. Imazapic applied to foliage for broomrape control is effective in sunflower (*Helianthus annuus*), carrot (*Daucus carota*), parsley (*Petroselinum crispum*), faba bean (*Vicia faba*), celery (*Apium graveolens*) and vetch (*Vicia spp.*).

Seed treatments with imidazolinones have proven to be effective for controlling *O. crenata* in faba bean (Sanchez *et al.*, 2003). The great advantage of this method is the low cost of application. The herbicide is incorporated as a coating on the seeds and distributed with them at the time of planting. This replaces a pre-emergence treatment and saves mechanical application costs. In addition, the application of imidazolinones reduces the herbicide rate required by two to three folds, hence being less harmful to the environment. However, under favorable environmental conditions for broomrape attack, the treatment must be supplemented to obtain high broomrape control (Sanchez *et al.*, 2003).

Seed dressing with herbicides using acetolactate synthesis inhibitor slow release formulations of fertilizers, pesticides, and drugs are common. The principle of this technique is the development of biodegradable formulations for seed dressing with small amounts of herbicide for broomrape control. The slow release herbicide formulations will achieve longer control of *O. crenata* with the acetolactate synthesis inhibitor imazapyr. The seed dressing allows imazapyr to spread throughout the crop root zone as the roots grow, prevents imazapyr from leaching away from the host rhizosphere, and requires less herbicide (Kanampiu *et al.*, 2002).

2.5.5. Biological Methods

Like all other plants, broomrapes have natural enemies, which can affect their growth and can potentially be used as agents for their control. Biological control of weeds is defined as the use of natural antagonists to exert pressure on the population of their host to reduce it to

levels below economic importance. Unlike chemical compounds, biological control agents have the advantage of being specific to the weed, and do not directly contribute to environmental pollution. This technique utilizes living organisms (insects, fungi, viruses and bacteria.) to suppress or reduce broomrape infestation. Pathogenicity toward non-target plants is a major constraint; therefore, it is very important that host-specificity and risk assessment should be made before the release of a control organism into the environment.

The 'bio-herbicide approach' employs virulent strains of pathogens (viruses, bacteria or fungi) which naturally occur on the weed and enhances their destructive activity. The infection build-up of the pathogen is manipulated to the extent of causing significant damage to the parasitic weed. Pathogens can be used as sole agents or as part of a complex integrated control strategy (Sauerborn *et al.*, 2007). The antagonistic agents are numerous (Del Serrone and Quacquarelli, 1988; Sauerborn, 1993; Sauerborn and Kroschel, 1996; Klein and Kroschel, 2002; Zermane *et al.*, 2004 and Sauerborn *et al.*, 2007) and there is continuing interest in some phytopathogenous fungi (*Trichoderma harzianum*, *Fusarium oxysporum*, *Urocladium botrytis*, *Mirothecium verrucasia*, and *T. viride*) and bacteria isolated from the rhizosphere of faba bean, available to counteract *O. crenata* (Mabrouk *et al.*, 2007).

Numerous microorganisms potentially useful for the biocontrol of *Orobanch*e species have been isolated and reported, but none has been subject to continuous wide-spread use. About 30 fungal genera were reported to occur on *Orobanch*e spp. (Boari and Vurro, 2004). Results of surveys for fungal pathogens of *Orobanch*e revealed that *Fusarium* species were the most prominent ones associated with diseased broomrapes. Of these, *F. oxysporum* was the predominant species. To date about six *Fusarium* species are reported to be associated with *Orobanch*e (*F. arthrosporioides*, *F. nygamai*, *F. oxysporum*, *F. oxysporum* f.sp. *orthoceras*, *F. semitectum* var. *majus* and *F. solani*) and have shown significant disease

development in selected species of *Orobanche* (Shabana *et al.*, 2003) when tested under controlled and/or field conditions.

Although data on the efficiency of *Fusarium* spp. to control *Orobanche* in the field are rare, the results already indicate that *Fusarium* spp. in most cases do not provide the level of control desired by farmers. Thus there has been no successfully demonstrated control of this weed using potential inundative bio-herbicides. The question arises, how the efficacy of the pathogens can be improved under field conditions in order to fight the target organism (Sauerborn *et al.*, 2007).

Most of the insects which have been reported to occur on *Orobanche* species are polyphagous without any host-specificity and thus damage to these parasitic weeds is limited (Klein and Kroschel, 2002). For biological control, oligo- and monophagous herbivorous insects are of interest. The fly *Phytomyza orobanchia* (Diptera: Agromyzidae) is reported to be host-specific attacking only *Orobanche* species. Its distribution is related to the natural occurrence of *Orobanche* spp. and *Smicronyx* spp. (Coleoptera: Curculionidae) (Klein and Kroschel, 2002).

Myrothecium verrucaria isolated from faba bean roots has been found to inhibit germination of *O. crenata* seeds due to the production of the macrocyclic trichothecene, verrucarins A (El-Kassas *et al.*, 2005). Preliminary results demonstrated control of infection of faba bean by *O. crenata* by the addition of spores of *M. verrucaria* to soil, raising the possibility that this approach might be applicable in the field.

Colonization by *Rhizobium leguminosarum* has been reported to decrease *O. crenata* seed germination (Mabrouk *et al.*, 2007; Fernandez-Aparicio *et al.*, 2009). Arbuscular mycorrhizal fungi (AMF) colonize roots of most agricultural and horticultural species and that AMF symbiosis reduces Strigolactone production by the host plant opens avenues for

its exploitation as an environmentally friendly bio-control strategy (Fernandez-Aparicio *et al.*, 2010).

Altered patterns in interactions between *O. crenata* and symbiotic mutants of *M. truncatula* and pea suggest that parasitic plant infection is controlled by both the conserved symbiotic pathway that mediates symbiotic recognition and by the establishment and autoregulation mechanisms that regulate the extent of colonization by rhizobia and AMF (Kubo *et al.*, 2009; Fernandez-Aparicio *et al.*, 2010). The *M. truncatula* genes "doesn't make infections", DMI2 and DMI3, which are required for development of nodulation with *Sinorhizobium meliloti* (Catoira *et al.*, 2000), play a negative function during *O. crenata* infection of *M. truncatula* and pea by limiting the number of infection events.

2.5.6. Crop Resistance

Resistance to broomrapes is a multi-faceted response in faba bean and legumes. Several defence mechanisms have been detected in plants resistant to broomrape attack, mainly involving cell wall reinforcement (Perez-de-Luque *et al.*, 2005, 2006a, 2007), production of toxic compounds (Lozano *et al.*, 2007).

In legumes, resistance to *O. crenata* associated with low induction of parasitic seed germination has been reported in several species (Perez-de-Luque *et al.*, 2005). It is not known whether this is based on production of germination inhibitors or on reduced production of stimulants. In pea, quantitative trait loci analyses revealed genomic regions involved in the control of low stimulation of *O. crenata* germination. Two quantitative trait loci were associated with pea roots that had low activity on inducing *O. crenata* seed germination (Fondevilla *et al.*, 2010).

Another pre attachment mechanism could be related to the chemotropism responsible for the correct guidance of the *O. crenata* seedling towards the host root. Misdirected growth

of germinated *O. crenata* seeds within the potentially infective distance has been observed in pea (Perez-de-Luque *et al.*, 2005), probably owing to changes in the concentration of compounds exuded from host roots responsible for the chemotropic response of the seedlings. Excess root or seedling exudation could also represent a resistance mechanism in which parasite radicle contact with the host root is avoided. Breeding programs focused on selection of host genotypes for high root exudation levels of germination stimulants could identify better candidates for trap crops to be used in a suicidal germination control strategy (Perez-de-Luque *et al.*, 2005).

2.5.7 Use of Trap Crops

Trap crops are commercially valuable crops that are able to reduce the seed bank of parasitic plants. They are false hosts owing to their ability to fool the parasite by triggering germination but not being compatible in the downstream infection process. Promising results for trap crops have been reported for many host parasite interactions, including pepper against *O. aegyptiaca* and *O. cernua* Loebl. barley, birds foot trefoil, common vetch, creeping bent grass, cucumber, field corn, red fescue, flax, Italian ryegrass, nasturtium, oats, orchard grass, perennial ryegrass, snap bean, sugar pea, sunflower, sweet corn, tall fescue, tomato, and wheat against *O. minor* (Lins *et al.*, 2006).

2.5.8 Integrated Methods

Integrated control of broomrape weeds means to combine and to integrate different preventive measures and control instruments into the given farming system. The integrated use of the resistance inducer benzothiadiazole with the bio-herbicidal pathogen *F. oxysporum* f. sp. orthoceras has shown to provide successful control of *O. cumana* (Muller-Stover *et al.*, 2005). The combined treatment of the two agents resulted in highly reliable control of *O. cumana* and reduced the parasite's emergence by up to 100% (Muller-Stover *et al.*, 2005). The excellent control level in the combined treatments resulted from a lower

number and reduced dry weight of *O. cumana* shoots, indicating that the combination of control strategies takes effect already in the early developmental stages of the parasite. This could be either due to an enhanced activity of the fungus against the early underground stages of the parasite or to an enhanced induced resistance within the sunflower plant.

The effect of combination of bacterial strains and chicken manures on broomrape of faba bean, the results displayed that among the treatments of faba bean inoculated with *R. leguminosarum* (TAL 1399) alone or in combinations with *Bacillus megatherium* var *Phosphaticum* or *Azospirillum brasiliense* plus chicken manure at 35 g pot⁻¹ displayed no *Orobanch*e emergence (above the ground). Furthermore, crop treated with TAL 1399 plus chicken manure at 35 g pot⁻¹ was significantly higher in root, shoot and total dry weight as compared to control and other treatments.

Three isolates of *Trichoderma* species including *T. harzianum*, *T. harzianum* and *T. viride* were tested for control of *Orobanch*e species in peas, faba bean and tomatoes under field conditions in Egypt. Results of field studies showed that soil treatment with these three fungal agents alone or soil treatment with fungal agents plus aerial spray of glyphosate (50 ppm) was efficient and cost-effective method in reducing infection, minimizing the number of spikes parasitic on host plants and increasing yields of peas, faba bean and tomatoes (Mokhtar *et al.*, 2009).

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted in the Southern Zone of Tigray, Ethiopia (Figure 1). The experimental site is located in Oflla district, Adigolo farmers association (FA), at 12°57'37"North latitude and 39°31'41"East longitude with an average elevation of 2552 meter above sea level. The zone includes five districts namely, Oflla, Enda-Mekoni, Emba-Alaje, Raya-Alamata and Raya-Azebo. The first four districts are the major faba bean growing areas in the zone. The district is classified into three agro-ecological zones, namely, highland, midland and lowland. The midland accounts for about 42% of the total 133,296 ha in the district while both the highland and lowland cover 29% each (Oflla district BoANRM, 2007). The average land holding per household is about 0.5 ha (Oflla district BoANRM, 2007).

The mean annual rainfall of the area is 806.5mm and the maximum and minimum temperature ranges from 22.57°C to 6.8°C, respectively. The major soils include clay (28%), loam (57%) and sand (15%) with a pH of 6.8 (BoARD, 2009).

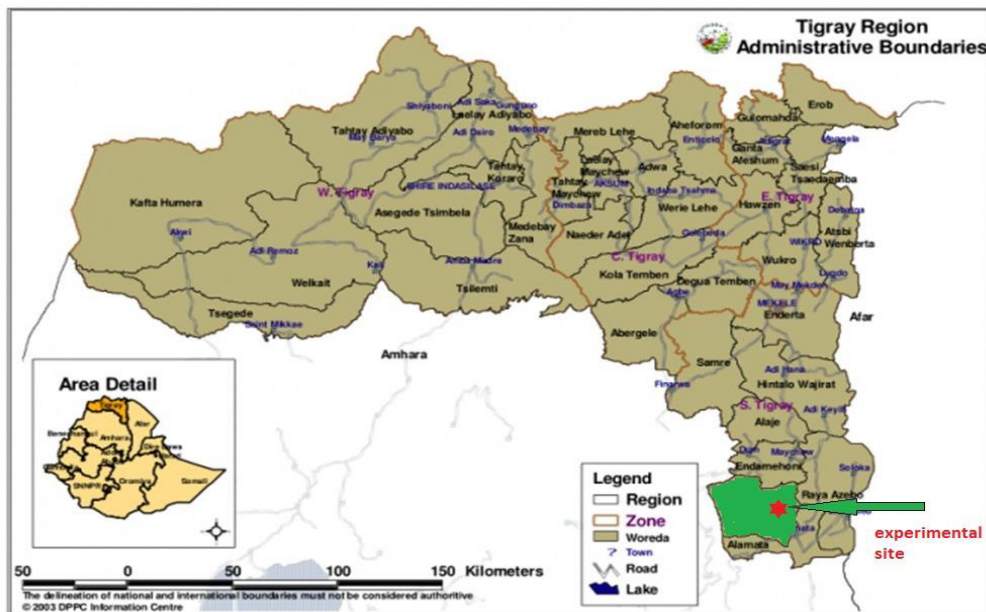


Figure 1: Map showing the study area

3.2. Field survey of *Orobanche crenata* on faba bean in southern zone of Tigray

Field surveys were conducted during 2015 main cropping season in Southern zone of the Tigray Regional State in four major faba bean growing districts. A total of 92 faba bean fields were selected depending on the availability of the crop along the road and accessible routes. Five counts were taken per field in an 'X' fashion using 1m x 1m quadrants. The prevalence or distribution of the *O. crenata* was computed by using the number of fields affected divided by total number of fields assessed and expressed as percentage (Abbes *et al.*, 2007) as:

$$\text{Prevalence (\%)} = \frac{\text{Number of fields infested by Orobanche} \times 100}{\text{Total number of fields assessed}}$$

Incidences were estimated using a 0 to 100% scale. On this scale, 0% represents a row in which no *O. crenata* had emerged and 100% represented a row in which all the host plants carried emerged spikes of *O. crenata* (Mokhtar *et al.*, 2009).

