CHAPTER ONE

INTRODUCTION

Faba bean (*Vicia faba* L.*)*, a Fabaceae, has the Near East, mainly Iraq, and Iran as centre of origin. However, secondary centres in Afghanistan and Ethiopia evolved later in the late Neolithic period (Cubero,1974). The crop is planted mainly in the Mediterranean regions, the Nile Valley, Ethiopia, Central Asia, and Northern Europe (Bond *et al*., 1985).The crop is a main source of edible proteins for human and animals (Bond *et al*., 1985). The protein contents varies widely (20 – 41%) (Chaven *et al*., 1989). In addition, faba bean contributes to soil fertility through nitrogen fixation and thus minimizes the need for chemical fertilizers and their adverse impact on the environment (Gasim *et al*., 2011).

In Sudan faba bean is the most important cool season food legume. It is the major source of protein for a major sector of the populace, particularly in urban areas (Babiker *et al*., 2007). Moreover, the crop is an important source of income to farmers and plays an important role in improving soil fertility and increasing productivity of subsequent crops. The crop is planted in northern Sudan since time immemorial along the fertile strip of the alluvial soils of the Nile valley extending north, on both banks of the Nile, from Khartoum to Wadi Halfa (*Ca*.2800 km) on the Egyptian borders. The crop is also produced in limited areas in Northern and Southern Darfur, the Gezira and the bank of the River Rahad (Babiker *et al*., 2007). In Sudan the total area under leguminous crops {Faba bean, Lentil (*Lens culinaris* S Medik), and Chickpea (*Cicer arietinum* L.)} is about 80 thousand hectares. Faba bean, planted in over 70% of the area, yields about 70 thousand metric tons of grains annually and thus constitute about 70% of the country's needs (Babiker *et al*., 2007). Productivity and yield stability of cool season legumes are constrained by many biotic and abiotic factors. Parasitism by broomrapes (*Orobanche* spp) is by far the most important in West

Asia, North Africa and Southern Europe (Linke *et al*.,1989). The yield losses, range from 5-100%, pending the level of parasite infestation (Linke *et al*.,1989).

Plate 1. *Orobanche crenata* on faba bean

Plate 2. Faba bean displaying complete damage by *O. crenata*

Orobanche crenata Forsk. was recently introduced into Sudan and was first reported in 2000/2001at Ed Debiba in Merowe governorate in an area of about 2 ha (Babiker *et al*., 2007). It was speculated that the parasite seeds were introduced, involuntarily, as contaminants of faba bean seeds from Egypt. Since

then the parasite has spreads rapidly. A survey undertaken at Ed Debiba area, one year after the parasite was reported, revealed that 94% of the area (158 ha) under faba bean was heavily infested. Subsequent, limited, annual surveys undertaken in 2003-2005 indicated spread of the parasite into new areas and the presence of incipient infestation foci all over the faba bean area extending from Mirnat, in the suburbs of Khartoum, to Wadi Halfa on the Egyptian borders (Babiker *et al*., 2007). A survey undertaken in 2004/2005 revealed that the area increased to over 2000 ha. Furthermore, the parasite had invaded large areas in both the Northern and River Nile States (Babiker *et al*., 2007). At present the parasite has become a national problem.

The present study comprising a survey and a series of laboratory and field experiments was designed to i) determine *O. crenata* distribution and infestation levels in Berber area in the River Nile State, ii) investigate under laboratory conditions, the effects of urea and imazethapyr on *O. crenata* germination and radicle extension and iii) study effects of the herbicide imazethapyr (Pursuit) alone and in combination with urea on *O. crenata* infestation in faba bean and faba bean growth and yield.

CHAPTER TWO

LITERATURE REVIEW

2.1. Parasitic plants

Plant parasitism is a phenomenon of extreme intimate plant-to-plant interactions. Parasitic plants are those which obtain some or all of their water and nutrients from autotrophic ones (hosts) (Nickrent and Musselman, 2004 Parasitic plants have a wide environmental tolerance. They occur in all climatic zones and on all continents except Antarctica. They have been able to adapt to all types of plant communities in all environments where flowering plants occur, except the aquatic environment. Parasitic plants are among the most problematic pests of agricultural crops worldwide, and cause serious problems for farmers in many parts of the world (Parker and Riches, 1993; Musselman *et al.,* 2001).

In vascular plants, parasitism is found only in the eudicotyledonous angiosperms. Parasitic plants have evolved independently about a dozen times (Heide-Jørgensen, 2013). Parasitic plants are distributed in about 12 orders and 28 dicotyledonous families and are represented by about 4500 species, in 270–275 genera. That is about 1 % of all known (260,000) seed plants (Runyon *et al.,* 2009).

Parasitic angiosperms may be classified into two broad categories, hemiparasites or holoparasites depending on the presence or absence of chlorophyll. Hemiparasites are chlorophyllous and photosynthetic (at least during some portion of the life cycle). Parasitic plants can be facultative and obligate, depending on their degree of dependence on the host. Facultative hemiparasites do not require a host to complete their life cycle. Obligate hemiparasites must attach to a host plant to complete their life cycle (Nickrent, 2002). The majority

of parasitic plants (90%) are hemiparasites. The remainder are Holoparasites which are totally achlorophyllous or nearly so. Parasitic angiosperms may be also classified depending on their site of attachment to the host as root or shoot parasites depending on the position of the haustorium (organs of attachment) either above or below ground (Parker and Riches, 1993; Sun, 2008). Over 60% of the total parasitic plants are root parasites. They can be photosynthetic or non- photosynthetic (Sun, 2008). Holoparasitism has evolved independently in at least seven lineages: Balanophoraceae, Cynomoriaceae, Hydnoraceae, Rafflesiales, Cuscutaceae, Lennoaceae and Orobanchaceae (Nickrent, 2002).

2.1.1. Orobanchaceae

Orobanchaceae is the largest family of parasitic plants after inclusion of the root hemiparasites, which were earlier placed in Scrophulariaceae (Heide-Jørgensen, 2013). The family Orobanchaceae includes the genera *Striga* (witchweeds), *Orobanche* and *Phelipanche* spp., which cause the most crop damage (Nickrent and Musselman, 2004). The main centers of distribution Orobanchaceae are the Mediterranean, Northern Africa, Sub.Saharan Africa and western North America (Musselman, 1980, 1986; Young *et al.,* 1999). Orobanchaceae is morphologically a diverse family of predominantly herbaceous, parasitic plants (Young *et al.,*1999). The majority of species are facultative or obligate root parasites, which may be hemiparasites or holoparasites. The family consists of 89 genera, containing 2061 species of which *Orobanche* is the largest (Nickrent, 2008).

2.1.2. *Orobanche* and *Phelipanche* (broomrapes)

Plants of the genera *Orobanche* and *Phelipanche*, Orobanchaceae, are root holoparasitic plants commonly known as broomrapes. They constrain production of many crops, mainly in the Mediterranean region (North Africa, the Middle

East and southern Europe) and Western Asia **(**Labrada, 2008; Runyon *et al.,* 2009). The genera *Orobanche* and *Phelipanche*, includes about 170 species, however only seven are considered economically as major biotic limiting factors to the production of major crops (Elzein and Kroschel, 2003; Gevezova *et al*., 2012).The species are *O. crenata*, *O. ramosa* L. (recently known as syn. *Phelipanche ramosa* L.), *O. aegyptiaca* Pers.(recently known as syn. *Phelipanche aegyptiaca* (Pers.) Pomel] (Fernandez-Aparicio *et al.,* 2007), *O. cernua* Loefl., *O. minor* Sm., *O. foetida* Poir. and *O. cumana* Wallr. (Parker and Riches, 1993; Joel, 2000; Nickrent and Musselman, 2004). Of these species *Orobanche crenata* (crenate broomrape) is the most serious biotic problem to grain and forage legumes production being widely distributed in the Mediterranean region including North Africa and into the Near East and Western Asia, with quite recent introductions into Sudan and Ethiopia (Rubiales *et al*., 2009a; Parker, 2013). *Orobanche crenata* attacks faba bean, chickpea, pea *(Pisum sativum L.*), lentil and carrot (*Daucus carota* L.) (Rubiales *et al.,* 2009b). *Orobanche* and *Phelipanche* (broomrapes), are among the most agronomically destructive parasitic plants globally (Parker, 2009; Westwood *et al.,* 2010). They cause great loss to crops worldwide and are consistently listed as the major constraint to legume food and vegetable crops production. In 1991*Orobanche* and *Phelipanche* species were considered to be a major threat to 16 million hectares in the Mediterranean and West Asia regions (Labrada, 2008; Parker, 2009).

The broomrapes belong to phloem-feeding parasites that abstract their nutrition predominantly from the phloem of their host plant (Gevezova *et al*., 2012) and consequently, lead to considerable yield reduction and in case of heavy infestations, complete crop failure (Jacobshon, 1986). Moreover, infestations by broomrape have been reported to decrease the area under cultivation. At present, over 73 million hectares of farmland under

cultivation in the Middle East, Southern and Eastern Europe, and regions of North Africa are infested with broomrapes. The yield losses range from 5-100% depending on host susceptibility, level of infestation and environmental conditions (Amsellem *et al*., 2001; Abang *et al*., 2007). The parasites affects the livelihoods of 100 million farmers and in monetary terms the annual losses in yield are estimated to be hundreds of millions of dollars (Amsellem *et al*., 2001; Abang *et al.,* 2007). In addition to yield losses and reduction in cultivated areas, broomrapes also reduce crop quality. The presence of broomrape plant materials in harvested crops reduces products values and often make them unmarketable (Nandulla, 1998). Furthermore, broomrapes reduce the number of crop alternatives available to farmers. The presence of broomrape in a field may force farmer to plant less economical, non-host crops or to leave the field fallow.

In Sudan three broomrape species, *P. ramosa*, *O. cernua* and *O. minor*, were reported as early as 1948. *O. crenata* was first reported in 2000/2001. Of the four broomrape species *P. ramosa* and *O. crenata* are naturalized and have become pests of economic importance (Babiker *et al.,* 2007). Tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.) are the main hosts of *P. ramosa*. However, the parasite has a wide host range among vegetables and weedy plants (Babiker *et al*., 1994, 2007). *P. ramosa* has spread from Rosarious on the Blue Nile and Kosti on the White Nile to Wadi Halfa on the southern borders of Egypt (Babiker *et al.,* 2007). Heavy tomato and potato infestations by *P. ramosa,* in Khartoum and Al Gezira States on the alluvial soils of the Nile soils, were reported to inflict over 30% in yield losses. Large areas were put out of tomato production (Babiker *et al.,* 1994).

Faba bean is the main host of *O. crenata* (Babiker *et al*., 2007). *O. crenata* was first reported in Merowe governorate in the Northern State in 2000/2001 and

subsequently in 2001/2002 in the River Nile State, which constitute the domains where the bulk of faba bean production is practiced. The parasite is spreading at an escalating rate. The number of infestation foci in the River Nile state increased from 1 in 2001/2002 to over 90 in 2005/2006. While in the Northern state, infestation spread within a 3 year period to cover over 28 sites constituting about 1% of the total area (21000 ha) under faba bean. The parasite was reported to inflict an average yield loss ranging from 6-90% (Babiker *et al*., 2007).

2.1.3. Distribution and host range

Broomrapes, comprises two genera of approximately 170 species that inhabit, mainly, the northern hemisphere (Gevezova *et al*., 2012). The species, except *O. crenata,* are distributed worldwide from temperate climate to the semi-arid tropics. *O. crenata* distribution is restricted to the Mediterranean regions, the Middle East and East Africa (Parker and Riches,1993). Of the species, only seven are considered economically significant. The species are *O. crenata*, *O. cernua*, *O. minor*, *O. foetida*, *O. cumana*, *P. ramosa*, and *P. aegyptiaca* (Parker and Riches, 1993). *O. crenata* (crenate broomrape) is widely distributed in the Mediterranean region including North Africa, the Near East and Western Asia, with quite recent introductions into Sudan and Ethiopia (Parker, 2013). It has a moderately wide host range including species, mainly in Fabaceae (faba bean, chickpea, pea and lentil) and Apiaceae including carrot and parsley (*Petroselinum crispum* Mill.). However, the parasite is of sporadic occurrence on crops in few other families such as, Asteraceae, Solanaceae, Cucurbitaceae, Lamiaceae and Ranunculaceae. The native distribution of *P. ramosa* and *P. egyptiaca* is Europe, Middle East, West Asia, North Africa, Ethiopia and Somalia. New infestations are being recorded, in Australia. They have the widest host ranges and, attacks a range of crops in the Solanaceae, especially tomato, eggplant (*Solanum melongena L.*) tobacco (*Nicotiana spp.*) pepper

(*Capsicum annuum* L.) and potato (Parker, 2013). *O. cumana* has a host range limited to Asteraceae, and it is an important pest of cultivated sunflowers (*Heliantus annuus* L.) (Parker and Riches, 1993; Press and Graves, 1995). *O. cernua* was reported on carrot, tomato and tobacco in the Mediterranean basin extending eastward into Europe and Asia. *O. minor* is widely distributed but it is only of economic importance on clover (*Trifolium* sp.) (Eizenberg *et al.,* 2005). *O. foetida*, in contrast, is of importance only on faba bean in Beja region of Tunisia (Kharrat *et al.,* 1992) although it has recently also been found in Morocco infecting common vetch (*Vicia sativa* L.) (Rubiales *et al.,* 2005).