The severity was determined based on the number of emerged spikes of the parasitic plant per host plant. The severity index was calculated using the formula described by Mokhtar *et al.*, (2009) as follows:

$$DS = \frac{\sum(n \times c)}{N}$$

Where, DS= disease severity index

n = Number of infected plants per category

c = Category number

N = Total plants examined

Yield loss on farmers' fields was estimated following the method of Mesa-Gacia and Garcia-Torres (1984) as follows:

$$L = 100 \times 0.124 \times ON$$

L= percent yield loss; ON= emerged *Orobanche* number per plant

3.3. Occurrence and Incidence of Diseases Associated with *O. crenata*

For the assessment of diseases affecting the *O. crenata* plants, samples were collected before the parasitic plant's maturation (October - November 2015). Five randomly selected farmers associations based on previous reports of faba bean infestation with *O. crenata* were assessed (Teklay *et al.*, 2013). Disease assessments in the field were based on symptoms. In each field 1 m² quadrant were thrown five times in an "X" pattern. The incidence of the diseases associated with *O. crenata* plants were estimated according to the following formula:

$$\text{Disease incidence(\%)} = \frac{\text{Number of diseased plant}}{\text{Total number of plants inspected}} * 100$$

The frequencies of occurrence of *O. crenata* plants with each disease symptom (wilting, lesions, spots and blights) in a square meter quadrant were determined. Diseased plant samples were collected, tagged, and carefully pressed in a standard plant press with a thick bundle of newspapers. The samples were packed in double plastic bags and transported to the Ambo PPRC laboratory for further examination. Observed symptoms on diseased *O. crenata* plants were recorded.

3.4. Isolation and Identification of Fungal Pathogens Associated with *O. crenata*

Fungal isolation was made on Potato Dextrose Agar (PDA) And Slight Nutrient Poor Agar (SNA): KH₂PO₄ (1gm), KNO₃ (1gm), MgSO₄.7H₂O (0.5gm), KCl (0.5gm), glucose (0.2gm), sucrose (0.2gm) and agar (17gm) in 1000 ml of distilled water) culture media. Antibiotics (streptomycin sulfate 50 mg/lit, chlortetracycline 10 mg/lit and Penicillin 100 mg/lit) were added to the culture media.

Orobanche plant samples were gently washed in running tap water to remove adhering soil particles and air dried in the laboratory. Then the roots and stems were split lengthwise and disinfected with 70% alcohol and 10% chlorox. Three pieces of the disinfected plant parts

were laid per plate in a circular arrangement on PDA. Examination of the cultures was made five days after incubation at 25 °C and subsequent sub-culturing was performed to obtain pure cultures of the suspected fungal pathogens on PDA and SNA.

To isolate fungi from aerial parts of *O. crenata* plants, infected *O. crenata* plants were surface sterilized using 10 % chlorox and 70% alcohol. Several small sections (5-10 mm square) were taken from the margin of the infected lesion to include both diseased and healthy looking tissue with sterile scalpel. Then three pieces were placed aseptically on a Petri dish containing PDA and incubated at 25 °C for seven days. Isolates were purified on PDA and SNA and pure cultures were maintained in test tubes containing PDA in the refrigerator at 4 °C.

Growing cultures of each isolate were visually observed with the intention of detecting distinct colony growth, colony color, and growth habit. This was followed by microscopic observation to determine the type of the fungus based on the morphology of its mycelium, fruiting structures, and spores. Comparisons of morphological characteristics were made using appropriate guides of fungi.

3.5. Pathogenicity test

3.5.1. Inoculum Production for Fungal isolates collected from *O. crenata* plants

Fungi isolated from *O. crenata* plants were mass produced on wheat grain. The water used to cook the grain had a pH of 6.5-7. Citric acid at a rate of 0.6ml/l was added to lower the pH so as to limit bacterial growth. The wheat grain was transferred into autoclave bags and cooked for 1 hour in water with a grain to water volume ratio of 2:1. Again, the cooked grain was autoclaved at a temperature of 121 °C for one hour.

Spores were harvested from the petri plates containing pure cultures of each fungi isolated and preserved as described under 3.4.using sterile spatula and placed into 250ml flask. A

drop of sterile 0.01% Tween 80 and 5 ml sterile distilled water were added into the flask. The solution was mixed using vortex mixer to form a suspension. Bags were inoculated by pouring the suspension in and agitated thoroughly. The mouth of the bag was covered by paper towel and cheesecloth and sealed with rubber band. After thoroughly mixing the contents of the bag, the mouths were covered with aluminum foil. Finally all bags were placed on shelves at 20-25 °C for twelve days under dark condition.

3.5.2. Preparation of planting medium for pathogenicity testing of fungal isolates on *O. crenata*

Soilmixture of black soil, red soil and sand in 2:1:1 ratio was sterilized for two hours at 150-200 °C. *O. crenata* seeds collected from healthy looking plants from the field during the survey and air dried at room temperature were mixed with part of the sterilized soil mixture. Pots of 25 cm diameter were half filled with the sterilized soil mixture and the rest half was filled with soil infested with *O. crenata* seeds. Seeds of a susceptible local faba bean cultivar was planted, five seeds per pot, and covered with soil mixture.

3.5.3. Inoculation of Fungal Isolates

From among the 12 fungal isolates obtained from the root and stem of symptomatic *O. crenata* plants, fast growing isolates, one from each fungal species, were selected for the pathogenicity testing. The fungal isolates grown on wheat grains were used as source of inoculum. Fifteen grams (10^8 cfu/ml) of wheat upon which *F. oxysporum* isolate was cultured, incorporated into the soil in each of the plots assigned for *F. oxysporum* treatment. The inoculant for *T. viridae* and *Alternaria* were prepared from the wheat grains upon which the fungi were cultured by washing the spores from the grains. The spore concentration for *T. viridae* and *Alternaria* was adjusted to 6×10^6 CFU using plate count method and sprayed on *O. crenata* plants. After inoculation, treatments were arranged in randomized complete design (CRD) with three replications. Treatments were watered as

deemed necessary. Data on number of *O. crenata* plants emerged, *O. crenata* dry biomass, plant height, and disease score were recorded.

3.7. Field Experiment

The study was conducted on soils with known history of severe *O. crenata* infestation. Treatments included four faba bean varieties (Hashenge, an improved variety with known resistance to *O. crenata*, Walki, Moti, and Ofla local susceptible cultivar) and two bioagents (*Rhizobium leguminosarum* and *Trichoderma harzianum*).

3.7.1. Inoculum preparation

3.7.1.1. *Trichoderma harzianum* inoculum production

T. harzianum was obtained from Mekelle University, previously collected from the study area. For the production of *T. harizianum* Potato Dextrose Agar (PDA) medium was used (Dhingra and Sinclair, 1986). The pure culture of *T. harzianum* colonies were plated on PDA and incubated at $24\pm1^{\circ}\text{C}$ for 96 hrs. The spores were washed and collected then after. The spore concentrations were adjusted to a concentration of 5×10^6 conidia/ml with a haemocytometer (Mokhtar *et al.*, 2009).

3.7.1.2. *Rhizobium leguminosarum* inoculum production

The bacterial strain AL38 namely *Rhizobium leguminosarum* was obtained from Mekelle Soil Research Center (MSRC). The strain was previously adjusted to the concentration of 10^8 cfu/ml by MSRC.

3.7.2. Bio-agent application

The bioagents were applied either as sole or in combination as follows: sole *Rhizobium* (*R. leguminosarum*), sole *Trichoderma* (*T. harzianum*), *Rhizobium* + *Trichoderma*, and uninoculated control. The experiment was arranged in factorial randomized complete block design (RCBD) with three replications on a plot size of 4m^2 . The rate of inoculation for *R.*

leguminosarum strain was 7 gram (10^8 cfu/ml) per 1000g of seeds of faba bean while *T. harzianum* was applied at a rate of 5×10^6 conidia/ml (Mokhtar *et al.*, 2009). Treatments were applied on the bean seeds by priming the seeds one hour before sowing. After planted, plots receiving *T. harzianum* with 95 ml spore suspension (5×10^6 conidia/ml) were sprayed four times at 15 days interval and started from 15 days after faba bean seedlings emergence as followed the method of Mokhtar *et al.*, (2009). Spore concentrations were determined with a haemocytometer.

3.8 Data Collected

Data were collected/ recorded on both plant basis and plot basis

3.8.1. Data collection on plant basis

Data on plant bases was recorded for the following characters on randomly taken 10 plants from each middle row of experimental unit (plot). These data were expressed as average of randomly taken plants in each experimental plot.

- Plant height: The height in centimeters from the ground level to the tip of the plant at maturity.
- Number of branches per plant
- Number of pods per plant: The number of pods per plant at harvest after maturity.
- Number of seeds per pod at harvest.
- Nodule numbers: The number of nodules per plant at flowering atage .
- Number of un-emerged *O. crenata*: The number of un-emerged *O. crenata* shoot on selected plants in each plot at plant harvesting.
- *O. crenata* severity: The numbers of *O. crenata* spikes that are attached to the roots of host plants were recorded at harvesting.

3.8.2. Data collection on harvestable plot basis

- Plant emergence date: The date on which 50% of the seedlings in each plot emerged.
- Days to maturity: The number of days from emergence to the stage when 90% of the plants in a plot reach physiological maturity.
- Crop biomass: The weight in grams of the total above ground biomass from each experimental plot.
- Grain yield per plot (g): The seed yield in grams of experimental plot.
- Number of emerged *O. crenata*: The number of emerged *O. crenata* shoots in each plot at harvest after crop maturity.
- *O. crenata* Incidence (%): number of un-infected and infected plant per plot.
- *O. crenata* dry biomass: the shoot dry biomass of *orobanche* per plot.

3.9 Statistical Analysis

Statistical analyses were performed using analysis of variance (ANOVA) using GEN-STAT software and SPSS software. Treatment means were separated by least significant difference (LSD) at $p = 0.05$.

4. RESULTS AND DISCUSSION

4.1. Survey of *Orobanche crenata* in South Tigray

In all four districts in the Southern Zone covered by the survey, *O. crenata* was found at varied infestation levels. In the surveyed area *O. crenata* is known by three different local names: ‘*Mestsalem*’, ‘*Betri- Zbia*’ and ‘*Akenchira*’. The names, in the local language, imply its nature and the symptom on affected crop. Symptoms of *O. crenata* damage observed on faba bean fields in the surveyed area were stunting and premature death.

4.1.1. Incidence and severity of *O. crenata*

The farm level incidence of the *O. crenata* was 72%, 25%, 20% and 4.7% in Ofla, Raya-Alamata, Emba-Alaje and Enda Mekoni districts, respectively. At the district level, the highest mean incidence of 49.67% and severity of 2.23 were recorded in Ofla district (Table 1). The prevalence and level of *O. crenata* infestation (incidence and severity) varied among districts, fields within districts, Farmers Associations (FAs) and fields within FAs (Table 1 and Appendix Table 2).

Faba bean infestation with *O. crenata* reached 100% in three of the surveyed farmers’ fields (Table 2). Six FAs i.e. Tsalgo, Adigolo, Hashenge, Fala, Menkere, and Hayalo had more than 75 % *O. crenata* prevalence.

Table 1: Infestation level of *O. crenata* in faba bean fields in Southern zone of Tigray in 2015

District	Altitude	Number of fields observed	Prevalence (%)	Incidence (%)	Severity
Emba-Alaje	2463-2767	17	20	3.7	0.81
Enda-Mekoni	2423-2938	16	4.76	0.23	0.12
Ofla	2125-2729	50	72.5	49.67	2.23
Raya-Alamata	2200-2517	9	25	2.35	0.5
Total		92			

In Aiba, Atsela, Bora, Embahasti, Meswaeti, Senay, Smret, Teklehaymanot, Tsibet, and Tsetsera FAs, no *O. crenata* was observed in any of the sampled faba bean fields (Appendix Table 2). Where as in Adigolo, Fala, Tsalgo peasant associations, all fields visited were severely infested with the parasitic weed. Incidence of the parasitic weed was high in the Adigolo, Fala, Hashenge, Tsalgo, Menekere and Wenberet where more than 50% of the faba bean plants in sampled plots were infested by the parasitic weed.

At the FA levels, *O. crenata* prevalence ranged from 0 to 100 % while the incidence and severity of the parasitic weed varied between 0 and 100 % and 0 and 14 %, respectively (Table 2).