2.1.4. Morphological characters and classification

The genus *Orobanche* was initially divided into four sections: Gymnocaulis, Myzorrhiza, Trionychon and Orobanche (Gevezova *et al*., 2012)). Sections of Orobanche and Trionychon contain the noxious weedy species. Trionychon includes economically important species such as *O. aegyptiaca*, *O. ramosa*, *O. nana*, and *O. mutelii* while Section Orobanche contains *O. crenata*, *O. cernua*, and *O. cumana*.

Broomrape species have reduced leaves, almost uniform inflorescences, microscopic and uniform seeds, and very little variations in corolla color and shape. Orobanche and Trionychon sections are mainly identified by the structure of the calyx and the presence or absence of bracteoles. Only Trionychon has bracteoles. Additional important distinguishing characteristics of the Section Trionychon are branched stems, an entire and campanulated (bell shaped) calyx, a blue or purple corolla, white anthers and usually white stigmas. The Section Orobanche has a single stem (unbranched stem); a calyx with two lateral segments; a white, yellow, brown, amethyst or red corolla; yellow, brown-grey or grey anthers; and yellow, orange, red, or purple stigmas (Pujadas-Salvà, 2002; Plaza *et al.,* 2004). The Section Orobanche includes *O. crenata*, *O. cernua* and

O. cumana. As expected, these species share many morphological characteristics such as the unbranched stem, absence of bracteoles, and calyx with two lateral segments. Among the three species *O. crenata* has a unique morphology and easily separated from the other two species. *O. crenata* has corolla lobes with erose or denticulate (irregularly notched) margins (Plate 4) in contrast to the entire margins in *O. cernua* and *O. cumana*. Also, *O. crenata* has dense inflorescence, fragrant flowers, and a long corolla (> 20 mm) which is usually white with purple veins (Plate 3). *O. cumana* is often confused with *O. cernua*. *O. cumana* is almost exclusively confined to cultivated sunflower, *O. cernua* is known from many hosts including wild plants. In the most recent taxonomic treatments, these sections were recognized as separate genera: *Aphyllon*, *Myzorrhiza*, *Phelipanche* and *Orobanche* (Gevezova *et al*., 2012).

2.1.5. Biology

O. crenata plant is pale, completely lacking chlorophyll, has fleshy, glandular, robust Stem, up to 12 mm in diameter and 1 m high with lanceolate, acute or acuminate scale leaves. The Inflorescence occupying 50–75 % of emerged stem, moderately dense with stem visible only in the lower half (Plate 3). Bracts, narrow lanceolate. Calyx variable, the lateral pairs of lobes narrow, almost subulate, deeply divided to more than half way or simple, undivided, but the lateral lobes separated by sinuses to the base dorsally and ventrally. Corolla, whitish with purple veins, glabrous to slightly glandular-hairy outside (Plate 4). Stamens, inserted 2–4 mm from the base of the corolla tube, glabrous or hairy towards the base. Anthers, glabrous, capsule, 10–12 mm long, splitting into two, releasing several hundred seeds about 0.3 mm long 'each', coarsely pitted. *O. crenata* normally out crossing but is facultatively autogamous (Parker, 2013).

Plate 3. *O. crenata* Inflorescence

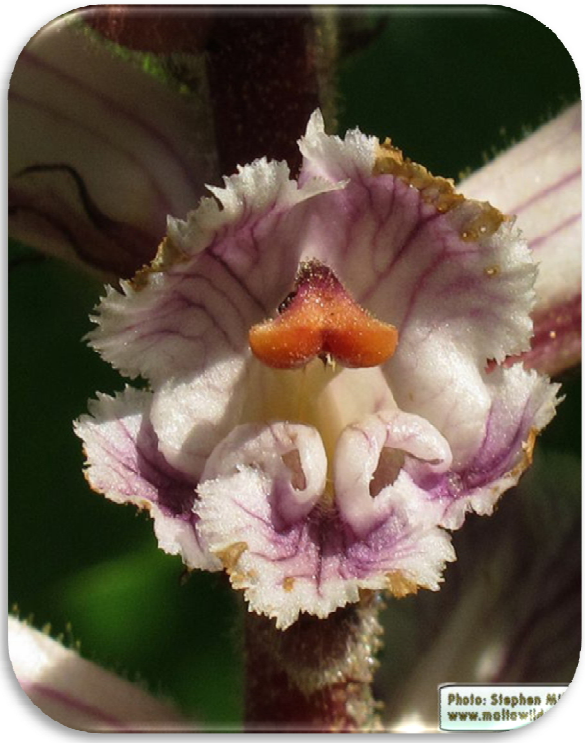


Plate 4. *O. crenata* corolla

2.1.5.1. The seed

Orobanche plants are annual parasitic plants which disperse and regenerate by seeds. The seeds are small and numerous with very little nutrient reserves. (Heide-Jørgensen, 2013). The seed of *O. crenata* extremely small, dust like, about 0.2–0.6 mm long, 0.1-0.5 mm wide (Plate 5), weighing about 3 to 7 µg (Linke and Vogt, 1987; Parker and Riches, 1993). The seed is oval, convex on one side and flat on the other with corresponding micropyle, with undifferentiated embryo made of a group of cells surrounded by others rich in reserve substances (endosperm). The seed is coarsely pitted. Normally outcrossing but is facultatively autogamous (Parker, 2013).The seeds are produced in capsules. The number of seeds per capsule is more than 4000 seeds. Since the average number of capsules is about 50, the total seeds per plant can be over 200,000 (Parker and Riches, 1993).

Plate 5. Seed of *O. crenata*

The seeds are generally spread by wind, water, man, contamined crop seeds, animals, vehicles and farm machinery (García-Torres, 1998; Joel *et al.*, 2007). The seeds remain viable in the soil for decades and are capable of exerting their damaging effects after 30 years or more (Joel *et al.*, 2007). Seeds in the laboratory have been reported to be viable after 8-10 years of storage (Parker and Riches, 1993). The whole life cycle from seed germination to seed production requires about 3-5months (Kroschel, 2001).

2.1.5.2. After-ripening

For parasitic weeds of the Orobanchaceae to emerge successfully above the soil surface, the seeds must first be after-ripened, conditioned, stimulated and germinate. The seedlings must then locate, attach to and penetrate a host root (Kebreab and Murdoch, 2001). *Orobanche* seeds after they have reached maturity, they are dormant and require a period of after-ripening or so called post harvest ripening period (Rubiales and Fernández-Aparicio, 2012). This period is defined as the time between shedding of the seeds and their preconditioning (Musselman, 1980). To release primary dormancy, generally a period of "dry" storage (dry after-ripening) is required. The mechanism of after-ripening in seeds probably relates to "degradation of mRNAs and proteins for positive regulation of dormancy and for negative regulation of germination. (Murdoch and Kebreab, 2013).The length of the after ripening period vary from, several days to months depending on species and environment (Nun and Mayer, 1993). With respect to environmental factors, seed moisture content is critical, but the rate of loss of primary dormancy during after-ripening varies with both temperature and seed moisture content. Moisture contents (fresh weight basis) of 5–18 % are required as the rate of dormancy alleviation during dry storage generally decreases with decrease in moisture content (Murdoch and Kebreab, 2013).

2.1.5.3. Conditioning

For germination to proceed, the ripened seeds require specific preconditioning period, which seems to be a prerequisite for germination. (Uematsu *et al.,* 2007). During germination of the parasite, several steps are mediated or regulated by signaling molecules that are exchanged between the parasite and its host. The chemical stimuli that initiate germination are exuded by host roots (Press and Graves, 1995; Bouwmeester *et al*., 2003). However, for the parasite seeds to respond to these stimuli and germinate, the ripened seeds require specific pre-treatment at a suitable temperature under moist conditions for some days, a treatment known as pre-treatment, conditioning or pre-conditioning (Joel *et al*., 1995; Matusova *et al*., 2004). However, some recent observations showed that non-conditioned seeds of both *O. cumana* and *P. aegyptiaca* were able to germinate in response to chemical stimulation by GR24 without prior conditioning (Gevesova *et al*., 2012). During conditioning, metabolic pathways in the seed are activated, for example, respiration and synthesis of DNA, protein and hormones (Joel *et al.,* 1995; Ejeta, 2005). Furthermore, seeds become increasingly sensitive to germination stimulants over periods of up to about 14 days. The period required for conditioning decreases with increase in temperature. The length of the conditioning period differs from species to another. *Orobanche* seeds need a conditioning period of 1-2 weeks under moist conditions. The length of the pre-conditioning period is affected by temperature that prevails during the pre-conditioning period (Joel *et al.,*1991; Matusova *et al*., 2004). Prolonging conditioning beyond the optimum leads to an induction of secondary dormancy (Murdoch and Kebreab, 2013). The optimum pre-conditioning temperature for *O. crenata* was found to be 15ºC - 20ºC (Kasasian, 1973a; van Hezewijk *et al*., 1993). Higher *O. crenata* seed germination occurred after 11days of conditioning at 18-20 ºC (García-Torres L. and Mesa-García,1991). Seed coat permeability may increase during

conditioning and /or changes in the levels or activities of endogenous germination promoters or inhibitors may occur (Song *et al*., 2005).

2.1.5.4. Germination and early development

For the seeds germination of *Orobanche*, a host-derived signal, germination stimulant, is needed. This is of great importance for the obligate parasites since they will not be able to survive for more than just few days after germination unless a contact with a host is established. Hence, seeds will only germinate within the host rhizosphere so that after germination they have a better chance to rapidly attach to the host roots (Yoneyama *et al*., 2013). Seeds germination occur only when conditioned *Orobanche* seed is exposed to sufficiently high concentrations of germination stimulants, hence assuring that seeds only germinate when they are in close vicinity (3-4 mm) of their host root (Sun, 2008). Since the seeds contain limited reserves, the germiling which grows by cell elongation to only a few millimeters (Joel and Bar, 2013), will die within a few days after germination unless a host root is invaded (Butler, 1995). The radicle grows towards the host root and the process is possibly directed by the concentration gradients of the germination stimulants (Plate 6. A) (Dube and Olivier, 2001., Joel and Bar, 2013). Once the radicle encounters a host root, apical cells extend outwards giving rise to the attachment organ and some of them become the intrusive cells, resulting in formation of a special attachment and penetrating organ, the haustorium (Plate 6. B) . The haustorium invades host tissues and serves as the structural and physiological bridge that allows the parasites to withdraw water and nutrients from the conductive systems of the host (Joel and Bar, 2013). The haustorium penetrates the host and connects the vascular system of the host with that of the parasite. However, the formation of this organ requires another host-derived chemical signals termed xenognosins or haustorium-inducing factors to initiate and guide this developmental transition

(Bandaranayake and Yoder, 2013). Following the establishment of connection with the host, the parasite develops a tubercle (Plate 6. B), that is a swelling in the young parasite seedling just above the surface of the host root. The tubercle accumulates starch which is utilized during later growth stages, mainly during flower and seed development (Joel, 2013). The tubercle forms crown roots (Plate 6. C). One to two weeks subsequent to tubercle formation, a bud encircled by scale leaves appears in the upper part (Plate 6. C). The bud gives rise to the flowering shoot. The carbohydrates reserves enable shoot elongation, emergence from the ground, production of shoots, flowering and seed setting within a very short period, after which the lifecycle starts again (Miller, 1994; Sun, 2008).