In an earlier survey conducted by Teklay *et al.*, (2013), the parasitic weed was reported from only five of the farmers associations but not from Tsalgo FA.

Table 2: Prevalence, incidence and severity of *O. crenata* in Southern Zone of Tigray on 2015 cropping season.

No	FAs	District	Prevalence (%)	¹ Incidence (%)	¹ Severity
1	Abeda	Emba-Alaje	50	0-45 (15)	0-4.4(3.4)
2	Tekea	Emba-Alaje	50	0-5 (3.5)	0-2(0.67)
3	Mekhan	Enda-Mekoni	33.3	0-5 (1.6)	0-3(.85)
4	Adigolo	Ofla	100	80-100 (93)	3-14(6.8)
5	Fala	Ofla	100	60-100 (76.5)	0-8(2.8)
6	Hashenge	Ofla	83.3	0-100 (48.5)	0-9(1.8)
7	Hayalo	Ofla	75	0-100 (32.5)	0-4(1.4)
8	Higumbrda	Ofla	50	0-45 (16.2)	0-4(1.54)
9	Menkere	Ofla	83.3	0-100 (67.5)	0-7(2.56)
10	Sessela	Ofla	66.7	0-80 (46.7)	0-6(1.08)
11	Tsalgo	Ofla	100	0-100(63.3)	0-5(2.16)
12	Wenbert	Ofla	50	0-100 (50)	0-7(2)
13	Zata	Ofla	16.7	0-15 (2.5)	0-2(0.55)
14	Merewa	Raya-Alamata	50	0-5 (4.7)	0-2(1)

¹Values outside parenthesis are ranges while those in parenthesis are mean values

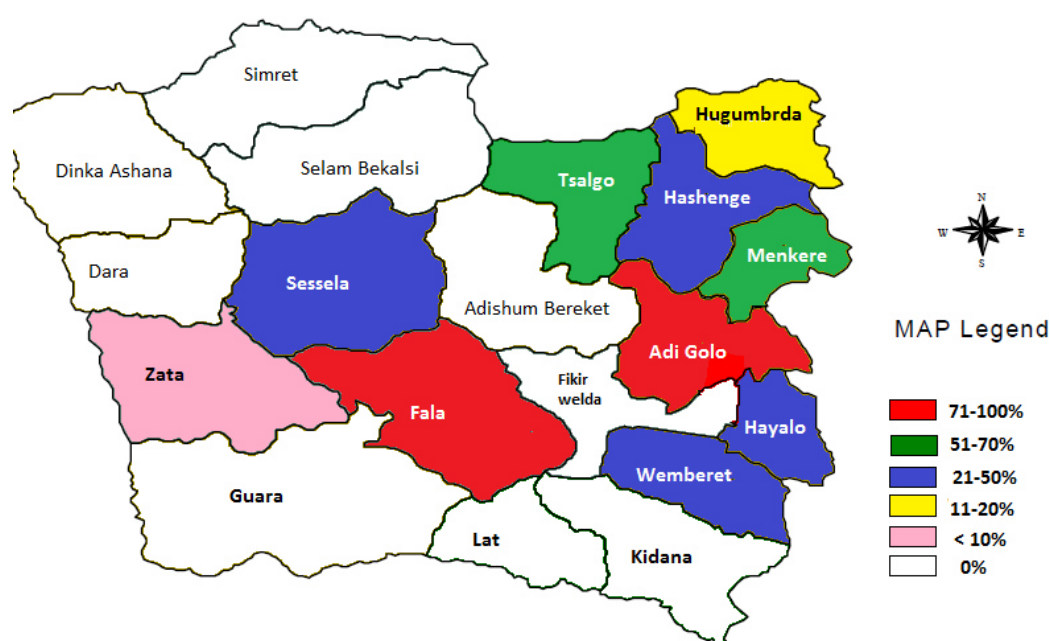


Figure 2: Map showing distribution and percent incidence of *O. crenata* in Ofla District, 2015

4.2. Yield Loss Assessment on Farmers' Fields

Yield loss of faba bean due to *O. crenata* across survey districts was estimated to range between 0 and 99.2%. The mean estimated yield loss across farmers' plots among districts varied depending on the intensity of infestation. Accordingly, the estimated mean yield losses in faba bean fields in Ofla, Raya-Alamata, Emba-Alaje and Enda Mekoni districts were 27.6%, 6.2%, 10.0% and 1.5, respectively (Figure 3). The estimated yield loss at district level was much lower as the intensity of *O. crenata* infestation was concentrated in some FAs while some had little or no infestation (Figure 4 and Appendix Table 3).

In some of the FAs including Aiba, Atsela, Bora, Embahsti, Meswaety, Senay, Smret, Teklehaymanot, Tsebet and Tsetsera no *O. crenata* infested field was observed and thus no yield loss due to the parasitic weed was expected to occur. Where as in the remaining 14 FAs, the estimated mean yield loss ranged from 7% to 84% (Table 3). The highest

estimated mean yield loss (84%) was recorded from the Adigolo followed by Abeda, Menkere, Fala , Tsalgo and Wenbert FAs that had estimated yield losses of 42.2%, 31.7%, 29.8%, 26.9% and 24.8%, respectively. In some of the faba bean fields at Adigolo, Hashenge and Fala, the estimated faba bean seed yield loss was nearly 100%. Adigolo, Hashenge and Fala are FAs where *O. crenata* is believed to have been observed for the first time in the Southern Zone of Tigray (Rezene and Gerba, 2003; Teklay *et al.*, 2013).

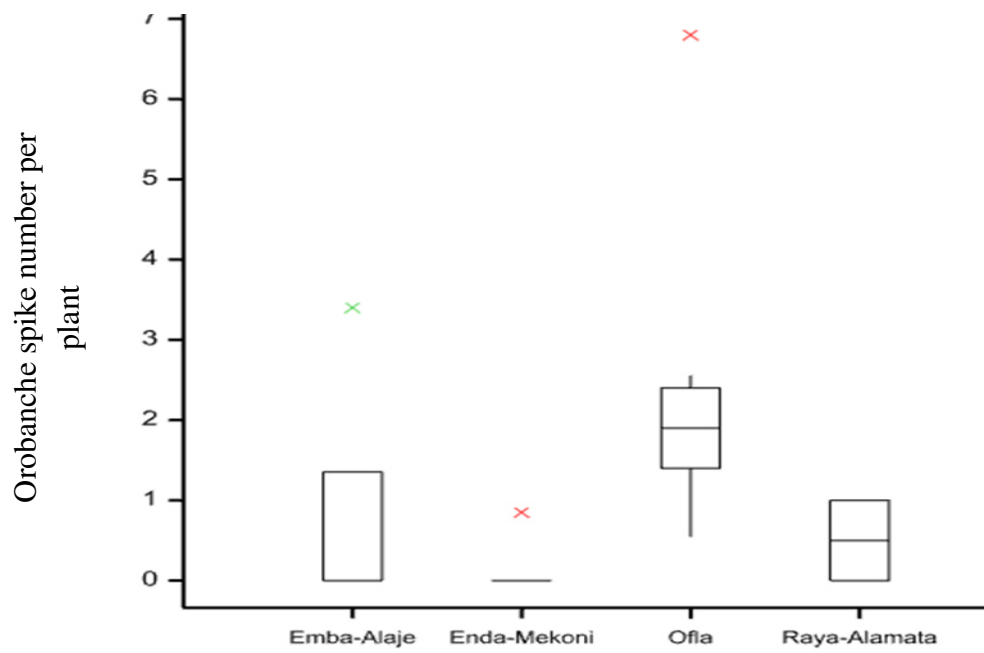


Figure 3: Orobanch spike number per plant on district levels of southern zone of Tigray in 2015 by boxplot.

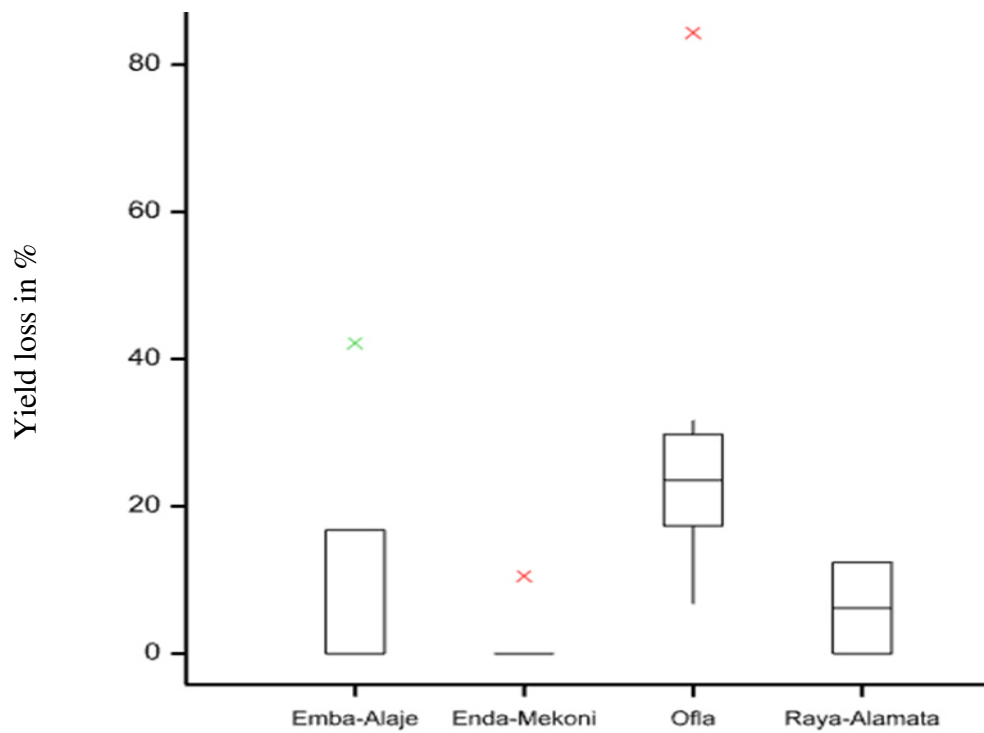


Figure 4: Yield loss on district levels of southern zone of Tigray in 2015 by boxplot.

Table 3: Estimated Yield loss in selected farmers associations of Ofla District in the Southern Zone of Tigray in 2015

FAs	<i>Orobanch</i> e number/ plant		¹ Estimated Yield loss (%)	
	Range	Mean	Range	Mean
Abeda	0-4.4	3.4	0 - 54.6	42.16
Adigolo	3-14	6.8	37 - 99.2	84.32
Fala	0-8	2.4	0 - 99.2	29.76
Hashenge	0-9	1.8	0 - 99.2	22.32
Hayalo	0-4	1.4	0 - 49.6	17.36
Higumbrda	0-4	1.54	0 - 49.6	19.09
Mekhan	0-3	0.85	0 - 37.2	10.54
Menkere	0-7	2.56	0 - 86.8	31.74
Merewa	0-2	1	0 - 24.8	12.4
Sessela	0-6	1.08	0 - 74.4	13.39
Tekea	0-2	0.67	0 - 24.8	8.31
Tsalgo	0-5	2.16	0 – 62	26.78
Wenbert	0-7	2	0 - 86.8	24.80
Zata	0-2	0.55	0 - 24.8	6.82

¹Yield loss (%) was estimated using the formula $100 \times 0.124 \times \text{ON/P}$ following Mukhtar *et al* (2009).

4.3. Incidence of Diseases Associated with *O. crenata*

Different disease symptoms, including black rot on roots and stem, necrotic lesions on the stem and wilting of the whole plant were observed on *O. crenata* plants in the field during the survey period in FAs where the parasitic weed was abundantly growing. Black root rot infection resulted in total death of the plant before the plants reaches flowering.

The incidence of diseases with symptoms including the wilting, black root rot and necrotic lesions, in the faba bean fields was quite low. From among the faba bean fields sampled, 1.4% of the fields at Adigolo and less than 1% of the fields at Menkere, Hashenge and Fala had *O. crenata* plants with one or more of the disease symptoms (Table 4).

In the current study, the frequency of occurrence of the diseases on the parasitic plant was also quite low (Table 5). Black root rot and wilting were the most important diseases associated with *Orobancha* with frequencies of occurrence of 8.5 and 20%, respectively. Necrotic lesions on *O. crenata* were also observed though of low frequency. According to an earlier survey conducted on Egypt by Ibrahim *et al.*, (2007) up to 72.7% % of emerged *O. crenata* plants were observed to die before reaching the reproductive stage due to natural mortality factors including soil borne pathogens.

Table 4: Per field mean incidence of *O. crenata* with disease symptoms in faba bean fields in the Southern Zone of Tigray in 2015.

FAs	Number of fields sampled	Percent fields with <i>O. crenata</i> plants showing disease symptoms
Adigolo	8	1.4
Hashenge	5	0.9
Fala	6	0.4
Menkere	7	0.1
Tsalgo	9	0.5

Table 5: Frequency of occurrence of disease symptoms on *Orbanche* plants in Oflla district, Southern Zone of Tigray in 2015.

Symptoms	Percent occurrence
Wilting	20
Black rot on the root	8.5
Necrotic lesion	5.8

4.4. Identification of fungal disease associated with *O. crenata*

Twelve fungal isolates belonging to three different fungal species were isolated from *O. crenata* plants with symptoms of wilting, black rots on stem and roots and necrotic lesions on the stem. The dominant species, in terms of number of isolates identified, was *Fusarium oxysporum*. Out of the total 12 isolates identified, 7 isolates belonged to *F. oxysporum*, 3 to *Trichoderma viridae* and 2 to *Alternaria* species (Table 6). In a similar study conducted in Jordan, *Alternaria alternata*, *F. oxysporum*, *Dendrophoma* sp., *Chaetomium* sp., *Penicillium* sp, and *Rhizoctonia* spp. were isolated from the stem of *O. crenata* plants that had disease symptoms (Hameed *et al.*, 2001).

Table 6: Type and number of fungal isolates obtained from different parts of infected *O. crenata* plant.

Fungal isolates	Number of isolates	Symptoms and infected <i>O. crenata</i> plant part
<i>F. oxysporum</i>	7	Wilting of the whole plant and necrotic black lesions on the stem
<i>T. viridae</i>	3	Black rot on the root
<i>Alternaria species</i>	2	Necrotic lesion on the stem

Cultures of fungal pathogens isolated from *O. crenata* showed variation in colony characteristics including rate of colony growth and colony surface morphology. The linear diameter of 96 hours old colonies of the isolates ranged from 33 to 87 mm. *T. viridae* isolate collected from Fala (Tv2) had the fastest growth and had a diameter of 87 mm (Appendix Table 1 and Fig. 5). The *T. viridae* isolates collected from Hashenge (Tv1) and Menkere (Tv3), had a colony linear growth of 85 and 82 mm, respectively. The *Alternaria* isolates collected from Hashenge (Al1) and Fala (Al2) had the lowest linear growth with 28 and 33 mm, respectively, (Fig. 5).

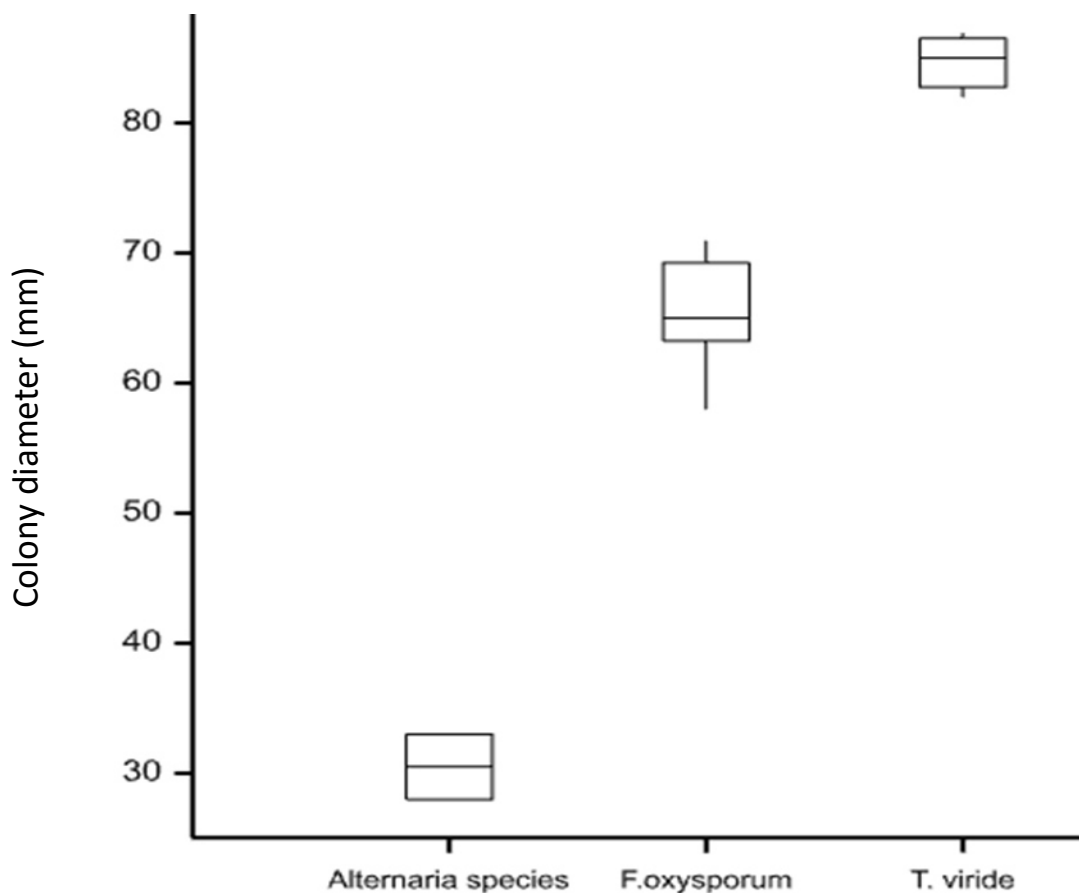


Figure 5: Linear colony diameter (mm) of fungal isolates 96 hours after incubation on PDA at 25°C

From among the *Fusarium* isolates, Isolates Fo1 and Fo7 had the fastest colony linear diameter of 70 and 71 mm, respectively. The slowest diameter colony was F05 with 58mm colony linear growth. While all *T. viridae* had more or less similar rate of growth, ranging between 85- 87mm, the *Alternaria* species had within species comparable linear growth (Figure 5).

All *Fusarium* isolates were completely white on Slight Nutrient Poor Agar (SNA). While on PDA some colonies had whitish colony growth, other variants had pink center and white periphery (Table 7). On the other hand, two of the *T. viridae* isolates had yellowish color with cottony growth on PDA and greenish colony with no cottony growth on SNA, one isolate had whitish colony on both PDA and SNA.

Table 7: Colony morphological characteristics of the fungal isolates obtained from diseased *O. crenata* plants and cultured on PDA and SNA

Isolate	Collection site	Fungal species Identified	Colony characteristics On PDA	Colony characteristics on SNA	Conidial Septation
Al1	Hashenge	<i>Alternaria species</i>	White, cottony	White, cottony	+
Al2	Fala	<i>Alternaria species</i>	White, cottony	White, cottony	+
Fo1	Adigolo	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Fo2	Adigolo	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Fo3	Adigolo	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Fo4	Hashenge	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Fo5	Fala	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Fo6	Menkere	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Fo7	Menkere	<i>F. oxysporum</i>	Pink, cottony	White, cottony	+
Tv1	Hashenge	<i>T. viride</i>	Yellow, flat	Green, flat	+
Tv2	Fala	<i>T. viride</i>	Yellow, flat	Green, flat	+
Tv3	Menkere	<i>T. viride</i>	White, fibrous	White, erect	-

4.5. Pathogenicity Test of fungal isolates on *O. crenata*

4.5.1. Pathogenic fungal isolates on *O. crenata*

4.5.1.1. Effect on *O. crenata* number and symptom development

Isolates of *Fusarium*, *Trichoderma* and *Alternaria* species that were isolated from symptomatic *O. crenata* plants in the study area were pathogenic to *O. crenata* but the isolates varied significantly ($p < 0.05$) in their effect on the number of *O. crenata* spikes emerged (Table 8). Significantly higher number of *O. crenata* plants emerged in pots treated with *Alternaria* and from untreated pots as compared to pots treated with *Fusarium* and *Trichoderma* suggesting the superiority of these two fungi in inhibiting the emergence of the parasitic weed. Similar trend was observed in terms of the dry biomass of the

parasitic weed. Those *O. crenata* shoots that were able to emerge in each of the inoculation treatments, had developed disease symptoms similar to what was observed under natural infestation in the field, but significantly higher percent of the plants had disease symptoms in the Fo7 treated pots than in the *Trichoderma* and *Alternaria* treated pots. There were significantly more *O. crenata* plants with disease symptoms in the *Trichoderma* treated pots than in the *Alternaria* treated pots (Table 8). There was no symptom development on the *O. crenata* plants in the untreated control pots. On the other hand, the *Alternaria* isolate tested in the current study was not able to significantly reduce the number of emerging *O. crenata* plants than that of the untreated control.

Table 8: Percent spikes with disease symptoms and number and dry biomass of *O. crenata* spikes in pots treated with fungal isolates

Fungi isolates	Percent infected spikes	<i>O. crenata</i> number/ pot	<i>O. crenata</i> dry biomass (g)
<i>F.oxysporum</i> (Fo7)	74.33 ^d	0.47 ^a	40.54 ^a
<i>T. viride</i> (Tv2)	54.00 ^c	0.72 ^a	43.63 ^a
<i>Alternaria</i> spp.	24.60 ^b	3.27 ^b	51.00 ^b
uninoculated	0.00 ^a	3.00 ^b	52.67 ^b
LSD	11.58	1.80	4.96
CV (%)	16.80	31.70	5.50

Means in column followed by the same letter are not significantly different according to LDS P=0.05

In a similar study conducted by Ghannam *et al.*, (2007), *F. oxysporum* isolates were highly effective in reducing the emergence of *O. crenata*. In another study, the tubercle number of emerged *O. crenata* in faba bean inoculated with *Fusarium* was reduced by 70% to 87% compared to the un-inoculated controls (Boutiti *et al.*, 2008). Similar to the current findings, *T. viride* isolates reduced the emergence of *O. crenata* and enhanced pea biomass yield in pot experiment (Mabrouk *et al.*, 2007).

The observed inhibition of *O. crenata* emergence in the *Fusarium* and *Trichoderma* treated soils could possibly be explained by the findings of the Kader *et al.*, (2001) and Muller-Stover and Kroschel (2005). According to these authors, the inhibition of *O. crenata* emergence following exposure to fungal propagules suggests that *O. crenata* seed structures were possibly altered to render the germination stimulant from faba bean ineffective in triggering germination; besides, the structural modification to the seed coat could be of a physical and/or of a chemical nature, thereby blocking the chemical binding of the stimulant to the seed (Abdel-Kader *et al.*, 2001; Muller-Stover and Kroschel, 2005).

4.5.1.2. The Effect of inoculation with fungal isolates on *O. crenata* dry biomass

The dry *O. crenata* shoot biomass in pots treated with *T. viride* (40.5g) was not significantly different from that of *F. oxysporum* (43.6g) treated pots. But the *O. crenata* dry shoot biomass in both the Fo7 and Tv2 treated pots was significantly lower than those of the untreated control and the AL1 treated pots (Table 8). However, there was no significant difference in the *O. crenata* dry shoot biomass between the latter two treatments (Table 9). Boutiti *et al.*, (2008), Thomas *et al.*, (1999) and Zermane *et al.* (1999) reported similar findings. Boutiti *et al.*, (2008) that inoculation with *Fusarium* reduced the dry matter weights of parasitic plants. Similarly, Thomas *et al.*, (1999) found that *Fusarium oxysporum* colonized seeds of *O. cumana* and the shoot biomass of *O. Cumana* plants resulting from *F. oxysporum* inoculated seeds was significantly reduced. Zermane *et al.* (1999) also found that the germination of *O. crenata* was reduced by 50.3% when inoculated with *F. oxysporum*. Thus the differences in *O. crenata* shoot dry weight observed among the different treatments could be explained by the effect of the different fungal isolates in causing varying level of disease development on *O. crenata* shoots.

4.5.2. Effect of inoculating faba bean seeds with fungal isolates pathogenic to *O. crenata*

4.5.2.1. Effect on Faba bean biomass

Inoculation of faba bean with *T. viride* (isolate Tv2) and *F. oxysporum* (isolate Fo7) resulted in enhanced faba bean dry shoot weight. Faba bean plants in pots treated with *T. viridae* had significantly higher dry shoot biomass (168.8g) than the faba bean plants in un-inoculated control as well as in pots treated with *F. oxysporum* and *Alternaria* species (Table 9). The dry shoot weight of faba bean plants grown in pots treated with *F. oxysporum* and *Alternaria* species was not significantly different but the *F. oxysporum*, had significantly higher dry shoot biomass than that of the un-inoculated control. None of the isolates seemed to have any negative effect on the faba bean plants. Ghannam *et al.*, (2007) observed that the use of *Fusarium* isolates pathogenic to *O. crenata* enabled to control *O. crenata* and increased the tomato biomass yield.

4.5.2.2. Effect on faba bean plant height

The different fungal isolates showed varied effects on faba bean plant height. The faba bean plant height was significantly higher in both *F. oxysporum* (64.3cm) and *T. viridae* (71 cm) treated plots than those of the *Alternaria* treated (56.6cm) and in the un-inoculated control (51.87 cm) (Table 9). Similar to the current findings, Boutiti, *et al.*, (2008) reported that inoculation with *Fusarium* isolates resulted in 36% to 38% increase in the height of faba bean compared to the un-inoculated control plots.