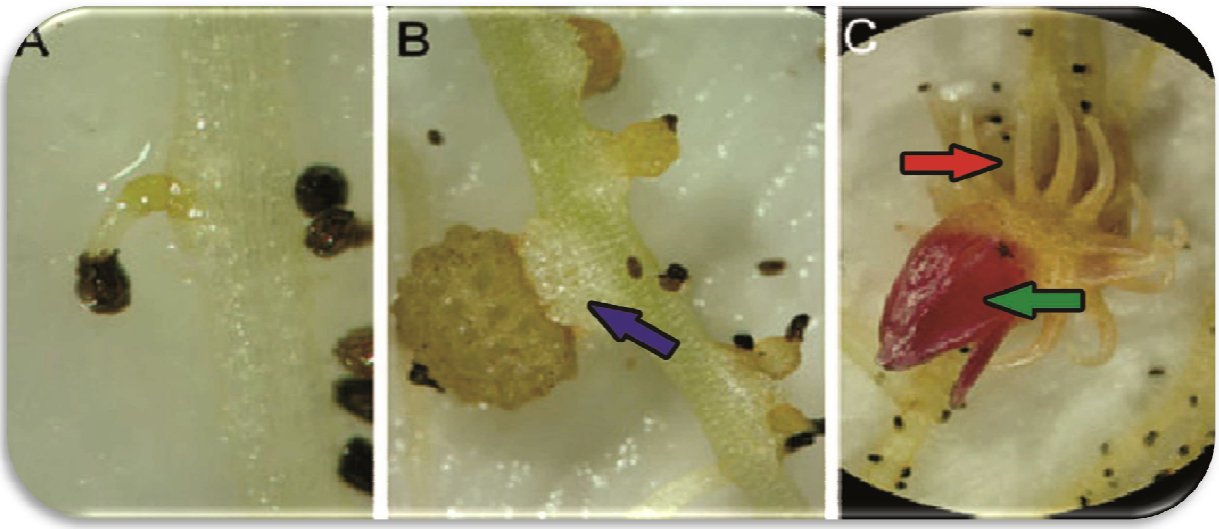


Plate 6. Infection process of *O. crenata*. A: *O. crenata* germiling contacting the root; B: Haustorium formation resulting in tubercles, blue arrow indicates the haustorium; C: Bud formation, red arrow indicates crown roots, green arrow indicates the bud

2.2. Means of Control

The immense number of seeds produced, minute seeds size, extreme seeds longevity, the ease of seeds dispersal, the intimate physiological interaction of the parasites with their host plants and the subterranean nature of early developmental stages are the main obstacles that limit development of successful

control measures that can be accepted and used by subsistence farmers (Goldwasser and Rodenburg, 2013). However, several control methods including "cultural and chemical, use of resistant varieties, and biological methods" have been tried for the control of the parasite (Elzein and Kroschel, 2003). So far most of the available means of control are either not satisfactory, have inconsistent performance, have no effects on current crop or expensive for subsistence farmers (Idris *et al.,* 2013). Today there is no single control method that can effectively solve the problem (Sun, 2008). Therefore the need for a management approach that provides a greater level of protection, does not involve a high level of skill and at the same time environmentally friendly, cost effective and sustainable is imperative (Idris *et al.,* 2013). Of the numerous interventions adopted to date, some are implemented before the infestation sets in (preventative), others afterwards (curative or therapeutic). Setting aside initiation, they are usually divided into agronomic, chemical, physical and biological (Restuccia *et al.,* 2009)

2.2.1. Preventative means

Containment of infested areas, prevention of seed distribution and curtailment of seed reserves replenishment should be the major objectives of parasitic weed management strategies, in addition to direct control interventions against the parasites (Goldwasser and Rodenburg, 2013). A small seed bank rapidly increases in subsequent seasons when suitable host plants (crops or weeds) grow in the field (Lopez-Granados and Garcia-Torres, 1993). The annual soil seed bank multiplies more than three fold in eight years due to high seed production from each successful *O. crenata* plant (Lopez-Granados and Garcia-Torres,1993). Only integrated measures are expected to lead to successful containment of the parasitic weeds problem (Rubiales and Fernandez-Aparicio, 2012). Such measures should be targeted through reduction of existing seed

banks, prevention of further seed production and avoiding seed dissemination. Reduction of the parasite reproduction and increase of seed bank demise while preventing seed dispersal to other places can all be achieved by cultural measures.

2.2.1.1. Phytosanitary

To prevent new infestations, movement of seeds out of an infested field must be avoided. Hence, vehicles, farm machinery and planting material must not carry infested soil when moving from one field to another. Farm animals should be prevented from becoming vectors for parasitic weeds by limiting their movements between fields, cleaning them to avoid transfer of infested soil or seeds on their body and avoiding use of fodder originating from infested fields. Parker and Riches (1993) reported that seeds of *O. aegyptiaca*, *O. cernua* and *O. crenata* could all survive passage through the digestive tracts of sheep. Furthermore , it is advisable that crop seeds come only from certified sources, irrigation or flooding should not come from contaminated water and erosion of infested soil, by wind or water, should be prevented (Goldwasser and Rodenburg, 2013). National and international trade of crop seeds contributes to the parasite seed dispersal over long distances. Contaminated crops seeds were reported to be the main vehicle for long distance transport of *Striga hermonthica* (Berner *et al*., 1995). Invoking strict quarantine measures, at various levels, national and international, helps in preventing the introduction of the parasite into parasite-free areas. (Abu Irmaileh, 2008).

2.2.2. Cultural Means

2.2.2.1. Land preparation

It has been realized that zero and minimum tillage increase broad bean infestation by *O. crenata* (Kukula and Masri, 1984). Deep ploughing of 40-50

cm deep reduced infestation by 80-90% (Christopher, 2011). Similar results were obtained with *O. crenata* where the surface seeds placement resulted in the lowest infestation (2 plants pot^{-1}). Seeds placed at 5 cm depth gave the highest infestation (11 plants pot^{-1}). When *Orobanche* seeds were placed at 10 cm soil depth or more, most of the *Orobanche* seedlings remained subterranean (Eltayeb, 2010).

2.2.2.2. Sowing date

Infestation of faba bean and other crops, such as lentil and chickpea, by *O. crenata* in the Mediterranean basin is extremely affected by date of planting (Parker and Riches, 1993**)**. Several researchers have studied, indirectly, the effect of temperature on broomrape parasitism by changing host sowing date. Early planted cool season legumes are more severely infected by the parasite. Delayed sowing is the best documented traditional method for *O. crenata* avoidance (Rubiales and Fernandez-Aparicio, 2012). Delayed sowing of broad bean and lentil, from autumn to winter, reduced the infection levels of *O. crenata* and *P. aegyptiaca,* probably because of unfavorable conditions for the parasite development under low winter temperatures (Joel *et al*., 2007). *Orobanche cumana*, *O. minor*, and *O. aegyptiaca* parasitism were positively correlated to temperature in the germination, attachment, and tubercle production stages (Eizenberg *et al*., 2003).

2.2.2.3. Cropping Systems

2.2.2.3.1. Trap crops

Trap crops 'sometimes known as false hosts' are plants that stimulate germination of the parasite seed, but do not become infected (they trick the parasite into suicidal germination). Crop rotation with trap and catch crops has long been practiced as control measure for parasitic weeds (Christopher, 2011).

Crop rotation, including trap crops, gradually depletes *Orobanche* seed bank. However, complete control will be achieved after a very long period (Abu-Irmaileh, 2008). The most promising trap crops reported for *Orobanche* and *Striga* species are those grown for fodder production e.g. Egyptian clover (*Trifolium alexandrinum* L.) as those crops are planted densely and extensively penetrate the soil with their roots (Kroschel and Klein, 2002). Integration of trap crops in a rotation was shown to reduce infestation of *Orobanche* considerably (Al-Menoufi, 1991; Schnell *et al*., 1994). Examples of trap crops for *O. crenata* include sorghum (*Sorghum biocolor* L.), barley (*Hordeum vulgare* L.) and smooth vetch (*Vicia dasycarpa* Ten.) (Parker and Riches, 1993). Exudates of flax (*Linum usitatissimum* L.) induced 75% germination of *O. crenata* and 16.6% germination of *P. ramosa* (Khalaf, 1992). Al-.Menoufi (1991) reported that Egyptian clover reduced broomrape emergence by more than 85% and 95% in faba bean fields when it was sown for three and four successive winter season, respectively.

2.2.2.3.2. Catch crops

Catch crops are true hosts plants, planted at high density but are removed from the field after the parasite seeds germinated and before the flowering and seed dispersal stages of the parasite (Christopher, 2011). By this method, the parasite seed bank is reduced in a manner similar to that of trap crops (Goldwasser and Rodenburg, 2013). Catch crops are rotational crops and usually have economic disadvantage, but have sometimes been technically more successful than trap crops (Parker and Riches, 1993).

2.2.2.3.3. Host Plant Resistance

Host plant resistance is most effective at protecting yield if it acts early to counteract the parasitic association and similarly able to reduce parasitic weed infestation rates because they act as trap crops (Pe´rez-Vich *et al*., 2013).

Resistance is a multicomponent event and a result of a series of escape factors or resistance mechanisms acting at different levels of the infection process. Host plants might escape infection by reduced root biomass and by root architecture that avoids the soil layer in which the seeds of the parasite are more common (Joel *et al.,* 2007). Low induction of germination has recently been found to play a role in resistance to *Orobanche* in a range of legumes, including vetches, peas, and chickpea and sunflower (Joel *et al.,* 2007). Necrosis and/or the development of protective layers that block the development or the intrusion of the haustorium inside host tissues have been reported in sunflower to *O. cumana* (Labrouse *et al.,* 2001), carrot to *P. ramosa* (Zehhar *et al.,* 2003), *Vicia atropurpurea Desf.* to *P. aegyptica* (Goldwasser *et al.,* 1997) and common vetch *Vicia sativa* L. (Pérez-de-Luque *et al.,* 2001), faba bean and chickpea to *O. crenata* (Rubiales *et al.,* 2003). This resistance has been correlated with an increase in the level of phenolics and peroxidase activity (Joel *et al.,* 2007) The accumulation of secretions at the infection site is another defense mechanism that may cause further necrosis of established tubercles (Pérez-de-Luque *et al.,* 2006). Resistance against most parasitic weeds is difficult to access, scarce, of complex nature, and of low heritability, making breeding for resistance a difficult task (Rubiales, 2003). However, significant success has been achieved in some crops. *Orobanche* resistance of simple inheritance, acting after parasite penetration, has been identified and widely exploited in breeding. This has been particularly important allowing a rapid progress in sunflower breeding against *O. cumana* (Joel *et al.,* 2007). In Russia, certain varieties of sunflower were found to be resistant to *O. cernua* in 1912, but the resistance broke down in the late 1920 due to existence of different races of *O. cernua* (Parker and Riches,1993; Joel *et al.,* 2007). Breeding of legume crop cultivars resistant to *O. crenata* was initiated with faba bean improvement in the early 1960s (Joel *et al.,* 2007). Only low levels of resistance to *O. crenata* was available in faba bean

until the appearance of the Egyptian line F402 (Nassib *et al.*, 1982). The first significant of resistance was the selection of the resistant line F402) that showed a high level of field resistance as well as agronomically favorable characteristics, and gave origin to 'Giza 402' that was widely used in Egypt (Nassib *et al.,* 1982). The line W1071 and various other cultivars were selected out of 'Giza 402', and are collectively identified as 'family 402'. A well adapted, high yielding faba bean cultivar Baraca has been developed in Spain under field conditions, with a high level of resistance to *O. crenata* (Cubero, 1994). Broomrape resistance in legumes is generally polygenic and non-race specific (Rubiales and Ferna´ndez-Aparicio, 2012). Several genetic studies on resistance to *O. crenata* in faba bean and common vetch (*Vicia sativa* L.) concluded that the genetic system controlling this trait is quantitative, with a very strong additive component (Pe´rez-Vich *et al.,* 2013). However, it has to be mentioned that development of resistant cultivars is an expensive option, and is useful only as part of an integrated management programme (Cooke, 2002).

2.2.2.3.4. Host Plant Tolerance

Tolerance involves the ability to endure inflicted damages caused by the parasite. Tolerant crop varieties are able to reduce the negative effects of parasitic weed infestation on crop yields, but do not prevent seed production by the parasite (Goldwasser and Jonne Rodenburg, 2013). The host may limit damage (tolerance) by factors that influence source-sink relationships, such as osmotic pressure (Joel *et al.,* 2007). Najeh, a small seeded faba bean variety (*Vicia faba* var. minor) was developed and released by the Field Crops Laboratory of the Institut National de la Recherche Agronomique de Tunisie (INRAT). Najeh was released on the basis of its high yield potential and its tolerance to broomrapes (*O. foetida* and *O. crenata*) (Kharrat *et al.,* 2010).

2.2.2.4. Nitrogen Fertilization

It is a general observation that root parasitic plants have adapted to prefer less fertile soils (Sauerborn, 1991; Abu-Irmaielh, 2008). Farmers, based on experience, used manure and compost to reduce broomrape growth (Nandula, 1998). Nitrogen compounds and manure fertilization has potential for control of broomrape species. Nitrogen in ammonium form affects negatively root parasitic weed germination (van Hezewijk and Verkleij, 1996) and/or elongation of the seedling radicle (Westwood and Foy, 1999). Fertilization of faba bean with a high rate of nitrogen (92.5 kg N/ha) reduced broomrape infestation by 13% and increased faba bean seed yield by11% (Zahran, 1973). On the other hand the use of nitrogenous fertilizers in faba bean at a lower rate of 47.6 kg /ha increased parasitism by enhancing development of broomrape by 25% and decreased bean yield by 15% in comparison to higher rates. Different nitrogen sources were tested, ammonium fertilizers were slightly more effective than urea, whereas the effect of nitrate is low. Nitrogen in form of ammonium sulphate (8 mM) reduced germination of seeds from 46 to 26% when applied during conditioning. A lower concentration of 4 mM ammonium sulphate did not inhibit germination. However, when applied during the germination phase, 4 mM ammonium sulphate strongly inhibited germination to less than 5%. Nitrogen as 8 mM urea or 16 mM nitrate did not inhibit germination during conditioning. However, when applied during the germination phase urea (8 mM) reduced germination to only a limited extent (from 58 to 40%) while nitrate had no effect (van-Hezewijk and Verkleij, 1996). Similar results were obtained with *P. ramosa* where the reduction in germination and radicle length were proportional to ammonium concentration (Abu-Irmaileh, 1994). The mechanism by which urea brought about reduction in *O. crenata* infestation is not yet ascertained (Linke, 1999; Abu-Irmaileh, 2008). The high cost of nitrogen fertilizers limited their practical use as a control measure for *O. crenata* (Cooke, 2002). However, the cost may

be offset by increased yield, and economic evaluation is imperative for decision making.

2.2.3. Physical Means

2.2.3.1. Solarization

Solarization is the heating of soil by sunlight trapped under a mulch of black, or more usually clear, polyethylene film (Habimana *et al.,* 2014). The temperatures of 48-57°C kill *Orobanche* seeds that are in the imbibed state; therefore soil must be wet at the time of treatment. Seeds of *P. ramosa* can survive 35 days at 50°C in dry air, but are quickly killed by temperatures of 40°C when wet (Habimana *et al.,* 2014). This technique has been used successfully on cropping land in many countries around the world with an endemic *Orobanche* problem, as a pre-planting treatment for tomato, carrot, faba beans and lentils. Soil solarization has proven to be the most effective methods in controlling broomrape in open crops fields (Habimana *et al.,* 2014). Reduction and devitalisation of broomrape seeds is achieved in the upper soil levels if the soil is covered for 4–8 weeks, allowing peak soil temperatures to reach 50°C or more during the day. The higher temperature leads to high mortality of *Orobanche* seeds (Joel *et al.,* 2007; Ashrafi *et al.,* 2009). Solarization achieved a total control of *O. crenata*, and improved faba bean growth and consequently grain yield in comparison with non-solarized soil (Mauromicale *et al.,* 2001). Solarization for 36 to 50 days gave 90-100% reduction in emergence of *P. ramosa* and *P. aegyptiaca* in crops planted immediately after treatment (Joel *et al.,* 2007; Ashrafi *et al.,* 2009). Furthermore, solarization has residual effects, which persist over several years (Abu-Irmaileh, 2008). However, the most important limitation to the solarization method is the high cost of polyethylene and the technique can only be used in the hot months of the year (Cooke, 2002).

2.2.3.2. Irrigation or Flooding

Flooding of broomrape-infested fields causes decay of parasitic weed seeds, leading to a decrease in infestation. Long-period flooding significantly reduced *O. crenata* infestation in subsequent host crops, but the effect of shorter flooding periods was not consistent (Parker and Riches 1993; Linke, 1999). In a recent laboratory study, *P. aegyptiaca* seeds placed in flooded soil containers completely lost their viability after 9 days of submergence (Goldwasser and Rodenburg, 2013). Flooding practice proved useful in some countries, where host crops are planted after rice. *Orobanche* infestation was drastically reduced in such a rotation (Abu-Irmaileh, 2008). Kebreab and Murdoch (1999) showed that seeds maintained at high moisture and high temperatures lose viability at a relatively rapid rate. van-Hezewijk *et al*., (1993) found that prolonged period of moistening induced secondary dormancy in *O. crenata* seeds.

2.2.4. Biological means

Biological control of weeds is the use of natural antagonists to exert pressure on the population of their host to reduce it to levels below economic importance. Bioagents include insects, fungi and bacteria. The fly *Phytomyza orobanchia* Kalt. is one of the few insects that are reported to be host-specific, only attacking *Orobanche* species (Joel *et al*., 2007). The insect larvae mine in stems and capsules of *P. ramosa* and other *Orobanche* spp., reducing seed production. Its short life cycle allows it to complete up to three generations in one season (Norambuena *et al.,* 2001). Seed production in *Orobanche* was found to be reduced significantly in many countries (Kroschel and Klein, 2002). However, several limitations restrict beneficial effects of *Phytomyza*. Tillage may bury broomrape stalks, containing *Phytomyza* pupa, deeper in soil, thus preventing emergence of the adults. Insecticides severely decrease the insect population.

Moreover, parasitoids reduce the fly population considerably. Crop rotation may also negatively impact survival of *Phytomyza* (Joel *et al*., 2007).

Several fungi have been reported to be virulent on *Orobanche*. The main advantage of fungal biocontrol agent is their high specificity. Approximately 30 fungal genera were reported to occur on *Orobanche* spp. (Joel *et al*., 2007). *Fusarium* species were the most promising candidates for the biocontrol in terms of their characteristic mycoherbicidal activities against different species of weeds specially the broomrapes (Gevezova *et al.,* 2012). Sauerborn *et al.,* (2007) confirmed that *Fusarium* species were dominant with diseased broomrape and witchweeds and that *Fusarium oxysporum* Schlecht. was the predominant one. They obtained an excellent control with *F. oxysporum* f. sp. orthoceras against *O. cumana* on sunflower under laboratory and greenhouse conditions. The total number of *O. cumana* was reduced to 80 % when soil was treated with a simple granular formulation of the fungus (Abouzeid, 2009). *Fusarium oxysporum* Schlecht. *var. orthoceras* gave some control of *O. cernua* and *O. cumana* on sunflower (Müller-Stöver *et al*., 2004). Data on the efficacy of *Fusarium* spp. on *Orobanche* in the field are rare . However, the results already indicated that *Fusarium* spp. in most cases do not provide the level of control desired by farmers (Joel *et al*., 2007). The fungus *Ulocladium atrum* has been used experimentally to control *O. crenata* on faba beans in Syria (Linke, 1992). Rhizobacteria from faba bean and *Orobanche* spp. were evaluated for their potentials as biocontrol agents for parasitic weeds. Among five bacterial isolates selected for pot trials, strain Bf 7- 9 of *Pseudomonas fluorescens* showed high biocontrol activity against *O. foetida* and *O. crenata* and positively influenced faba bean growth (Zermane *et al*., 2007). Faba bean inoculated with Rhizobial bacterial strain TAL 1399 alone or in combinations with *Bacillus megatherium* var phosphaticum (BMP) or *Azospirillum braziliense* (Ab) plus chicken manure at 35 g/pot completely inhibited *Orobanche crenata* emergence (Hassan *et al*.,

2012). Similar result were obtained with Faba bean inoculated with bacterial strains TAL 1399 plus *A. brasilense* and TAL 1399 plus BMP *B. megathirium* var phosphaticum in combination with arbuscular mycorrhiza fungi (AMF) (Hassan and Abakeer, 2013).

Insects or pathogens, each alone, may not provide adequate control of *Orobanche* spp. Therefore, insects and pathogens such as fungi may be deployed as part of an integrated management for *Orobanche* spp.

2.2.5. Chemical means

Measures such as soil fumigation, germination stimulants, and certain pre-plant or pre-emergence herbicides act on early stages of development . However, some other herbicides viz glyphosate suppresses the parasite after attachment and penetration of the host roots (Nandulla, 1998).

2.2.5.1. Soil Fumigation

Soil disinfection by fumigation is used for reducing the parasite seed banks. Fumigation aims at eliminating the seed bank in 1-2 years. It is assisted by burning straw in the first year only to destroy seeds at the soil surface. Several chemicals are used to destroy buried seeds. Methyl bromide has high effectiveness when correctly applied. This fumigant was widely used for broomrape control mainly in cash crops and in eradication programmes such as the US national eradication project of *P. ramosa* in California (Wilhelm *et al*., 1959; Goldwasser and Kleifeld, 2004). A rate of 350 kg/ha is generally adequate to control *Orobanche* (Foy *et al.,* 1989). One application kills virtually 100% of the seed bank, as demonstrated by experience (Parker & Riches, 1993). Metham sodium (sodium methyl dithiocarbamate) releasing the active ingredient methyl isothiocyanate, applied as a liquid product such as Vapam® is the preferred soil fumigation method in the USA. It gives 50% kill of *Orobanche* seed, but is

rapidly lost from soil by volatilization (Cooke, 2002). Other fumigants such as ethylene dibromide (EDB), methyl iodide, and telone (1,3-dichloropropene) were tested as possible substitutes for methyl bromide on an experimental or limited commercial scale (Joel *et al*., 2007). Soil fumigation with methyl bromide has so far been the most effective fumigant, being highly successful in killing broomrape seeds in soil. Nevertheless, it may no longer be used due to its harmful environmental effects. Other fumigants are rarely used because of their high cost, complicated application procedures, and limited efficacy (Eizenberg *et al*., 2013).

2.2.5.2. Germination stimulants

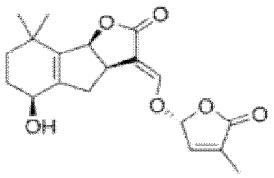
Parasitic plants exert much of their damage to host crops during the early phases of attachment, thus control approaches should target the initial steps in the host-parasite interaction, of which germination is the first (López-Ráez *et al.,* 2009). As germination stimulants play an essential role in the life cycle of parasitic plants this might be an important target for the development of new control strategies for parasitic weeds. Seeds of parasitic weeds such as *Striga* and *Orobanche* spp. remain dormant in the soil until exudates (germination stimulants) from host plants induce germination. Seed bank depletion and death of weed germlings could be achieved by suicidal germination which is regarded as the induction of germination in the absence of or away from hosts roots. Suicidal germination could be achieved by introducing either natural or synthetic germination stimulants into the soil in the absence of a suitable host leading to both seed bank depletion and death of weed germlings because of complete dependence on the host for their sustenance (Parker and Riches, 1993). Germination stimulants, both natural and synthetic, have good potential as effective tools of management of broomrape. Brown *et al.* (1951,1952) demonstrated that various plant species produce germination

stimulants for seeds of *O. minor* and *Striga hermonthica* (Del.) Benth. They also predicted, that these germination stimulants contain a lactone group. In 1966, this was proven to be correct as the structure of the first *Striga* germination stimulant (strigol) is a bislactone (Fig. 2.1) (Cook *et al.,* 1966). These compounds are now collectively called strigolactones (SLs) (Butler, 1995). Strigolactones (SLs) are the major germination stimulants for root parasitic plants in the rhizosphere of host plants. However, natural germination stimulants other than the SLs have also been identified (Yoneyama *et al.,* 2013). Strigol and strigyl acetate, the first reported SLs, were isolated as *Striga asiatica* (syn. *S. lutea*) germination stimulants from root exudates of cotton (*Gossypium hirsutum* L.) (Yoneyama *et al.,* 2013). Orobanchol (Fig. 2.1) was isolated from root exudates of red clover, a host of *O. minor*, as the first *Orobanche* germination stimulant (Yokota *et al*., 1998).

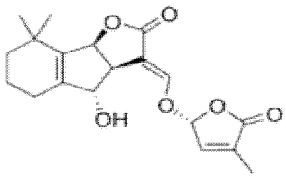
A number of different classes of secondary metabolites, including dihydrosorgoleone, strigolactones and sesquiterpene lactones) have been described to have germination stimulant activity (Sun, 2008). Galindo *et al.,* 2002) demonstrated that several sesquiterpene lactones induced germination of *O. cumana*, but not of *O. crenata* and *P. ramosa*. *O. cumana* is a specific parasite of sunflower, and sunflower is known to contain large amounts of sesquiterpene lactones (Bouwmeester *et al.,* 2003).

Several synthetic stimulants including strigol analogues and ethylene have been tested for their stimulatory effects on in vitro seed germination. Synthetic analogous of strigol including GR7 and GR24 (Fig. 2.1). have been used worldwide in parasitic weed research to stimulate germination (Wigchert *et al.,* 1999). However, strigol or its synthetic analogous did not provide practical control of broomrape due to their short stability in soils (Babiker and Hamdoun,1983; Nandula, 1998). Ethylene stimulates germination of many

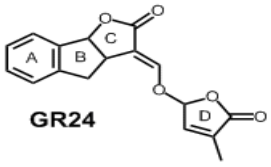
weed seeds and was found very effective in stimulating germination of witchweed (*Striga* spp.) seeds, but there was limited success when ethylene was used to stimulate broomrape seeds germination (Parker and Riches, 1993; Galindo *et al.,* 2004). Edwards *et al.,* (1976) reported that ethylene had no effect on germination of *O. crenata* seeds, but gibberellic acid (GA3) increased response to germination stimulant (Yoneyama *et al.,* 2013). *Striga* and *Orobanche* germination stimulants, strigol and orobancol isolated from both hosts and non-host species, showed striking structural similarities (Matusova *et al.,* 2005).