Table 9: Faba bean shoot dry weight and height as influenced by inoculation with different fungal isolates

Treatment (isolates)	Faba bean dry biomass(g)	Plant height(cm)
<i>F. oxysporum</i> (Fo7)	159.90 ^b	64.33 ^b
<i>T. viride</i> (Tv2)	168.80 ^c	71.00 ^b
<i>Alternaria</i> species(All)	152.70 ^{ab}	56.60 ^a
Un-inoculated control	151.00 ^a	51.87 ^a
LSD	7.74	6.69
CV (%)	2.50	5.80

Means in a column followed by the same letter are not significantly different at LSD P=0.05

4.6. Synergistic Effect of Host Plant Resistance, *Rhizobium leguminosarum* and *Trichoderma harzianum* on *Orobanche crenata* Infestation and Faba bean yield

4.6.1 Effect of varieties and bio-inoculants on incidence and severity of *O. crenata*

The main effect of varieties and bio-inoculants on the incidence and severity of *O. crenata* infestation was highly significant (Table 10). The faba bean variety Hashenge had the lowest percent of *O. crenata* incidence (25.98%) and severity of infestation (1.07) that were significantly lower than that of Walkie, Moti and the local variety. The local variety had the highest incidence (97.7%) and severity (5.17) of *O. crenata* that were significantly higher than that of Hashenge, Walkie and Moti. Varieties Walkie and Moti had similar incidence and severity of *O. crenata*. The percent incidence and severity of the parasitic weed infestation were lower in varieties Hashenge, Walki and Moti than was recorded in the local variety. The difference in *O. crenata* severity in different varieties was attributed to the differences in the level of resistance between faba bean genotypes (Mokhtar *et al*, 2009).

Among the bio-inoculation treatments, the untreated control had significantly highest incidence and severity of the parasitic weed as compared to that of the *T. harzianum*, *T. harzianum* + *R. leguminosarum* and *R. leguminosarum* treatments (Table 10). The latter three treatments did not vary significantly in the incidence and severity of the weed among themselves. However, they had 11.46%, 8.42% and 7.62%, less *O. crenata* incidence and 39.45, 47.85, and 35.16%, less severity of infestation than that of the untreated control, respectively. Mokhtar *et al.* (2009) similarly found significant reductions in the severity of *O. crenata* infection in *T. harzianum* and *T. viride* treated plots than the untreated control.

Table 10: Main effect of varieties and bio-inoculants on the incidence and severity of *O. crenata*

Treatments	Incidence	¹ Reduction (%)	Severity	¹ Reduction (%)
Varities				
Hashenge	25.98 ^a	74.41	1.07 ^a	79.30
Walkie	87.65 ^b	10.30	3.62 ^b	29.98
Moti	86.78 ^b	11.19	4.35 ^b	15.86
Local Variety	97.72 ^c	0	5.17 ^c	0
Mean	74.53		3.55	
Cv (%)	7.9		27.6	
LSD	4.938		0.821	
P. value	<.001		<.001	
Bio-inoculants				
<i>T. harzianum</i>	70.86 ^a	11.46	3.10 ^a	39.45
<i>R. leguminosarum</i>	73.93 ^a	7.62	3.32 ^a	35.16
<i>T. harzianum</i> + <i>R. leguminosarum</i>	73.29 ^a	8.42	2.67 ^a	47.85
Uninoculated control	80.03 ^b	0	5.12 ^b	0
Mean	74.53		3.55	
Cv (%)	7.9		27.6	
LSD	4.938		0.821	
P-value	0.005		<.001	

Means in column followed by the same letter are not significantly different according to LSD P=0.05

¹ **Percent reduction** is the difference in percent incidence/severity in relation to the Percent incidence/severity recorded in the local variety/un-inoculated treatment

4.6.2 Effect of varieties and bio-inoculants on the number of emerged and un-emerged juvenile shoots and dry biomass of *Orobancha crenata* shoots

Varities and bio-inoculants affected the number of emerged *O. crenata* shoots significantly (P<0.05) while only varieties showed significant variation in the number of juvenile un-emerged shoots and bio-inoculants showing no significant difference (Table

11). The dry biomass of the parasitic weed was significantly affected by both the varieties and the bio-inoculants ($P < 0.001$).

The number of juvenile un-emerged *O. crenata* spikes attached to the roots of faba bean plant, varied among the varieties significantly ($P < 0.05$) but not among the bio-inoculants (Table 11). Significantly higher number of un-emerged *O. crenata* shoots was recorded in the variety Walki (4.5) that was significantly higher by 78.80, 33.50, and 20.31% than that of Hashenge, the local variety and Moti, respectively. The lowest was recorded in Hashenge (about 1) which was significantly lower than that of the other three varieties.

The highest number of emerged *O. crenata* shoots/m² was recorded from the local variety (41.67 shoots/m²) that was significantly higher than the one was recorded in Moti (31.7 shoots/m²), Walki (29.8 shoots/m²) and Hashenge (10.8 shoots/m²) varieties. Similarly, the significant difference among the varieties in the both the emerged and un-emerged *O. crenata* shoots could be attributed to the difference in their genetic makeup of varieties as was also reported by Mokhtar *et al.*, (2009) where varieties varied in the number of emerged and un-emerged juvenile *O. crenata* shoots.

The analysis of variance for *Orobancha* shoots dry weight showed significant ($p < 0.001$) difference among the varieties and bio-inoculants with no Variety x bio-inoculant interaction effect (Table 11 Appendix Table 9). The highest *O. crenata* dry biomass weight was recorded in the local variety (611g) that was not significantly different from that of Walki (564g). The least was recorded in Hashenge (199.6g) which was significantly lower than those recorded from the other three varieties. However, Walki and Moti did not differ in the *Orobancha* dry biomass weight.

Among the bio-inoculants, only *T. harzianum* + *R. leguminosarum* treated plots had significantly lower *O. crenata* dry biomass (334g) than that of the untreated control (559g)

and the sole *T. harzianum* (473g) and sole *R. leguminosarum* (492g) treated plots. The latter three bio-inoculants were not statistically different in the *O. crenata* dry biomass.

Table 11: Main effect of varieties and bio-inoculants on *Orobanche* dry biomass, number of emerged and un-emerged *Orobanche* shoots.

Treatments	<i>O. crenata</i> shoot number / m ²		<i>O. crenata</i> Dry biomass (g)
	Emerged shoots	Un-emerged shoots	
Varieties			
Hashenge	10.82 ^a	0.95 ^a	199.6 ^a
Walki	29.83 ^b	4.483 ^c	564.3 ^{bc}
Moti	31.69 ^b	3.567 ^b ^c	484.3 ^b
Local variety	41.67 ^c	2.983 ^b	611.2 ^c
Mean	28.5	3.00	465.
Cv(%)	25.1	56.7	26.1
LSD	5.509	1.416	104.1
P-value	<.001	<.001	<.001
Bio-inoculants			
<i>T. harzianum</i>	23.44 ^a	2.85	473.8 ^b
<i>R. legumniosarum</i>	26.81 ^a	3.55	491.7 ^b
<i>T. harzianum</i> + <i>R. leguminosarum</i>	27.92 ^a	2.867	334.5 ^a
<i>Untreated control</i>	35.83 ^b	2.717	559.4 ^b
Mean	28.5	3.00	465.
Cv(%)	25.1	56.7	26.1
LSD	5.509	NS	104.1
P-value	<.001	0.629	0.001

Means in column followed by the same letter are not significantly different according to LSD
P=0.05

4.6.3. Effect of bio-inoculants and varieties on seed and biomass yield of faba bean

The faba bean seed yield was significantly different only among varieties ($P < 0.001$) with no significant effect of the bio-inoculants and no variety x bio-inoculant interaction effect (Table 12 and Appendix Table 9). The highest (2220.10kg/ha) seed yield was obtained from Hashenge variety while the least was from Walkie (165.40kg/ha). The seed yield obtained from the Hashenge variety was significantly higher than those of the other three varieties. Though Walki had statistically similar seed yield with the local variety, it had

significantly lower seed yield than Moti. Hashenge variety had 1245, 593, and 952% more seed yield than varieties Walki, Moti and the local variety, respectively. From among the bio-inoculants, only *T.harzianum* + *R. leguminosarum* treated plots had higher seed yield than that of the untreated control that was higher by 27% even though it was not statistically significant.

The biomass yield of faba bean was significantly different among varieties ($p < 0.001$) with no significant difference among the bio inoculants and no variety x bio inoculant interaction effect. Only Hashenge had significantly higher dry biomass yield (4642 kg/ha) than that of Walkie (2125kg /ha), Moti (2333kg/ha) and the local variety (1812kg/ha). Nevertheless, the later three varieties were not different in the dry biomass yield.

Among the bio-inoculants, though not significant, plots treated with sole *T. harzianum* had higher dry biomass yield than plots treated with sole *R. leguminosarum* which was higher by 24%.

Table 12: Main effect of varieties and bio-inoculants on faba bean seed yield, dry biomass yield, 100 seed weight and days to maturity

Treatments	Seed yield (kg/ha)	Biomass yield (kg/ha)	100 Seed weight	Days to maturity
Varieties				
Hashenge	2220.10 ^c	4667 ^b	67.39 ^c	116.60 ^d
Walkie	165.40 ^a	2125 ^a	47.39 ^b	99.80 ^b
Moti	320.20 ^b	2333 ^a	48.28 ^b	105.30 ^c
Local variety	210.90 ^{ab}	1812 ^a	31.94 ^a	93.50 ^a
Mean	729.00	2734	48.75	103.81
Cv (%)	24.00	23.20	6.70	2.50
LSD	149.2	5.279	2.799	2.171
P-value	<.001	<.001	<.001	<.001
Bio-inoculants				
<i>T. harzianum</i>	772.00	3042	47.93 ^b	103.10 ^{ab}
<i>R. leguminosarum</i>	709.10	2450	49.77 ^{bc}	105.20 ^{bc}
<i>T. harzianum</i> + <i>R. leguminosarum</i>	803.50	2854	51.06 ^c	105.40 ^c
<i>untreated control</i>	632.00	2592	46.24 ^a	101.50 ^a
Mean	729	27.34	48.75	103.81
Cv (%)	24.0	23.20	6.7	2.50
LSD	NS	NS	2.799	2.171
P-value	0.126	0.122	0.008	0.002

Means in column followed by the same letter are not significantly different according to LSD P=0.05

Mokhtar *et al.*, (2009) found increased yield in pea plants with the inoculation of *Trichoderma* spp which they explained could be a result of significant increase in the accumulative yield of green pea pods compared to the untreated controls. Their findings, however, is partly not in agreement with the findings of the current study where the

untreated control and the sole *T. harzianum* treated plots were not significantly different. Improvement in seed yield was observed where there was a combined application of *R. leguminosarum* and *T. harzianum*.

4.6.4. Effect of varieties and bio-inoculants on hundred seed weight, days to maturity and plant height

Both varieties and bio inoculants varied in the faba bean 100 seed weight with no variety x bio-inoculant interaction effect (Table 12 and Appendix Table 9). The local variety had the least 100 seed weights (31.9g) that was significantly lower than those of the varieties Hashenge (67.4g), Walkie (47.4) and Moti (48g). Though Walkie and Moti did not differ in the 100 seed weight, both varieties had significantly lower 100 seed weight than that of Hashenge. Hashenge 100 seed weight was 110, 42, and 39% more than that of the local variety, Walkie and Moti varieties respectively.

Inoculation with *T. harzianum* + *R. leguminosarum* resulted in significantly higher seed weight that was different from those of the untreated control and the sole *T. harzianum* treated plots. The sole *R. leguminosarum* treated plots were not different in 100 seed weight from both the *T. harzianum* + *R. leguminosarum* and sole *T. harzianum* treated plots but was higher than that of the untreated control. The untreated control had 100 seed weight that was 10, 8 and 4% lower than that of the *T. harzianum* + *R. leguminosarum*, sole *R. leguminosarum* and sole *T. harzianum*, respectively. Mokhtar *et al.* (2009) similarly found improved seed weight by the application of *T. harzianum* though the percent improvement they reported, 21.8 % seed weight increment over the un-inoculated control was much higher than that of the current study.

Both the varieties and the bio-inoculants varied in the days to maturity with no interaction effect (Table 12 and Appendix Table 9). The varieties differed significantly in days to maturity with the local variety maturing 23, 6 and 12 days earlier than varieties Hashenge,

Walkie and Moti, respectively. Hashenge was a late maturing variety followed by Moti, Walki and the local variety. The bio-inoculants also showed significant variation in the days to maturity of the crop with plots treated with *T. harzianum* + *R. leguminosarum* maturing late by 4 days than the un-inoculated control. The latter matured significantly earlier (by 4 days) than plots treated with the sole *R. leguminosarum* and *T. harzianum* + *R. leguminosarum* but was similar with that of the sole *T. harzianum* treated plots.

The analyses of variance showed significantly ($p < 0.05$) different effect on plant height only among varieties but not among bio-inoculants and no interaction effect (appendix Table 9 and Table 13). The lowest plant height was recorded from the local variety (73.33cm) while the highest was recorded on Hashenge variety (85.82 cm). All three varieties were significantly shorter than followed by Moti which was taller than the local variety.

4.6.5. Effect of bio-inoculants and varieties on yield components of faba bean

4.6.5.1 Effect of varieties and bio-inoculants on number of pods and branches per plant and number of seeds per pod

The number of seeds per pod showed no significant variation among interaction effect and among bio inoculants. The number of branches and the number of pods per plant were significantly different only among varieties but not among bio inoculants (Tables 13).

The number of branches per plant was significantly higher in the Hashenge variety (2.95 branches per plant) than that of Moti (2.47 branches per plant) and Walkie (2.45 branches per plant) but was similar to that of the local variety (2.68 branches per plant). The local variety on the other hand had similar number of branches per plant to those of the Moti and Walkie.

Similarly, Hashenge had significantly higher number of pods per plant (8.58 pods/plant) than that of the local variety (5.65 pods per plant), Walkie (2.33 pods per plant) and Moti

(3.05 pods per plant). The last two varieties had significantly lower number of pods per plant than the local variety.

Table 13: Main effect of varieties and bio-inoculant on faba bean nodule number (NdN), plant height (PH), seed number per pod (SN-PL), branch number (BN) and pod number per plant (PN-PL)

Treatments	NdN	PH	SN_PL	BN	PN_PL
Varities					
Hashenge	86.42 ^b	85.82 ^c	3.55 ^b	2.95 ^b	8.583 ^c
Walkie	43.17 ^a	77.15 ^{ab}	1.717 ^a	2.45 ^a	2.333 ^a
Moti	42.67 ^a	78.7 ^b	2.167 ^a	2.467 ^a	3.05 ^a
Local variety	22.17 ^a	73.33 ^a	2.2 ^a	2.683 ^{ab}	5.65 ^b
Mean	48.6	78.75	2.41	2.638	4.90
Cv(%)	58.2	7.3	59.4	20.4	59.9
LSD	23.57	NS	1.193	NS	2.450
P-value	<.001	<.001	0.023	0.101	<.001
Bio-inoculants					
<i>T. harzianum</i>	51.92	79.82	2.95	2.583	5.2
<i>R. leguminosarum</i>	45.92	76.67	2.05	2.733	4.25
<i>T. harzianum</i> + <i>R. leguminosarum</i>	44.42	81.7	2.383	2.617	5.85
<i>untreated control</i>	52.17	76.82	2.25	2.617	4.317
Mean	48.6	78.75	2.41	2.638	4.90
CV (%)	58.2	7.3	59.4	20.4	59.9
LSD(0.05)	NS	NS	NS	NS	NS
P-value	0.866	0.113	0.466	0.910	0.497

Means in column followed by the same letter are not significantly different according to LDS
P=0.05

4.6.5.2 Effect of bio-inoculants and varieties on number of nodules per plant

Only varieties differed in the number of nodules per plant. Variety Hashenge had the highest nodule number per plant (86.4) that was significantly higher than that of the other three varieties (Table 13). Varieties other than Hashenge were not different in the number of nodules per plant.

On the other hand, from among the bio-inoculants, *T. harzianum* + *R. leguminosarum* treated plots had nodule numbers (81.7 higher than that of the sole *R. leguminosarum* treated (76.7) and the untreated plots (76.8) though not statistically significantly different.

Ghannam *et al.* (2007) reported that higher root, shoot and total dry weight were obtained when faba bean plants were inoculated with *R. leguminosarum* plus compost as compared to the control and that shoot dry weight of faba bean combinations between *R. leguminosarum* plus compost was found to be three times greater than that of the control. In the current study as well, *T. harzianum* and /or *R. leguminosarum* in combination or singly decreased *O. crenata* infestation and increased faba bean yield, dry biomass, pod number, 100-seed weight and plant height.

4.6.6. Relationship between *O. crenata* Infestation and Yield of Faba bean

Faba bean grain yield showed highly significant difference ($p \leq 0.01$) and negative correlation ($r = -0.81$) with *O. crenata* severity. On the other hand, there was highly significant difference ($p \leq 0.01$) and positive correlation ($r = 0.79$) between *O. crenata* incidence and severity of the treatment tested. Highly significant difference ($p \leq 0.01$) difference and negative correlation ($r = -0.74$) was observed between *O. crenata* dry biomass and grain yield (Appendix Table 7). This indicates that the association between *O. crenata* dry biomass and grain yield were higher, which means low *O. crenata* biomass yield enhances the faba bean grain yield. Generally, based on Faba bean grain yield obtained from the tested faba bean varieties, the local variety was the most susceptible and Hashenge variety was the most resistant variety.

Teklay *et al*, (2013) reported that the susceptible genotypes had a higher percentage of infection with *O. crenata* than the resistant genotypes and similarly Mabrouk *et al*, (2007) and Mokhtar *et al*, (2009) reported that incidence was shown to be positively correlated with the severity and biomass yield of *O. crenata*.

5. SUMMARY AND CONCLUSION

Faba bean is one of the major food legumes crop in Ethiopia. Ethiopia is considered as one of the centers of diversity for faba bean. Faba bean is a major source of protein in almost every rural Ethiopian household and improving soil health. Faba bean yields are, however, low due to many factors of which the parasitic weed, *O. crenata*, is the major one. However, not enough research efforts have been directed to develop effective and sustainable options against *O. crenata* in Ethiopia, particularly in southern Tigray. This study was undertaken to assess *O. crenata* distribution and fungal antagonists associated with *O. crenata* and to test the pathogenicity of potential fungal isolates that could be used in the biological control of *O. crenata*. The work also aims to evaluate the combined effect of host plant resistance and bio-agents on *O. crenata*.

In the present work, *O. crenata* was studied in a more specific and intensive way. A survey was conducted in Southern zone of the Tigray National Regional State, which covered Ofra, Raya-Alamata, Emba-Alaje and Enda-Mekoni districts. Prevalence, incidence and severity were recorded from each field. *O. crenata* was prevalent in all the districts surveyed and is an important parasitic plant of faba bean in the zone. The parasite was more abundant and intense in Ofra district in which, the highest prevalence (100%), incidence (93%) and severity (6.8%) were recorded. Adigolo FA was the most affected one among the surveyed FAs in the area. Diseases were observed infecting roots and stems of the *O. crenata* plant. *F. oxysporum* was the most dominant species of the isolated fungi. Among the tested isolated fungi shows significantly different ($P < 0.05$) effect on *O. crenata* biomass and number of juvenile.

Field experiment was conducted on soils with known history of severe *O. crenata* infestation. Using four faba bean varieties including one resistant variety and a local check,

and bio-inoculants (*Rhizobium leguminosarum*) and bio-control agent (*Trichoderma harzianum*) that were applied singly or in combination to plots. Data on *O. crenata* infestation and faba bean yield were collected. The results from field experiment revealed that the highest (5.17%) *O. crenata* severity was recorded on local variety whereas the lowest severities (1.07 and 3.62 %) were recorded on Hashenge and Walkie varieties, respectively. The percent incidence and severity of the parasitic weed infestation was 74, 11, and 10% and 79, 30, and 16%, respectively, lower in varieties Hashenge, Walki and Moti than was recorded in the local variety. The highest *O. crenata* dry biomass weight was recorded in the local variety (611g) that was not significantly different from that of Walki (564g). The lowest was recorded in Hashenge (199.6g) which was significantly lower than those recorded from the other three varieties. However, Walki and Moti did not differ in the Orobanche dry biomass weight.

The Orobanche resistant variety, Hashenge, had 1245, 593, and 952% more seed yield than varieties Walki, Moti and the local variety, respectively. Hashenge had seed weight that was 110, 42, and 39% more than that of the local variety, Walkie, and Moti varieties, respectively. The untreated control had seed weight that was 10, 8 and 4% lower than that of the *T. harzianum* + *R. leguminosarum*, sole *R. leguminosarum* and sole *T. harzianum*, respectively.

There was no bio inoculant x variety interaction effect across all parameters assessed, which explains that the varieties had similar response to the bio inoculants. In addition, bio inoculant had no effect on branch number, total number of nodules, plant height, pod number, grain yield and biomass yield, emerged Orobanche number, seed number per plant. Bio inoculant affected the days to maturity, 100 seed weight, emerged *O. creanta* number, Orobanche dry biomass, incidence and severity of *O. crenata*.

Hashenge variety have good yield, good level of resistance against *O. crenata* and has better agronomic performance than the other tested varieties including the local. Therefore, the variety has to be made available to all the farming communities in the *O. crenata* infested areas, Moreover, extensive studies need to be conducted to evaluate the reaction of faba bean varieties to orobanche and to come up with bio-control methods of *O. crenata* to enhance faba bean production. Efforts on finding the possibility of mass production of these biological control agents and storage of the products should also be sought for.

6. REFEEENCES

- Abbes, Z., Kharrat, M., Delavault, P., Simier, P. and Chaïbi, W. 2007. Field evaluation of the resistance of some faba bean (*Vicia faba* L.) genotypes to the parasitic weed *Orobanche foetida* Poiret. *Crop Protection* 26(12): 1777-1784.
- Abbes, Z., Kharrat, M., Simier, P. and Chaïbi, W. 2007. Characterization of resistance to crenata broomrape (*Orobanche crenata*) in a new small-seeded line of Tunisian faba beans. *Phytoprotection* 88(3): 83-92.
- Abdel-Kader, M. and El-Mougy, N. 2009. Prospects of mycoherbicides for control of broomrapes (*Orobanche* spp.) in Egypt. *Journal of Plant Protection Research* 49(1): 63-76.
- Abdel-Kader, M.M. and El-Mougy, N.S. 2007. Applicable control measure against *Orobanche ramosa* in tomato plants. *Australasian Plant Pathology* 36(2): 160-164.
- Agrios, G.N., 2005. Plant Pathology. (5th ed.). Elsevier Academic Press.USA. pp. 831
- Asfaw, T, Tesfaye, G and Beyene, D. 1994. Genetics and breeding of faba bean. Pp .122-137. In: Asfaw Telaye (Eds). Cool-season food Legumes in Ethiopia. Proceedings:
- Ashrafi, Z.Y., Hassan, M.A., Mashhadi, H.R. and Sadeghi, S. 2009. Applied of soil solarization for control of Egyptian broomrape (*Orobanche aegyptiaca*) on the cucumber (*Cucumis sativus*) in two growing seasons (in Iran). *Journal of Agricultural Technology* 5(1): 201-212.
- Beck-Mennagetta G, 1930.Orobanchaceae. In: Engler HGA, ed. Das Pflanzenreich, 96(IV-261):1-275.
- Besufekad Tadesse, Admassu Tadesse, Rezene Fessehaie, 1999. Orobanche problem in south Wollo. *Research Review*, EARO.
- Boari, A. and Vurro, M. 2004. Evaluation of *Fusarium* spp. and other fungi as biological control agents of broomrape (*Orobanche ramosa*). *Biological Control* 30(2): 212-219.
- Bond, D.A, Laws, G. Hawtin, M.. Saxena, and J. S. Stephens, 1985. Faba bean (*Vicia faba* L.). pp. 199-265.

- Boutiti, M.Z., Souissi, T. and Kharrat, M. 2008. Evaluation of *Fusarium* as potential biological control against *Orobanche* on Faba bean in Tunisia. In *Proceedings of the XII international symposium on biological control of weeds*. CAB International, Wallingford, UK 238-244.
- Central Statistical Agency (CSA). 2015. Report on area and production of major crops (private peasant holdings, meher season). *Statistical bulletin* 578(1): 21-25.
- Crepona K, Marget P, Peyronnet C, Carrouéa B, Arese P, Duc G. 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research* 115: 329–339.
- Dhingra, O. D. and J. B. Sinclair. 1986. Basic Plant Pathology Methods. CRC Press, Inc. Boca Raton, Florida, 355p.
- Díaz-Sanchez, J., Jurado-Expósito, M., López-Granados, F., Castejón-Muñoz, M. and García-Torres, L. 2003. Pronamide Applied to Sunflower Seeds for *Orobanche cumana* Control . *Weed Technology* 17(2): 314-319.
- Dorr I, Kollmann R.1995. Symplasmic sieve element continuity between *Orobanche* and its host. *Botanica Acta* 108(1): 47-55
- Duc, G., 1997. Faba bean (*Vicia faba* L.) production in the world. *Field Crops Research*, 53(1): 99-109.
- Eizenberg, H., Colquhoun, J.B. and Mallory-Smith, C.A. 2006. Imazamox application timing for small broomrape (*Orobanche minor*) control in red clover. *Weed Science* 54(5): 923-927.
- El-kassas, R., karam el-Din, Z., Beale, M.H., Ward, J.L. and Strange, R.N. 2005. Bioassayed isolation of *Myrothecium verrucaria* and verrucarins A as germination inhibitors of *Orobanche crenata*. *Weed Research* 45(3): 212-219.
- Evidente, A., Cimmino, A., Fernández-Aparicio, M., Rubiales, D., Andolfi, A. and Melck, D. 2011. Soyasapogenol B and trans-22-dehydrocampesterol from common vetch (*Vicia sativa* L.) root exudates stimulate broomrape seed germination. *Pest Management Science* 67(8): 1015-1022.