A (+)-Strigol



B (+)-Orobanchol



C GR24

Fig. 2.1. Chemical structures of germination stimulants A) strigol; B) Orobanchol and C) GR24

2.2.5.3. Herbicides

Herbicides may be applied before weed emergence (PRE) or following weed emergence (POST). Weedy holoparasites, such as the broomrapes *Orobanche* and *Phelipanche* must be treated during their underground stages of development because they emerge above the ground only during flowering when most of the damage to the host has already been done (Eizenberg *et al.,* ٢٠06). Treatment

after emergence is usually too late to prevent yield losses, and herbicides applied on emerged parasites mainly help in limiting parasite seeds production and dispersal. Several systemic herbicides have so far been proposed for broomrape control in vegetables and field crops. These herbicides include the aromatic amino acid synthesis inhibitor glyphosate, which targets the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), and the branched-chain amino acid synthesis inhibitors imidazolinones, sulfonylureas, and pyrithiobacsodium, which target the enzyme acetolactate synthase (ALS), also known as acetohydroxy acid synthasase (AHAS) (Schloss, 1990). Glyphosate is commonly absorbed by foliage, while some ALS-inhibiting herbicides can be absorbed by the roots, others by the shoots, and in this latter case they are rapidly transferred to the roots or to meristematic tissues that act as sinks. Shoot-applied imidazolinones may be exuded from the plant roots (Kanampiu *et al*., 2001) which is relevant to the development of control strategies for ALS-sensitive root parasitic weeds. The leaching potential of any given ALS inhibitor, as well as the rates of its degradation in the soil and the crop, may influence the level of control and the period of control activity. Glyphosate was the first promising herbicide developed for *O. crenata* control on faba bean (Schmitt *et al.,* 1979). Glyphosate is commonly absorbed by foliage. Low rates of the systemic herbicide glyphosate, applied up to three times, were effective for broomrape control only on a few hosts that are less susceptible to the herbicide, i.e., members of the Apiaceae (carrot, celery, and parsley), Fabaceae (faba bean, vetch, pea, *Vicia narbonensis*, *V. sativa*), and various Brassicaceae (Eizenberg *et al.,* 2013). Two application of 80 g /ha glyphosate, gave almost complete control of broomrapes in faba bean, but with no yield increase (Sauerborn *et al.,* 1989). The exact dosage of glyphosate is important for better efficiency, and for reducing phytotoxic symptoms on the crop. The safety margin in non-herbicide resistant crops is low and increasing the glyphosate rate has a heavy penalty as it

reduces yields. In addition to glyphosate, sulfonylurea and imidazolinone herbicides proved to be effective on many host crops (Abu-Irmaileh, 2008). Chlorsulfuron gave better control both in faba bean and sunflower when applied to the soil surface than when incorporated (KotoulaSyka and Eleftheroninos, 1991). Triasulfuron at 7.5 g ha^{-1} applied as a single foliar spray caused unacceptable damage to potatoes (Goldwasser *et al.*, 2001). However, 3 applications of 12.5 or 25 g/ha triasulfuron (registered in Australia as Titus® for use on tomatoes) selectively controlled *P. ramosa* with no crop damage. Saber *et al.,* (1994) found that two applications of 20 g/ha imazaquin gave better control than 23 g/ha glyphosate with no crop damage. Imazethapyr was used selectivity in faba bean (Garcia-Torres and Lopez Granados, 1991). Imazethapyr and imazapyr resulted in 60-80% broomrape control when faba bean, are soaked for 5 min in 0.01-0.1% herbicide solutions or coating at 20-40 g ha^{-1} and did not affect seed germination and crop growth (Jurado-Expósito *et al.*, 1997). Good control of broomrape was obtained by coating or soaking the seeds of faba bean, lentil or pea in low concentration of imazethapyr, or imazapyr, respectively (Garcia-Torres *et al.,* 1999). Seed germination and crop growth were not affected by phytotoxicity of both herbicides. However, herbicides application is too technical and expensive in subsistent farming and intensive farmer training and effective extension services are required (Abu-Irmaileh, 2008).

2.2.5.3.1. Imazethapyr

Imazethapyr (RS)-5-ethyl-2- (4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid (Fig 2.2), an imidazolinone, is a selective systemic herbicide. It acts by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS) (Schloss, 1990), which is a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, lecucine, and valine

in plants. It controls a wide spectrum of grass and broadleaved weeds, effective at low application rates, and has low mammalian toxicity (Tan *et al.,* 2005). The herbicide is readily absorbed by the roots and leaves, with translocation in the xylem and phloem, and accumulation in the merristamatic region (Zabalza, *et al*., 2002).

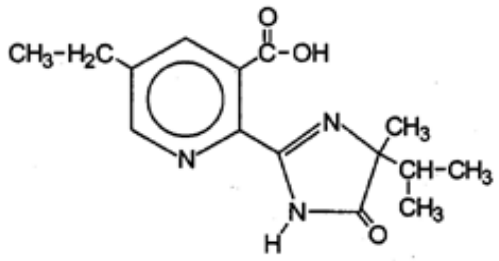


Fig. 2.2. Chemical structure of imazethapyr

2.2.6. Integrated Management

No single measure is sufficient to control parasitic weeds effectively (Joel *et al.,* 2007; Babiker *et al*., 2007; Parker, 2009; Rubiales and Fernández-Aparicio, 2012). The various means for management reviewed above, offer varying levels of *Orobanche* control. However, none of them proved to be as effective, economical and applicable as desired (Joel, 2000; Goldwasser and Kleifeld, 2004). Large areas of new territory are at risk of invasion if care is not taken to limit the introduction of the parasite seeds and to educate farmers and others to be aware for new infestations. The only way to cope with weedy root parasites is through an integrated approach (Rubiales and Fernández-Aparicio, 2012). The effective integrated approach combines tactics that protect or enhance crop yield, with those that reduce parasite seed production and hence reduce size of seed banks (Ransom *et al.,* 2007). Integrated management strategies need to combine variety of measures in a concerted manner, starting with containment and

sanitation, direct and indirect measures to prevent the damage caused by the parasites, and finally depleting the parasite seed bank in soil (Rubiales and Fernández-Aparicio, 2012). The most practical and feasible combination is the use of resistant cultivars together with chemical control or hand weeding. A similar approach should also be adopted in the absence of resistant crops, when chemical control seems to be successful for a particular parasite. In this case, hand weeding would help in eliminating the rare (but potentially existing) parasite individuals that survive following the chemical treatment and that would otherwise propagate and endanger the efficacy of the herbicide. Wisely combining these three control methods would significantly reduce or even completely prevent break down of resistance (Joel *et al.,* 2007).

Combining low cost control methods that enhance crop tolerance to the parasite through improvement of soil fertility, utilizing the most tolerant cultivars that are available, in addition to potential preventive measures and cultural methods such as manipulating seeding rate and planting date may help in reducing infestations. The aim of integrated management is to constantly reduce the parasite population leading to eventual reduction in the soil seed bank (Abu-Irmaileh, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1.General

Afield survey and a series of laboratory and field experiments were undertaken during this study. The field survey was undertaken to determine the spread of the parasite in Berber locality in the River Nile State (Fig. 3.1). The laboratory and field experiments were undertaken at the College of Agricultural Studies (CAS), Sudan University of Science and Technology (SUST) at Shambat (latitude 15° 35′ N and longitude 32° 30′ E). The laboratory experiments were conducted at the *Striga* research laboratory to investigate the effects of urea and imazethapyr on *O. crenata* germination and radicle extension. The field experiments were conducted in two consecutive seasons (2009/10 and 2010/11) to study the effects of the herbicide imazethapyr (Pursuit), alone and in combination with a nitrogen fertilizer (urea) on *O. crenata* control and faba bean growth and yield.

3.2. Materials

3.2.1. Plant material

The faba bean cultivar Hudieba 93 was obtained from the Agricultural Research Corporation (ARC), Hudieba Research Station. Seeds of *O. crenata*, collected in 2008/2009 from under faba bean at Koboshia in the River Nile State, were supplied by Dr Naser Eldeen Abdalla Khiry ARC Shendi Research Station.

3.2.2. Inoculum

Agadeen, a suspension of *Rhizobium leguminsorum* Frank. strain TAL1399 on charcoal, was obtained from the Biofertilization Department, Environment and

Natural Resources Research Institute (ENRRI), the National Centre for Research, Khartoum, Sudan.

3.2.3. Herbicide imazethapyr (Pursuit)

Imazethapyr (RS)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid as Pursuit(10% EC) was obtained from the local market.

3.2.4. Imazethapyr stock solution

A stock solution of imazethapyr (100µM) was prepared by mixing 29 µl pursuit in 10 ml of sterilized distilled water. The desired concentrations (10-100 µM) were obtained by serial dilutions (Appendix IV).

3.2.5. Urea stock solution

A stock solution of urea (400 mM) equivalent to 2N per feddan calculated based on filter paper size (9 cm) was prepared by dissolving 2.4 g of urea in 10 ml water and completing to volume (100 ml) with sterilezed distilled water to obtain the desired concentrations (20-200 mM) (Appendix V).

3.2.6. Strigol analogue (GR24) stock solution

GR24 was provided by Professor B. Zwanenberg, the University of Nimijhen, the Netherland. A Stock solution of the stimulant was prepared by dissolving 1mg in 1ml of acetone and completion to volume (100 ml) with sterilized distilled water to obtain the desired concentrations (0.1, 1 and10 ppm) (Appendix III).

3.3. Methods

3.3.1. Field survey

A field survey was conducted, in season 2009/2010, at Berber locality, western bank of the River Nile (west Berber area) in the River Nile State, 311 kilometers

north of Khartoum. The survey was undertaken just prior to harvest on the sixteenth of February 2009. The objectives of the survey were to i) outline founder and incipient populations of the parasite, ii) identify infested areas and distribution of the parasite, iii) assess farmer's awareness of the problem. The field survey was conducted through a questionnaire (Appendix I) distributed to farmers in the surveyed sites, Fotoar, Eljoal, Hilat Uonis, Elmaseed and Kadabas (Fig. 3.1). Fifty farmers were selected randomly. The farms were visually surveyed for, i) presence or absence of the parasite, ii) intensity of infestation classified as, nil, low, medium, or heavy. Farmers were asked about, i) the first time they saw *O. crenata* in their fields, ii) the crop on which the parasite was first seen, iii) source of the crop seeds on which the parasite was first seen, iv) *Orobanche* management practices.

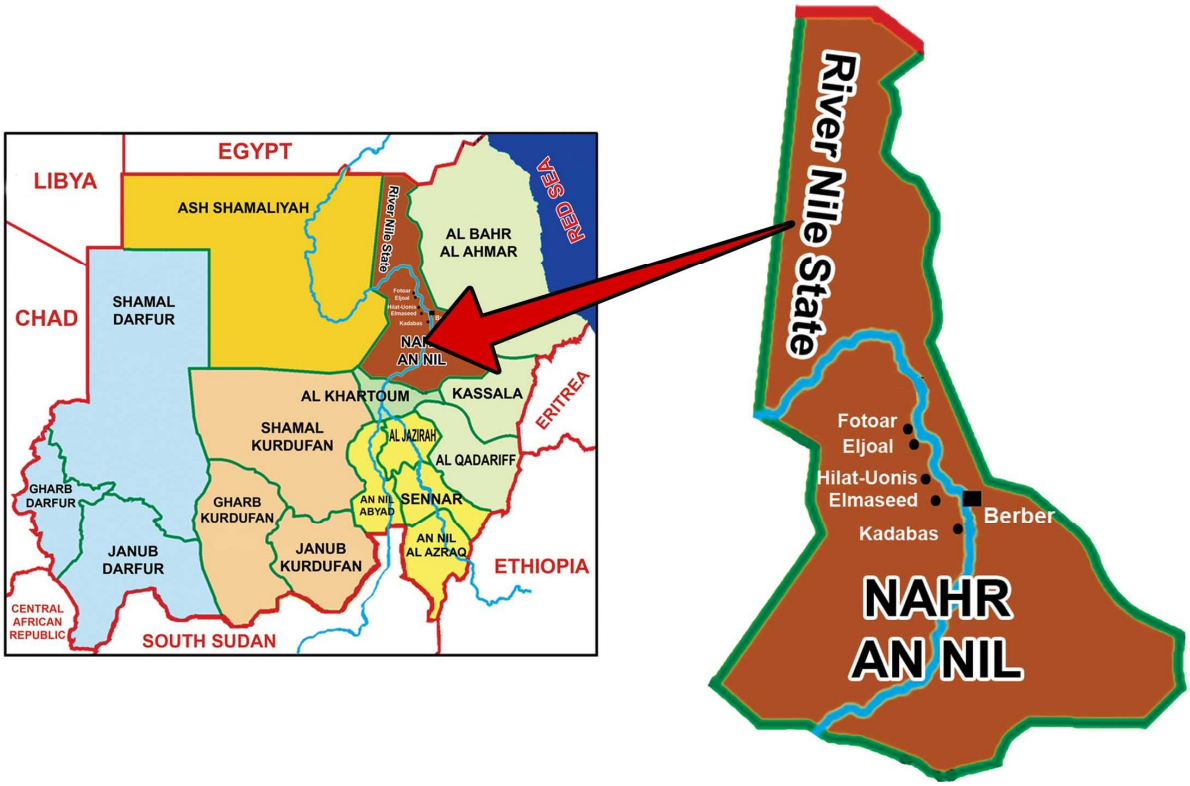


Fig. 3.1. Map of the Sudan States

3.3.2. Laboratory experiments

3.3.2.1. General

O. crenata seeds were surface sterilized by immersion, for 3 min, in sodium hypochlorite (NaOCI) solution (1%), obtained by appropriate dilution of commercial Bleach. The sodium hypochlorite was drained off. The seeds, thoroughly washed, under suction with sterilized distilled water, were plotted dry on Whatman No. 1 filter papers, air-dried in a laminar flow and stored at ambient temperature till used.