- Evidente, A., Fernandez-Aparicio, M., Andolfi, A., Rubiales, D. and Motta, A. 2007. Trigoazonane, a monosubstituted trioxazonane from *Trigonella foenum-graecum* root exudate, inhibits *Orobanche crenata* seed germination. *Phytochemistry* 68(19) : 2487-2492.
- FAO, 2008. Progress on farmer training in parasitic weed management. Rome-Italy.
- FAOSTAT, www.fao.org. Available at [http://www.rlc.fao.org/progesp/pesa/cari.com/pdf/](http://www.rlc.fao.org/progesp/pesa/cari.com/pdf/Overview%20of%20Food%20and%20Nutrition%20Security.pdf) Overview of/ Food and Nutrition Security.
- Fernandez-Aparicio, M., Andolfi, A., Evidente, A., Perez-de-luque, A. and Rubiales, D. 2008. Fenugreek root exudates show species-specific stimulation of *Orobanche* seed germination. *Weed Research* 48(2): 163-168.
- Fernandez-Aparicio, M., García-Garrido, J.M., Ocampo, J.A. and Rubiales, D. 2010. Colonisation of field pea roots by *Arbuscular mycorrhizal* fungi reduces *Orobanche* and *Phelipanche* species seed germination. *Weed Research* 50(3): 262-268.
- Fernandez-Aparicio, M., Yoneyama, K. and Rubiales, D. 2011. The role of strigolactones in host specificity of *Orobanche* and *Phelipanche* seed germination. *Seed Science Research* 21(01): 55-61.
- Fernandez-Aparicio, M., Flores, F. and Rubiales, D. 2009. Recognition of root exudates by seeds of broomrape (*Orobanche* and *Phelipanche*) species. *Annals of Botany* 103(3): 423-431.
- Fessehaie, R. and Neftci, K., 2006. Review of Weed Research in Weed Research in Highland and Lowland Pulses. *Increasing Crop Production through Improved Plant Protection I*, p.133.
- Fondevilla, S., Fernandez-Aparicio, M., Satovic, Z., Emeran, A.A., Torres, A.M., Moreno, M.T. and Rubiales, D. 2010. Identification of quantitative trait loci for specific mechanisms of resistance to *Orobanche crenata* Forsk. in pea (*Pisum sativum* L.). *Molecular Breeding* 25(2): 259-272.
- Ghannam, I., Barakat, and Al-Masri, 2007. Biological control of Egyptian broomrape (*Orobanche aegyptiaca*) using *Fusarium* spp. *Phytopathologia Mediterranea* 46(2): 177-184.

- Grenz, J.H., Manschadi, A.M., Uygur, F.N. and Sauerborn, J. 2005. Effects of environment and sowing date on the competition between faba bean (*Vicia faba*) and the parasitic weed *Orobanche crenata*. *Field Crops Research* 93(2): 300-313.
- Habimana, S., Murthy, K.N.K., Hatti, V. and Nduwumuremyi, A. 2013. Management of orobanche in field crops-a review. *Scientific Journal of Crop Science* 2(11): 144-158.
- Hassan, M.M., Osman, A.G., Sherif, A.M., Rugheim, A.M., Mohamed, I.S., Gani, M.E.S.A. and Babiker, A.G.E.T. 2012. Effects of bacterial strains and chicken manure on *Orobanche crenata* infesting Faba bean. *International Journal of Applied Science* 2(1): 59-63.
- Heraux, F.M., Hallett, S.G., Ragothama, K.G. and Weller, S.C. 2005. Composted chicken manure as a medium for the production and delivery of *Trichoderma virens* for weed control. *Journal of Horticultural Science* 40(5): 1394-1397.
- ICARDA. 2006. Technology Generations and Dissemination for Sustainable Production of Cereals and Cool Season Legumes. International Center for Agricultural Research in the Dry Areas, Aleppo, Syria. pp. 256.
- Joel, D.M. and Mayer, A.M. 2003. Changes in the activity of the alternative oxidase in *Orobanche* seeds during conditioning and their possible physiological function. *Phytochemistry* 64(1): 235-241.
- Joel, D.M., Hershenhorn, J., Eizenberg, H., Aly, R., Ejeta, G., Rich, P.J., Ransom, J.K., Sauerborn, J. and Rubiales, D. 2007. Biology and management of weedy root parasites. *Horticultural Review Journal* 267–349.
- Joel, D.M., Steffens, J.C., and Matthews, D.E. 1995. Germination of Weedy Root Parasites. Marcel Dekker: New York, USA Inc., 567-598.
- Kebreab, E. and Murdoch, A.J. 1999. Modelling the effects of water stress and temperature on germination rate of *Orobanche aegyptiaca* seeds. *Journal of Experimental Botany* 50(334): 655-664.

- Kiros Meles and Muluberhan Haile. 2005. Distribution of some orobanche and cuscuta species in Tigray. Poster presented at the 7th Annual Conference of the Weed Science Society of Ethiopia. 24-25 November 2005, Addis Abeba, Ethiopia.
- Klein, O., Kroschel, J. 2002. Biological control of *Orobanche* spp. With *Phytomyza orobanchia*. *A Review. Biological Control* 47(2): 245-277.
- Kohlschmid, E., Sauerborn, J. and Muller-Stover, D. 2009. Impact of *Fusarium oxysporum* on the holoparasitic weeds *Phelipanche ramosa*: biocontrol efficacy under field-grown conditions. *Weed Research* 49(51): 56-65.
- Kroschel, J. and Saxena, M.C. 1997. Effect of plant density on grain yield, root-length density and *Orobanche crenata* infestation in two faba bean genotypes. *Weed Research* 37(1): 39-49.
- Kroschel, J., 2001. A Technical Manual for Parasitic Weed Res. and Extension. Kluwer Academic Publishers, Dordrecht, the Netherlands, 256.
- Kuijt, J. 1969. The biology of parasitic flowering plants. . *Biology of parasitic flowering plants*. University of California Press, Berkeley.
- Letousey, P., De Zelicourt, A., Vieira Dos Santos, C., Thoiron, S., Monteau, F., Simier, P., Thalouarn, P. and Delavault, P. 2007. Molecular analysis of resistance mechanisms to *Orobanche cumana* in sunflower. *Plant Pathology* 56(3): 536-546.
- Lopez-Granados F, Garcia-Torres L. 1999. Longevity of crenate broomrape (*Orobanche crenata*) seed under soil and laboratory conditions. *Weed Science* 47: 161-166.
- Lozano, M.D., Moreno, M.T., Rubiales, D., Perez-De-Luque, A. 2007. *Medicago truncatula* as a model for non-host resistance in legume-parasitic plants interactions. *Plant Physiology* 145: 437-449.
- Maalouf, F., Khalil, S., Ahmed, S., Akintunde, A.N., Kharrat, M., El Shama'a, K., Hajjar, S. and Malhotra, R.S. 2011. Yield stability of faba bean lines under diverse broomrape prone production environments. *Field Crops Research* 124(3): 288-294.

- Mabrouk, Y., Zourgui, L., Sifi, B., Delavault, P., Simier, P. and Belhadj, O., 2007. Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitised by *Orobanche crenata*. *Weed Research* 47(1): 44-53.
- Metayer .2004. *Vicia faba* breeding for sustainable agriculture in Europe. Giefeverole.
- MoA (Ministry of Agriculture). 2011. Animal and plant health regulation directorate. Crop varieties register. Issue No. 14. Addis Ababa, Ethiopia. pp. 71-73.
- Ofla district BoANRM (Ofla district burro of Agriculture and Natural Resources Manegment). 2007. Natural resource annual report.
- Muller-Stover, D., Thomas, H., Sauerborn, J. and Kroschel, J. 2004. Two granular formulations of *Fusarium oxysporum* f. sp. orthoceras to mitigate sunflower broomrape *Orobanche cumana*. *Biological Control* 49(5): 595-602.
- Mussa, J., Dereje, G., Gemechu, K. 2008. Procedures of Faba Bean Improvement through Hybridization. 48p. Technical Manual No. 21, Ethiopian Institute of Agricultural Research.
- Mwakaboko, A.S. and Zwanenburg, B., 2011. Strigolactone analogs derived from ketones using a working model for germination stimulants as a blueprint. *Plant and Cell Physiology* 52(4): 699-715.
- Oswald, A., Ransom, J.K., Kroschel, J. and Sauerborn, J. 2002. Intercropping controls *Striga* in maize based farming systems. *Crop Protection* 21(5): 367-374.
- Panetta, F.D. and Lawes, R., 2005. Evaluation of weed eradication programs: the delimitation of extent. *Diversity and Distributions* 11(5): 435-442.
- Paran, I., Gidoni, D. and Jacobsohn, R. 1997. Variation between and within broomrape (*Orobanche*) species revealed by RAPD markers. *Heredity* 78(1): 128-131
- Parker, C. and Riches, C.R. 1993. Parasitic Weeds of the World. CAB International, Wallingford.
- Parker, C. 2009. Observation on the current statues of *Orobanche* and *Striga* problems worldwide. *Pest Management Science* 65(5):453-459.
- Paszkowski, U. 2006. A journey through signaling in *arbuscular mycorrhizal* symbioses 2006. *New Phytologist* 172(1): 35-46

- Perez-De-Luque, A., Eizenberg, H., Grenz, J.H., Sillero, J.C., Avila, C., Sauerborn, J. and Rubiales, D. 2010. Broomrape management in faba bean. *Field crops Research* 115(3): 319-328.
- Perez-de-Luque, A., Jorin, J., Cubero, J.I. and Rubiales, D. 2005. *Orobanche crenata* resistance and avoidance in pea (*Pisum* spp.) operate at different developmental stages of the parasite. *Weed Research* 45(5): 379-387.
- Perez-de-Luque, A., Lozano, M.D., Cubero, J.I., Gonzalez-Melendi, P., Risueno, M.C. and Rubiales, D. 2006. Mucilage production during the incompatible interaction between *Orobanche crenata* and *Vicia sativa*. *Journal of Experimental Botany* 57(4): 931-942.
- Perez-de-Luque, A., Moreno, M.T. and Rubiales, D. 2008. Host plant resistance against broomrapes (*Orobanche* spp.): defence reactions and mechanisms of resistance. *Annals of Applied Biology* 152(2): 131-141.
- Punia, S.S. 2014. Biology and control measures of Orobanche. *Indian Journal of Weed Science* 46(1): 36-51.
- Restuccia, A., Marchese, M., Mauromicale, G. and Restuccia, G. 2009. Biological characteristics and control of *Orobanche Crenata* Forsk., a review. *Italian Journal of Agronomy* 4(1): 53-68.
- Rezene F, Gerba L. 2003. Weed Research in High Land Food Legumes of Ethiopia. In: Ali K, Kenneni G, Ahmed S, Malhotra R, Beniwal S, Makkouk, K, Halila MH (eds)2003. Food and forage legumes of Ethiopia: Progress and prospects. Proceedings of the Workshop on Food & Forage Legumes 22-26 September 2003, Addis Ababa, Ethiopia pp 278-287.
- Riopel, J.L. and Timko, M.P. 1995. Haustorial initiation and differentiation. *Parasitic Plants* 39-79.
- Rubiales, D., Fernández-Aparicio, M., Wegmann, K. and Joel, D.M. 2009. Revisiting strategies for reducing the seedbank of Orobanche and *Phelipanche* spp. *Weed Research* 49(51): 23-33.

- Rubiales, D., Perez-de-Luque, A., Fernandez-Aparico, M., Sillero, J.C., Román, B., Kharrat, M., Khalil, S., Joel, D.M. and Riches, C. 2006. Screening techniques and sources of resistance against parasitic weeds in grain legumes. *Euphytica* 147(1-2): 187-199.
- Rubiales, D. and Heide-Jorgensen, H.S. 2011. Parasitic plants. *Wiley Online Library. Els.*
- Sahile S, Ahmed S, Fininsa C, Abang MM, Sakhuja PK. 2008. Survey of chocolate spot (*Botrytis fabae*) disease of faba bean (*Vicia faba* L.) and assessment of factors influencing disease epidemics in northern Ethiopia. *Crop Protection* 27: 1457-1463.
- Sahile, G., Abebe, G. and Tawaha, A.I. 2005. Effect of soil solarization on *Orobanche* soil seed bank and tomato yield in central rift valley of Ethiopia. *World Journal of Agricultural Science* 1: 143–147.
- Sauerborn, J. and Saxena, M.C. 1986. A review on agronomy in relation to *Orobanche* problems in faba bean (*Vicia faba* L.). In *Proceedings of a Workshop on Biology and control of Orobanche, Wageningen (Netherlands) 13-17 Jan 1986*.
- Sauerborn, J., Müller-Stöver, D. and Hershenhorn, J. 2007. The role of biological control in managing parasitic weeds. *Crop Protection* 26(3):246-254.
- Tashkoski, P. 2013. Fusarium rot on *Orobanche ramosa*-broomrape on tobacco in the republic of Macedonia. *Tütün/Tobacco* 63.
- Teklay, A., Hadas, B. and Yemane, N. 2013. Distribution and economic importance of broomrape (*Orobanche crenata*) in food legumes production of south Tigray, Ethiopia. *Journal of Crop Production* 2: 101-106.
- Teklay, A., Kiros, M., Yemane, N., Hadas, B. and Abrha, K. 2013. Interaction between broomrape (*Orobanche crenata*) and resistance faba bean genotypes (*Vicia faba* L.) in Tigray region of Ethiopia. *Canadian Journal of Plant Protection* 1(3): 104-109.
- Thomas, H., Heller, A., Sauerborn, J. and Muller-Stover, D. 1999. *Fusarium oxysporum* f. sp. orthoceras, a Potential Mycoherbicide, Parasitizes Seeds of *Orobanche cumana* (Sunflower Broomrape): a Cytological Study. *Annals of Botany* 83(4): 453-458.