Glass fiber filter papers (GFFP) discs (8mm diameter) were cut, wetted thoroughly with water and placed in an oven set at 100 ºC for one hour for sterilization just before use. For pre-conditioning the sterilized discs, placed in 9 cm Petri dishes lined with a single sheet of glass fiber filter papers, were moistened with 5ml of distilled water or the respective test solution. Surface sterilized, *O. crenata* seeds (*Ca*. 25-50) were sprinkled on each of the glass fiber discs. The Petri dishes, sealed with Parafilm to avoid moisture loss, were wrapped with aluminum foil and incubated in the dark at 20 ºC, for 14 days.

For germination glass fiber filter paper discs containing *O. crenata* seeds, were, dapped on a filter paper to remove excess water and transferred to sterilized Petri dishes. Each disc was treated with a 20µl aliquot, of the respective test solution. Apiece of filter paper moistened with sterilized distilled water was placed in the centre of each Petri dish to maintain moist conditions during the test period. The seeds, reincubated in the dark at 20ºC, were examined for germination and radicle extension 7 days later using a stereomicroscope equipped with an ocular micromete (ocular lens containing a micrometer disc) for radicle measurement. Treatments were arranged in a complete randomized design (CRD) with 5 replicates. All experiments were repeated three times and the data presented was from one typical experiment.

3.3.2.2. Effects of urea, applied during conditioning, on *Orobanche* seeds germination and radicle extension

O. crenata seeds, placed on glass fiber filter paper discs in Petri dishes, were moistened with 5 ml of DW or urea solution at the respective dilutions (20, 30, 40, 50, 60, 70, 80, 90, 100, 200 and 400 mM). The Petri dishes were sealed with parafilm, wrapped in aluminum foil and incubated at 20 ºC in the dark for 14 days as described in 3.3.2.1. The seeds were subsequently retrieved, dapped on a filter paper as in 3.3.2.1 and treated with GR24 at 10 ppm. The Petri dishies sealed with parafilm, wrapped in aluminum foil were incubated as in 3.3.2.1. The seeds were subsequently examined for germination and radicle extension as previously described in 3.3.2.1.

3.3.2.3. Effects of urea applied subsequent to GR24, on *Orobanche* seeds germination and radicle extension

O. crenata seeds were conditioned in sterilized distilled water as described in 3.3.2.1. Subsequent to conditioning the seeds were treated with GR24 at 10 ppm and reincubated, for 1, 4, 8 and 24 hours in dark at 20 C. Subsequent to incubation with GR24 for the prescribed period the discs, containing *O. crenata* seeds, were dapped on a filter paper to remove excess water and treated with urea at the respective dilution (0, 200 and 400 mM). The Petri dishes sealed with parafilm were reincubated as previously described in 3.3.2.1 and the seeds were subsequently examined for germination and radicle extension as described in 3.3.2.1.

3.3.2.4. Effects of urea, applied prior to GR24, on *Orobanche* seeds germination and radicle extension

O. crenata seeds, conditioned in sterilized distilled water, as described in 3.3.2.1, were subsequently treated with urea solutions (0, 200 and 400 mM) and reincubated for 1, 4, 8 and 24 hours in the dark at 20 ºC. Following the

prescribed time of urea treatment the seeds were retreived, dapped on a filter paper to remove excess water, treated with GR24 at 10 ppm and reincubated at 20 ºC in the dark for 7 days prior to examination for germination and radicle extension as previously described in 3.3.2.1.

3.3.2.5. Effects of urea and GR24, applied simultaneously on *Orobanche* seeds germination and radicle extension

O. crenata seeds conditioned in distilled water as in 3.3.2.1 were treated with 20 µl aliquots of urea at 0, 200 and 400 mM each mixed with GR24 at 0.1, 1 and 10 ppm. The petri dishes containing the seeds were incubated as in 3.3.2.1. Seeds conditioned in distilled sterilized water and similarly treated with GR24 were included as controls for comparison. The seeds were examined for germination and radicle extension as described in 3.3.2.1.

3.3.2.6. Effects of imazethapyr on *O. crenata* seeds germination and radicle extension

O. crenata seeds, on glass fiber filter paper discs in petri dishes, were moistened with 5ml of water or imazethapyr solution at the respective dilution (0, 10, 20, 40, 60, 80, and 100µM). The Petri dishes were sealed with parafilm, wrapped in aluminum foil and incubated at 20 ºC in the dark for 14 days as described in 3.3.2.1. The discs containing *O. crenata* seeds were treated with 20µl of the respective GR24 at 0.1, 1 and 10 ppm. A control treated with distilled water was included for comparison. Petri dishes were reincubated at 20 ºC in the dark for 7 days, as described in 3.3.2.1, prior to examination for germination and radicle extension.

3.3.3. Field experiments

3.3.3.1. General

The field experiments were conducted for two consecutive seasons (2009/10, and 2010/11) to study the effects of imazethapyr, nitrogen fertilization and their combinations on *O. crenata* incidence and faba bean growth and yield. The experimental area was disc ploughed, harrowed, leveled, ridged and divided into sub-plots (3 x 5 m each). Number of rows per sub-plot was 4 and the spacing between rows was 70 cm. Faba bean (cv: Hudieba 93), was used in the two experiments. The crop seeds were sown in holes, (2 seeds per hole). The holes, 4 cm deep each, made on both sides of each ridge, were spaced at an intra-row spacing of 20 cm. In the experiment involving fertilization, urea at 1N (40 kg fed^{-1}) was applied by broadcasting. Two controls were included, uninfested (*Orobanche* free control) and *Orobanche* infested control. All sub-plots, were artificially inoculated with *Orobanche* seeds excluding those used for the uninfested control each season. The inoculums were prepared by adding 1g of clean *Orobanche* seeds to1kg of Shambat soil, previously sieved through a 2 mm metal screen, followed by thorough mixing. The inoculums were applied to the soil before faba bean sowing (5 mg *Orobanche* seeds $hole^{-1}$). One mg of broomrape seeds contains about 200 seeds (Mesa-Garcia and Garcia Torres, 1986). Just before sowing faba bean seeds were moistened with an aqueous suspension of Arabic gum (400 g/L) and Agadeen (500 g/50kg faba bean seeds) was added and thoroughly mixed by hand. The treated seeds were sown early December. Subsequent irrigations were made every week. Two hand-weeding were made in each season. Treatments were laid out in a Randomized Complete Block Design (RCBD) with four replicates.

Data collected on faba bean growth attributes, included, i) number of leaves, ii) plant height (cm), iii) number of flowers, iv) number of pods, v) grain yield (kg

fed^{-1}) and weight of 100 seeds (g). Data on *O. crenata* included, i) number of *Orobanche* shoots and ii) number of capsules per plant. The later was counted in season 2010/2011 only.

3.3.3.2. Effects of imazethapyr, rate and application time, on *Orobanche* incidence and faba bean growth and yield

Faba bean was sown as described in 3.3.3.1. Imazethapyr at 20 and 30 g a.i.fed^{-1}, was applied 15, 30 and 45 days prior to and at sowing (AS) as aqueous spray at a volume rate 100L fed^{-1} , using a knapsack sprayer. An uninfested (*Orobanche* free) and *Orobanche* infested controls were included for comparison.

Treatment effects were assessed by, selecting 10 faba bean plants from the two central ridges at random, and i) counting number of leaves at 30 and 60 DAS, ii) measuring plant height 30 and 60 DAS, iii) counting number of pods 60 and 75 DAS, iv) counting *Orobanche* plants in the two central ridges in each plot 67 and 82 DAS and v) determining grain yield and 100 seeds weight at harvest.

3.3.3.3. Effects of imazethapyr, nitrogen, and their combinations on *Orobanche* incidence and faba bean growth and yield

Experimental design and crop planting were as in 3.3.3.1. Imazethapyr at 20 and 30 g a.i. fed^{-1}, was applied at sowing (AS). Urea at 40 kg fed^{-1}, (1N) was applied at sowing (AS) or 15 and 30 days later. An uninfested and *Orobanche* infested controls were included for comparison.

Treatment effects on broomrape and effects on faba bean growth and yield were assessed as in 3.3.3.2. However, in this season number of flowers on faba bean and number of capsules on *O. crenata* were also counted.

3.4. Statistical analysis

Data collected from laboratory and field experiments were subjected to statistical analysis {Analysis of Variance (ANOVA)}, using GenStat package release 10.3 DE and SAS 9.1 statistical package. Means were separated for significance using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1. Field Survey

The field survey undertaken as described in 3.3.1 revealed substantial differences in distribution of farmers according to age, education and land ownership as well as in *Orobanche* infestation.

4.1.1. Distribution of farmers by age

The frequency of distribution of farmers according to age showed that farmers' age ranged between 20 and 69 years. Most of the farmers (64%) were of age 30-49 (Table 4.1)

Table 4.1. Distribution of farmers by age group

Age group	Frequency	Distribution %
20 – 29	6	12
30 – 39	16	32
40 – 49	16	32
50 – 59	4	8
60 – 69	8	16
Total	50	100

4.1.2. Farmers' educational levels

The survey showed that small proportion (4%) of the farmers were illiterate, a considerable proportion (54%) received primary education (Fig. 4.1) and only 36% and 6% received secondary and university education, respectively.

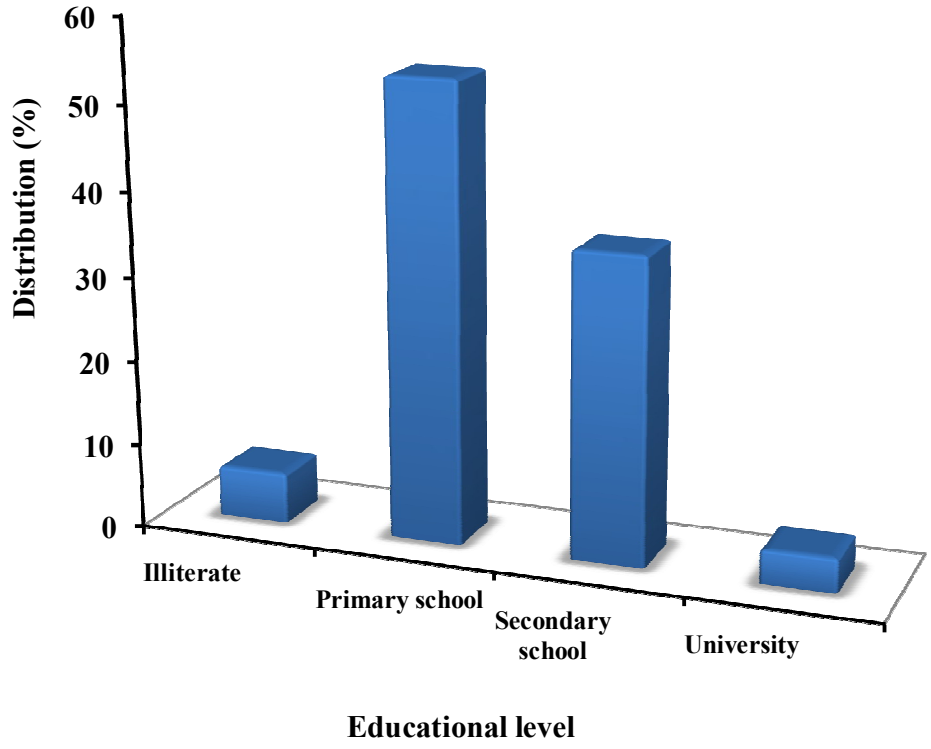


Fig. 4.1. Distribution of farmers by educational level

4.1.3. Land-tenure system

Out of the 50 participating farmers 56% were land lords and 32% were cultivating land through sharing with land owners (lease system) (Fig. 4.2). The rest of the farmers (12%) were hiring the land.

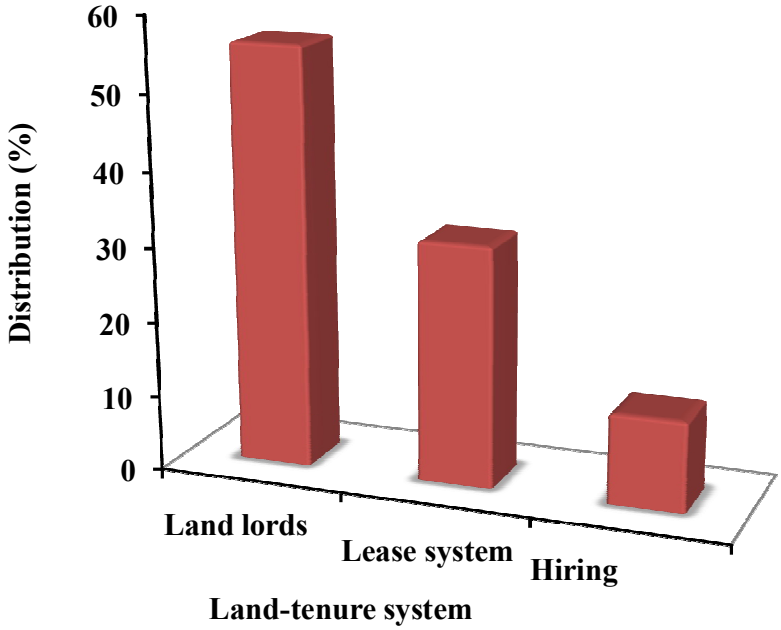


Fig. 4.2. Distribution of farmers according to Land-tenure system

4.1.4. Traditional cultural practices

A small proportion of the farmers (26%) grew summer crops, mainly forages. The rest (74%) did not grow summer crops. However, in general, there were no systematic crop rotations. A large proportion of the farmers (64%) get their seeds from their farms. The rest of the farmers ٢8 and 8% get their seeds from the market and neighboring farms, respectively. Only 12% of the farmers use organic manure from their own livestock (Table 4.2). A small proportion of the farmers (18%) use their own farm machinery, while a considerable proportion (82%) share machinery with others. Most of the farmers (78%) plant faba bean early in November where as the rest (22%) plant the crop late in November.

4.1.5. *O. crenata* incidence and distribution and farmers′ perception

All the participating farmers reported *O. crenata* in their fields. Most of the farmers (54%) reported that emergence of the parasite occurred at faba bean flowering, while 18% of the farmers reported emergence to occur after flowering

and only 8% of them claimed the occurrence of the parasite before flowering. Visual rating of *O. crenata* showed that infestation was very heavy in 74% of the surveyed farms, while moderate infestations were recorded in 26% of the farms. The parasite was restricted to the borders in 36% of the surveyed farms. However, wide spread of the parasite within the farm was reported by 64% of the participating farmers. However, despite the heavy infestation, faba bean production has not been abandoned completely. Of the respondents 12% are still producing the crop while 78% have been forced to change their cropping system and wheat and vegetables have become the main crops. Several management practices were adopted by the farmers. About 30% adopt hand-pulling of the parasite, allowing it to dry and subsequently burned. However 16% of the farmers hand-pulled the parasite, but leave it on the farm or threw it onto the road between farms (Plate 7). Of the participating farmers only 6% employed chemical control and the rest (48%) adopted no control measures. Following harvest, 80% of the farmer's dispose of crop residues as animal feed. Moreover, they allowed their farm to be grazed by animals. Only 20% of the studied farmers disposed of crop residues through burning.

Table 4.2. Some characteristics of farming and farming system in west Berber area (2009/2010)

Item	Frequency	Percent of responding farmers
Planting summer crops		
Yes	13	26
No	37	74
Total	50	100
Type of summer crops		
Forages	11	84.6
Forages and sorghum	2	15.4
Total	13	100

Adopting crop rotation		
Yes	13	26
No	37	74
Total	50	100
Source of seeds		
Own farm	32	64
Other sources	18	36
Total	50	100
Use organic manure		
Yes	6	12
No	44	88
Total	50	100
Manure source		
Own animals	6	12
Other farmers	-	-
Total	6	12
Missing	44	88
Total	50	100

Plate 7. *O. crenata* thrown onto the road between farms

4.2. Laboratory experiments

4.2.1. Effects of urea applied during conditioning on *O. crenata* seeds germination

Orobanche seeds conditioned in urea were less responsive to GR24 (10 ppm) than those conditioned in DW (Fig. 4.3). The response to GR24 progressively decreased with increasing urea concentration. Seeds conditioned in DW and subsequently treated with GR24, displayed 89.66% germination. Seeds conditioned in urea at 20 and 30 mM and similarly treated with GR24, displayed 58.74 and 37.32% germination, respectively. Increasing urea concentration to 40, 50 and 60 mM decreased germination to 15.93, 14.76 and 13.71%, respectively. Further increase in urea concentration to 70 mM or more resulted in negligible germination (0 - 4.6%) (Fig. 4.3).

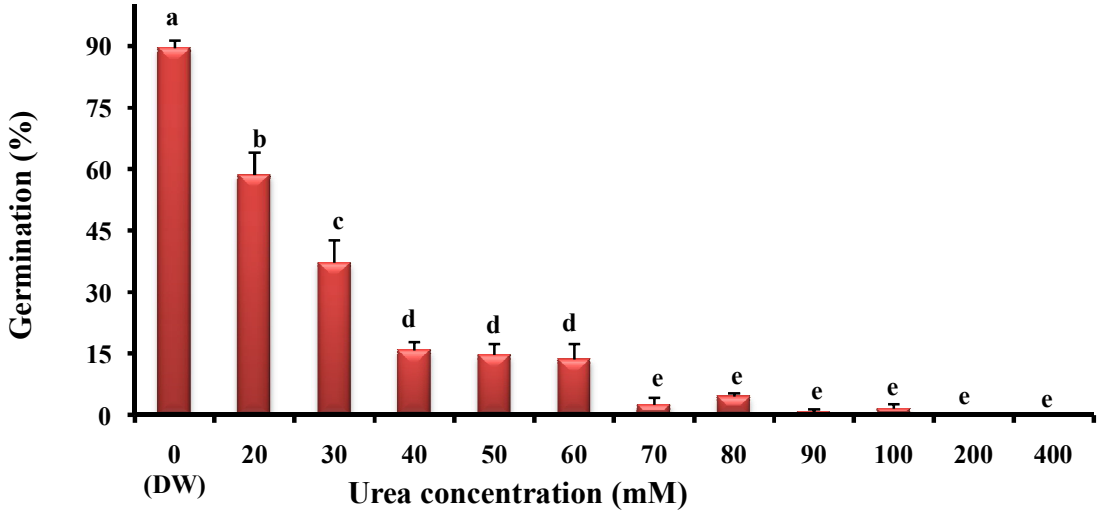


Fig. 4.3. Effects of urea, applied during conditioning, on *O. crenata* seeds germination. Bars are means ± standard errors according to DMRT; P ≤ 0.05. Bars marked with different letters are significantly different at P<0.05 (DMRT).

4.2.2. Effects of urea applied during conditioning on *O. crenata* radicle extension

Germilings from seeds conditioned in urea displayed reduced radicle extension. The radicle extension progressively decreased with increasing urea concentration in the conditioning solution (Fig. 4.4). Germilings from seeds conditioned in DW and subsequently treated with GR24 at 10 ppm, displayed mean radicle extension of 34 x 10^{-3}mm (Fig. 4.4). Conditioning in urea at 20 mM reduced radicle extension by 11.8%. Increasing urea concentration to 30-60 mM reduced redicle extension significantly by 42-69%. A further increase in urea concentration to 70 mM or more resulted in further reductions 85-100%.

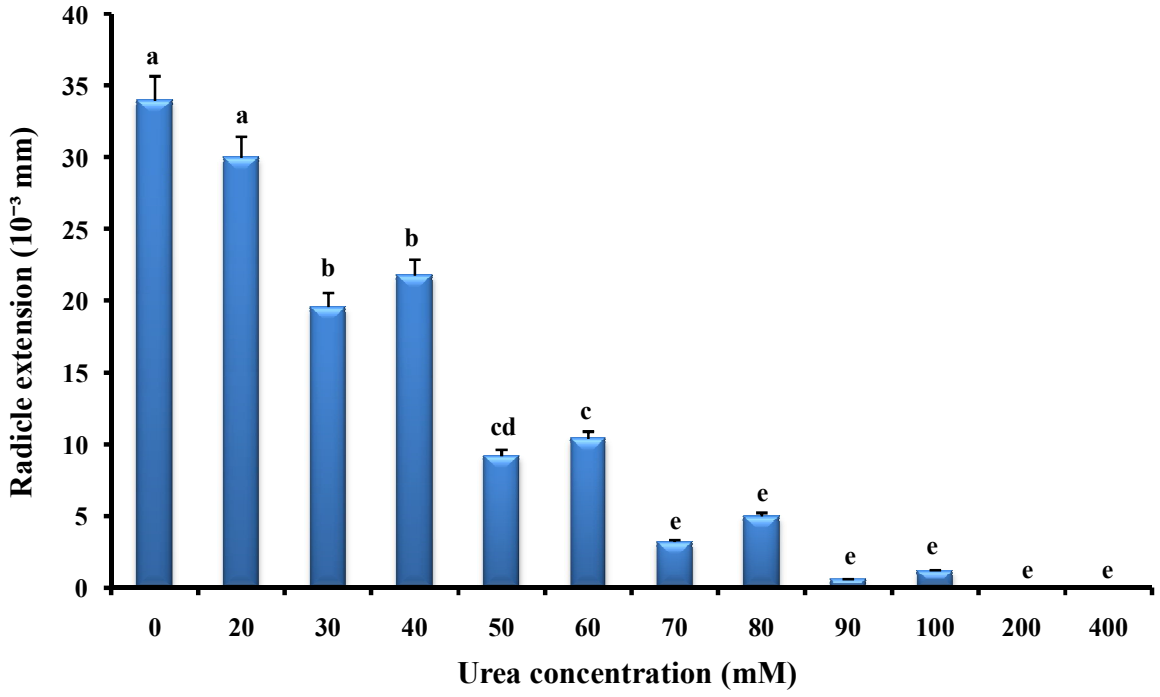


Fig. 4.4. Effects of urea applied during conditioning on radicle extention of *O. crenata.* Bars are means ± standard errors according to DMRT; P ≤ 0.05. Bars marked with different letters are significantly different at P<0.05 (DMRT).

4.2.3. Effects of urea applied subsequent to GR24, on *Orobanche* seeds germination

Orobanche seeds conditioned in DW and treated with GR24 at 10 ppm 1, 4, 8 and 24 h, displayed 80.81, 79.33, 79.65 and 80.62 % germination, respectively. *Orobanche* seeds conditioned in DW and treated with urea, irrespective of concentration, subsequent to GR24 at 10 ppm, showed germination comparable to those treated with GR24 only (Table 4.3).

Table 4.3. Effects of urea applied subsequent to GR24, on *Orobanche* seeds germination

Urea (mM)	Germination % GR24 application time (h)			
	1	**4**	**8**	**24**
Aqueous control	80.81	79.33	79.65	80.62
200	81.65	74.32	80.77	82.20
400	79.42	72.86	80.15	78.50
2-Way ANOVA				
Urea	n.s			
Time	n.s			
Urea^{vs}T	n.s			

n.s = not significant.

4.2.4. Effects of urea applied subsequent to GR24, on *O. crenata* radicle extension

Orobanche germilings from seeds conditioned in water and treated with GR24 at 10 ppm 1, 4, 8 and 24h, showed radicle extension of 42.14 x 10^{-3}, 45.86 x 10^{-3} , 40.36 x 10^{-3} and 45.86 x 10^{-3}mm, respectively (Table 4.4). Germilings from seeds treated with GR24 at 10 ppm and subsequently treated with urea, irrespective of concentration and timing of urea treatment, showed inconsistent

effects on radicle extension, which was often comparable to the corresponding control (Table 4.4).