- Timko, M.P., Florea, C.S. and Riopel, J.L. 1989. Control of germination and early development in parasitic angiosperms. In *Recent Advances in the Development and Germination of Seeds* 3: 225-240. Springer US.
- Torres, A.M., Román, B., Avila, C.M., Satovic, Z., Rubiales, D., Sillero, J.C., Cubero, J.I. and Moreno, M.T. 2006. Faba bean breeding for resistance against biotic stresses: towards application of marker technology. *Euphytica* 147(1-2): 67-80.
- Villegas Fernández, Á.M. 2012. Screening faba bean (*Vicia faba* L.) for chocolate spot resistance: evaluation methods and effects of age of host tissue and temperature. *European Journal of Plant Pathology* 132(3): 443-453.
- Westwood, J.H., Yu, X., Foy, C.L. and Cramer, C.L. 1998. Expression of a defense-related 3-hydroxy-3-methylglutaryl CoA reductase gene in response to parasitization by *Orobanch* spp. *Molecular Plant-microbe Interactions* 11(6): 530-536.
- Yohannes, T., Ngugi, K., Ariga, E., Abraha, T., Yao, N., Asami, P. and Ahonsi, M. 2016. Genotypic Variation for Low Striga Germination Stimulation in Sorghum “*Sorghum bicolor* (L.) Moench” Landraces from Eritrea. *American Journal of Plant Sciences* 7(17): 2470.
- Zermane, N., Souissi, T., Kroschel, J. and Sikora, R. 2007. Biocontrol of broomrape (*Orobanch* *crenata* Forsk. and *Orobanch* *foetida* Poir.) by *Pseudomonas fluorescens* isolate Bf7-9 from the faba bean rhizosphere. *Biocontrol Science and Technology* 17(5): 483-497.

7. APPENDICES

Appendix Table 1: Colony diameter (mm) of fungal species growth on PDA for 96 hours

Isolate Code	Fungal species identified	Colony diameter (mm)
Fo1	<i>F. oxysporum</i>	70
Fo2	<i>F. oxysporum</i>	65
Fo3	<i>F. oxysporum</i>	63
Fo4	<i>F. oxysporum</i>	67
Al1	<i>Alternaria</i> species	28
Tv1	<i>T. viride</i>	85
TV2	<i>T. viride</i>	87
Al2	<i>Alternaria</i> species	33
Fo5	<i>F.oxysporum</i>	58
TV3	<i>T. viride</i>	82
Fo6	<i>F.oxysporum</i>	64
Fo7	<i>F.oxysporum</i>	71

Appendix Table 2: Prevalence, incidence and severity of *O. crenata* in southern Tigray

No.	FAs	Altitude	¹ NOF	¹ Prevalence (%)	Incidence (%)		Severity	
					range	mean	range	mean
1	Abeda	2587-2696	4	50	0-45	15	0-4.4	3.4
2	Aiba	2463-2767	3	0	0	0	0	0
3	Atsela	2554-2590	3	0	0	0	0	0
4	Bora	2463-2543	3	0	0	0	0	0
5	Tekea	2582-2671	4	50	0-5	3.5	0-2	0.67
6	Embahasti	2801-2938	2	0	0	0	0	0
7	Mekhan	2423-2538	3	33.33	0-5	1.6	0-3	0.85
8	Meswaety	2504-2552	3	0	0	0	0	0
9	Senay	2504-2552	2	0	0	0	0	0
10	Smret	2642-2784	2	0	0	0	0	0
11	Teklhaymanot	2589-2658	3	0	0	0	0	0
12	Tsebet	2843-2939	1	0	0	0	0	0
13	Adigolo	2554-2576	7	100	80-100	93	3-14	6.8
14	Fala	2489-2673	6	100	60-100	76.5	0-8	2.4
15	Hashenge	2542-2589	6	83.33	0-100	48.5	0-9	1.8
16	Hayalo	2601-2678	4	75	0-100	32.5	0-4	1.4
17	Higumbrda	2506-2576	4	50	0-45	16.25	0-4	1.54
18	Menkere	2532-2578	6	83.33	0-100	67.5	0-7	2.56
19	Sessela	2216-2557	3	66.67	0-80	46.67	0-6	1.08
20	Tsalgo	2590-2816	6	100	0-100	63.33	0-5	2.16
21	Wenbert	2548-2594	2	50	0-100	50	0-7	2
22	Zata	2125-2729	6	16.67	0-15	2.5	0-2	0.55
23	Merewa	(2200-2517	6	50	0-5	4.7	0-2	1
24	Tsetsera	2359-2467	3	0	0	0	0	0
Tot			92					
al								

¹NOF: number of field

Appendix Table 3: Estimation of Yield loss on farmers' field in south Tigray

Fas	¹ ON/P		² Yield loss (%)	
	Range	Mean	Range Yield loss (%)	Mean Yield loss (%)
Abeda	0-4.4	3.4	0-54.56	42.16
Adigolo	3-14	6.8	37-99.20	84.32
Aiba	0	0	0	0
Atsela	0	0	0	0
Bora	0	0	0	0
Embahasti	0	0	0	0
Fala	0-8	2.4	0-99.20	29.76
Hashenge	0-9	1.8	0-99.20	22.32
Hayalo	0-4	1.4	0-49.60	17.36
Higumbrda	0-4	1.54	0-49.60	19.09
Mekhan	0-3	0.85	0-37.20	10.54
Menkere	0-7	2.56	0-86.80	31.74
Merewa	0-2	1	0-24.80	12.4
Meswaety	0	0	0	0
Senay	0	0	0	0
Sessela	0-6	1.08	0-74.40	13.39
Smret	0	0	0	0
Tekea	0-2	0.67	0-24.80	8.31
Teklhaymanot	0	0	0	0
Tsalgo	0-5	2.16	0-62	26.78
Tsebet	0	0	0	0
Tsetsera	0	0	0	0
Wenbert	0-7	2	0-86.80	24.80
Zata	0-2	0.55	0-24.80	6.82

¹ON/P=Orobanche number per plant

²Yield loss (%) was estimated by $100 \times 0.124 \times \text{ON/P}$ formula

Appendix Table 4: ANOVA table for on incidence, Orobanche dry biomass (ODB), emerged Orobanche number (EON/M²), severity and un-emerged Orobanche number (UON)

Sources of variation	Degrees of freedom	₁ Incidence	ODB	EON/M ²	₂ Severity	UON
Mean square values						
Replication	2	2.75	28397	263.54	2.9200	4.516
Variety(V)	3	12868.86	408126	1992.00	37.7000	26.896
Bio-inoculants(B)	3	182.34	106910.	330.36	13.9667	1.692
V*B	9	23.54	11577.	76.18	0.9244	2.638
Error	30	35.07	15603.	43.65	0.9707	2.885
P-value						
Variety(V)		<.001	<.001	<.001	<.001	<.001
Bio-inoculants(B)		0.005	0.001	<.001	<.001	0.629
V*B		0.728	0.668	0.122	0.497	0.526

Appendix Table 5: ANOVA table for faba bean grain yield (FGY), dray biomass (FDB), 100 seed weight (HSW) and faba bean days to maturity (FMD)

Sources of variation	Degrees of freedom	FGY	FDB	HSW	FMD
Mean square values					
Replication	2	37453.	23.83	17.25	19.312
Variety(V)	3	11946248.	2046.31	2528.95	1150.354
Bio-inoculants(B)	3	66107.	84.01	53.31	42.076
V*B	9	23758.	16.74	8.13	5.113
Error	30	32008.	40.09	11.27	6.779
P-value					
Variety(V)		<.001	<.001	<.001	<.001
Bio-inoculants(B)		0.126	0.122	0.008	0.002
V*B		0.668	0.916	0.685	0.658

Appendix Table 6: ANOVA table for faba bean nodule number (FNdN), plant height (FPH), seed number per pod (FSN-PL), branch number (FBN) and pod number per plant (FPN-PL)

Sources of variation	Degrees of freedom	FNdN	FPH	FSN_PL	FBN	FPN_PL
Mean square values						
Replication	2	641.1	48.67	0.111	0.3175	0.306
Variety(V)	3	8774.2	327.36	7.534	0.6564	96.559
Bio-inoculants(B)	3	193.7	71.67	1.790	0.0519	7.021
V*B	9	760.7	22.47	1.185	0.1460	5.710
Error	30	799.3	33.13	2.049	0.2899	8.636
P-value						
Variety(V)		<.001	<.001	0.023	0.101	<.001
Bio-inoculants(B)		0.866	0.113	0.466	0.910	0.497
V*B		0.497	0.722	0.804	0.860	0.736

Appendix Table 7: Correlation between faba bean yield and *O. crenata* infestation (severity, incidence and biomass yield).

	Incidence	Severity	OBY	ON/M ²	FGY
Incidence	–	-			
Severity	0.7916 ^(***)	-			
OBY	0.7372 ^(***)	0.7547 ^(***)	-		
ON/M ²	0.7646 ^(***)	0.7677 ^(***)	0.6544 ^(***)	-	
FGY	-0.9520 ^(***)	-0.8143 ^(***)	-0.7224 ^(***)	-0.7452 ^(***)	-

*significant at (P<0.05), **significant correlated at (P<0.01) and *** highly significant correlated at (P<0.001).

Appendix Table 8: Interaction effect of varieties with bio-agent on *O. crenata* infestation

Treatments	Incidence	Severity	Orobanche biomass yield(kg/ha)	Un-emerged Orobanche number	Orobanche number /m ²
Bio-agent*varieties					
B1* V1	23.08	0.27	184.2	0.93	9
B1* V2	82.5	3.93	608.6	5.46	28.25
B1* V3	81.42	3.60	519.6	2.66	19.17
B1* V4	96.45	4.60	582.5	2.33	37.33
B2 *V1	26.12	1.13	273.1	0.53	9.5
B2 *V2	83.92	4.73	573.1	4.66	31
B2* V3	85.7	2.73	445.1	5.46	26.67
B2* V4	100	4.67	675.3	3.53	40.08
B3* V1	24.67	0.80	123.9	0.73	9.52

Continue

B3 *V2	89.58	3.13	386.8	3.66	22.25
B3* V3	84.5	2.73	407.5	3.93	36.67
B3* V4	94.42	3.73	419.9	3.13	43.25
B4 *V1	30.04	2.06	217.2	1.60	15.25
B4 *V2	94.58	5.60	688.5	4.13	37.83
B4 *V3	95.5	5.40	565	2.20	44.25
B4 *V4	100 ^c	7.40	767.1	2.93	46
CV (%)	4.3	24	24.7	56.7	43.3
LSD (5%)	ns	ns	ns	ns	Ns

V1=Hashenge variety, V2=Walkie variety, V3=Moti variety, V4=Local cultivar

B1=*T. harzianum*, B2= *R. leguminosarum*, B3= *T. harzianum* + *R. leguminosarum*,
B4=un-inoculated control

Appendix Table 9: Interaction effect of varieties and bio-control agent on faba bean grain yield (FGY), faba bean plant height (FPH),faba bean days to maturity (FMD),dry biomass (FDB) and100 seed weight (HSW)

Treatments	FGY	FPH	FMD	FDB	HSW
B1 V1	2246	83.87	67.2	53.33	8.67
B1 V2	168.5	80.4	47.33	23.33	11.83
B1 V3	442.3	80.73	46.89	24.17	9.1
B1 V4	235.3	74.27	30.29	20.83	15.25
B2 V1	2167	87.47	68.23	40.5	31.58
B2 V2	161.3	74.4	50.67	21.67	31
B2 V3	320.5	73.93	47.83	21.67	22.25

Continue					
B2 V4	180.8	70.87	32.33	14.17	34.67
B3 V1	2460.7	88	68.33	48.33	25.17
B3 V2	191.3	76.47	49.33	21.67	25
B3 V3	304.3	83.93	52.27	24.17	36
B3 V4	234.5	78.4	34.3	20	39.5
B4 V1	2010	83.93	65.79	44.5	56.33
B4 V2	143.7	77.33	42.23	18.33	54.08
B4 V3	214.7	76.2	46.13	23.33	47.25
B4 V4	159.7	69.8	30.82	17.5	65.18
CV%	21.4	7.3	2.2	17.2	43.3
LSD	ns	ns	ns	ns	ns

BIOGRAPHICAL SKETCH

The author was born on 13 September 1986 in Wukro district, Eastern zone of Tigray Region, from his father Brhane Aregawi Gebregergs and his mother Abadit Kebede Tafere. He completed his elementary in Abrha-we- Atsbha Primary School and Agazi- Wukro Secondary School from 1992-2004. After completing his Secondary School, he joined the Bahir Dar University in 2012 and graduated with B.Sc. degree in Plant Sciences. After his graduation, he was employed by the Tigray Agricultural Research Institute (TARI) and was involved in different research activities in the field of Plant Protection at the Alamata Agricultural Research Center. He joined the School of Graduate Studies at Hawassa University in September 2015 to pursue a study leading to M.Sc. degree in Crop Protection (specialization: Plant Pathology).