Table 4.4. Effects of urea applied subsequent to GR24, on *O. crenata* radicle extension

Urea (mM)	Radicle length (mm 10^{-3}) GR24 application time (h)			
	1	**4**	**8**	**24**
Aqueous control	42.14^{ab}	45.86^{a}	40.36^{abc}	45.86^{a}
200	42.94^{ab}	36.54^{abc}	42.40^{ab}	41.60^{abc}
400	42.54^{ab}	36.37^{bc}	42.66^{ab}	34.84^{c}
2-Way ANOVA				
Urea	*			
Time,(h)	n.s			
Urea ^{vs} T	*			

Means within columns followed by different letters are significantly different according to DMRT. n.s = non-significant. *= $P \leq 0.05$.

4.2.5. Effects of urea preceding GR24 treatment on *O. crenata* germination

Orobanche seeds conditioned in DW displayed differential response to GR24 at 10 ppm applied 1, 4, 8, and 24h subsequent to urea (Table 4.5). The magnitude of the response was dependent on time and the concentration of urea. Seeds conditioned in DW and subsequently treated with GR24 1, 4, 8, and 24h later, displayed 89.08, 74.7, 76.65 and 65.23% germination, respectively. Seeds treated with urea, irrespective of concentration or duration and subsequently treated with GR24, displayed inconsistent germination (Table 4.5).

Table 4.5. Response of conditioned *O. crenata* seeds to GR24 applied subsequent to urea (germination)

Urea (mM)	Germination %			
	Urea application time (h)			
	1	4	8	24
Aqueous control	89.08 [a]	74.7 [bc]	76.65 [abc]	65.23 [cd]
200	81.8 [ab]	74.15 [bc]	79.24 [ab]	64.72 [cd]
400	83.79 [ab]	59.96 [d]	77.97 [ab]	75.54 [bc]
2-Way ANOVA				
Urea	n.s			
Time, T	***			
Urea [vs] T	*			

Means within columns followed by different letters are significantly different according to DMRT. ***= $P \leq 0.001$. * = $P \leq 0.05$.

4.2.6. Effects of urea preceding GR24 treatment on *O. crenata* germilings radicle extension

Orobanche germilings from seeds treated with GR24 at 10 ppm 1, 4, 8, and 24h later, showed radicle extension of 44.8 x 10^{-3}, 42.4 x 10^{-3}, 37.06 x 10^{-3} and 26.8 x 10^{3} mm (Table 4.6). Germilings from seeds treated with urea irrespective of concentration 1 and 4 h prior to GR24 treatment, displayed reduced radicle extension, albeit not significantly (Table 4.6). Germilings from seeds treated with urea 8 h prior to GR24 treatment, irrespective of urea concentration, displayed radicle extension comparable to GR24 treated control (Table 4.6). However, germilings from seeds treated with urea 24 h prior to GR24, irrespective of the former concentration, displayed increased radicle extension (Table 4.6).

Table 4.6. Radicle extension of germilings from *O. crenata* seeds treated with GR24 subsequent to urea

Urea (mM)	Radicle length (mm 10^{-3})			
	Urea application time (h)			
	1	4	8	24
Aqueous control	44.8^{a}	42.4^{a}	37.06^{ab}	26.8^{c}
200	38.14^{ab}	39.46^{ab}	41.06^{a}	37.96^{ab}
400	41.6^{a}	42.66^{a}	37.96^{ab}	31.90^{bc}
2-Way ANOVA				
Urea	n.s			
Time, T	***			
Urea ^{vs} T	*			

Means within columns followed by different letters are significantly different according to DMRT. ***= $P \leq 0.001$, *= $P \leq 0.05$. n.s = not significant.

4.2.7. Effects of urea and GR24, applied simultaneously, on *Orobanche* seeds germination

Orobanche seeds conditioned in DW and subsequently treated with GR24 or mixtures of GR24 with urea showed differential germination (Table 4.7). The magnitude of the response showed dependence on concentration of both urea and GR24. Seeds treated with mixtures of GR24 with urea showed significantly less germination, compared with seeds treated with GR24 alone (Table 4.7). *Orobanche* seeds conditioned in DW and subsequently treated with GR24 at 0.1, 1 and 10 ppm, displayed 5.25, 37.74, and 83.63% germination, respectively (Table 4.7). *Orobanche* seeds treated with urea at 200 mM mixed with GR24 at 0.1, 1 and 10 ppm, displayed 0, 4.62 and 34.91% germination, respectively.

Orobanche seeds treated with urea at 400 mM in mixtures with GR24 at 0.1, 1 and 10 ppm displayed 0, 3.94 and 45.99% germination, respectively.

Table 4.7. Effects of urea and GR24, applied simultaneously on *O. crenata* seeds germination

Urea (mM)	Germination% GR24 concentration (ppm)		
	0.1	**1**	**10**
Aqueous control	5^{d}	37.74^{c}	83.63^{a}
200	0.0^{d}	4.62^{d}	34.91^{c}
400	0.0^{d}	3.94^{d}	45.99^{b}
2-Way ANOVA			
Urea	***		
GR24, G	***		
Urea^{vs}G	***		

Means within columns followed by different letters are significantly different according to DMRT. ***= $P \leq 0.001$.

4.2.8. Effects of urea and GR24, applied simultaneously, on radicle extension

Orobanche germilings from seeds treated with GR24 at 0.1, 1 and 10 ppm, showed radicle extension of 15.4 x 10^{-3}, 34.9 x 10^{-3} and 61.1 x 10^{-3}mm, respectively (Table 4.8). However, seeds treated with GR24 in mixtures with urea, displayed significantly less radicle extension. The reduction in radicle extension increased with increasing urea concentration. Seeds treated with urea at 200 mM in mixtures with GR24 at 0.1, 1 and 10 ppm displayed radicle extension of 0, 14.4 x 10^{-3} and 35.1 x 10^{-3}mm, respectively (Table 4.8). Seeds

treated with the urea at 400 mM in mixtures with GR24 at 0.1, 1 and 10 ppm displayed radicle extension of 0, 15.1 x 10^{-3} and 40.8 x 10^{-3}mm, respectively. (Table 4.8).

Table 4.8. Effects of urea and GR24, applied simultaneously on *O. crenata* radicle extension

Urea (mM)	Radicle length (mm 10^{-3}) GR24 concentration (ppm)		
	0.1	**1**	**10**
Aqueous control	15.4[c]	34.9[b]	61.1[a]
200	0.0[d]	14.4[c]	35.1[b]
400	0.0[d]	15.1[c]	40.8[b]
2-Way ANOVA			
Urea	***		
GR24, G	***		
Urea[vs]G	**n.s**		

Means within columns followed by different letters are significantly different according to DMRT. ***= $P \leq 0.001$. n.s = not significant.

4.2.9. Effects of imazethapyr applied during conditioning on *O. crenata* seeds germination

*Orobanch*e seeds conditioned in DW and treated with GR24 showed differential germination (Table 4.9). The magnitude of the response showed dependence on GR24 concentration. *Orobanch*e seeds conditioned in DW and treated with GR24 at 0.1ppm displayed 5.8% germination. Increasing GR24 concentration to 1 and 10 ppm increased germination significantly to 40 and 76.6 %, respectively (Table 4.9). *Orobanch*e seeds conditioned in imazethapyr displayed differential response to GR24. The magnitude of the response showed dependence on concentration of imazethapyr and GR24. Seeds conditioned in imazethapyr at 10 µM and subsequently treated with GR24 at 0.1, 1 and 10 ppm, displayed higher

germination than that achieved by seeds conditioned in DW, albeit not significantly (Table 4.9). Increasing imazethapyr concentration to 20-80 µM, resulted in non-significant inhibition of germination. A further increase in imazethapyr concentration to 100 µM significantly inhibited germination at the lower GR24 concentration (0.1 and 1 ppm), but not in the higher (10 ppm).

Table 4.9. Effects of imazethapyr applied during conditioning on *O. crenata* seeds germination

	Germination%		
Imazethapyr	**GR24 concentration (ppm)**		
(µM)	**0.1**	**1**	**10**
Aqueous control	5.8^{gh}	40^{cd}	76.6^{ab}
10	12.5^{fg}	44.7^{c}	82.9^{a}
20	$3.8^{hı}$	48.8^{c}	66.1^{b}
40	5.9^{gh}	29.1^{de}	70.2^{b}
60	4.1^{ghi}	28.7^{de}	66.2^{b}
80	4.7^{hi}	25^{de}	66.2^{b}
100	$0.77^{ı}$	21.7^{ef}	65.7^{b}
2 - Way ANOVA			
Imaz.	***		
GR24, G	***		
Imaz.^{vs}G	n.s		

Means within columns followed by different letters are significantly different according to DMRT. ***= $P \leq 0.001$. ns = not significant**.**

4.2.10. Effects of imazethapyr applied during conditioning on *O. crenata* radicle extension

Orobanche germilings from seeds conditioned in DW and treated with GR24 at 0.1, 1 and 10 ppm, showed radicle extension of 11.6 x 10^{-3}, 19.2x 10^{-3} and 40.2 x 10^{-3}mm, respectively (Table 4.10). *Orobanch*e germilings from seeds

conditioned in imazethapyr, displayed differential radicle extension, the magnitude of which showed dependence on concentration of imazethapyr and GR24 (Table 4.10). Conditioning in imazethapyr, irrespective of concentration showed inconsistent effect on radicles of germilings from seeds induced to germinate with GR24 at 0.I and 1 ppm. However, germilings from seeds induced to germinate with GR24 at 10 ppm showed significant reduction in radicle extension.

Table 4.10. Effects of imazethapyr applied during conditioning on *O. crenata* radicle extension

	Radicle length (mm 10^{-3})		
Imazethapyr	**GR24 concentration (ppm)**		
(µM)	**0.1**	**1**	**10**
Aqueous control	11.6^{e}	19.2^{bcd}	40.2^{a}
10	13.6^{de}	22.6^{bc}	20.8^{bc}
20	3.6^{f}	16.6^{cde}	18.8^{bcd}
40	4.8^{f}	12.8^{de}	23.0^{bc}
60	11.2^{e}	16.4^{cde}	24.2^{b}
80	4.8^{f}	16.8^{cde}	21.4^{bc}
100	0.8^{f}	11.4^{e}	19.4^{bcd}
2 - Way ANOVA			
Imaz.	***		
GR24, G	***		
Imaz.^{vs}G	***		

Means within a column followed by different letters are significantly different according to DMRT. ***= $P \leq 0.001$

4 .3. Field experiments

4.3.1. Season 2009/10

4.3.1. 1. Effects of imazethapyr, and its timing of application on *Orobanche* incidence and faba bean growth and yield

4. 3.1.1.1. Effects on *Orobanche* emergence

Imazethapyr, irrespective of rate and application time, reduced *O. crenata* emergence and differences between treatments were highly significant (Table 4.11).

Table 4.11. Effects of imazethapyr, rate and application time, on *O. crenata* emergence (season 2009/10)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Imazethapyr application time (DAS)	*O. crenata* plant m^{-2}	
			67 DAS	82 DAS
Imaz.	**20**	**0**	5 (2.43) ^{b}	21.34 (4.7)^{a}
Imaz.	**20**	**15**	0.78 (1.3) ^{c}	7.58 (2.7)^{b}
Imaz.	**20**	**30**	0.88 (1.4) ^{c}	9.40 (3.04)^{ab}
Imaz.	**20**	**45**	11.98 (3.6) ^{a}	14.91 (3.9)^{ab}
Imaz.	**30**	**0**	0.5 (1.2) ^{c}	12.53 (3.6)^{ab}
Imaz.	**30**	**15**	0.41(1.2) ^{c}	8.18 (2.8)^{b}
Imaz.	**30**	**30**	0.38 (1.2) ^{c}	6.42 (2.6)^{b}
Imaz.	**30**	**45**	4.28 (2.2) ^{b}	10.86 (3.4)^{ab}
Infested control	-	-	15.5 (4) ^{a}	20.41 (4.6)^{a}
P≤ 0.05			***	*
SE±			1.32	1.35
CV%			23.9	26.7

Imaz. = imazethapyr, DAS = days after sowing. Data between parentheses are square root transformed means. Means within a column followed by different letters are significantly different according to DMRT.***=P ≤ 0.001,* = P ≤0.05.

The observations made 67 DAS showed that imazethapyr, at 20 g a.i. fed^{-1} applied at sowing reduced the parasite emergence by 67.7% (Table 4.11). Treatments made 15, 30 and 45 DAS, reduced *O. crenata* emergence by 94.8, 94.3 and 22.7%, respectively. Observations made 82 DAS, showed that the herbicide, at 20 g a.i. fed^{-1} applied at sowing did not reduce emergence of the parasite. Treatment made 15 DAS showed a significant reduction (63%). Treatments made 30 and 45 DAS, reduced *O. crenata* emergence by 54 and 27%, respectively. At 67 DAS imazethapyr at 30 g a.i. fed^{-1}, applied at sowing and 15, 30 and 45 DAS reduced the parasite emergence by 96.8, 97.4, 97.6 and 72.4%, respectively. Observations made 82 DAS, showed that the herbicide at 30 g a.i. fed^{-1}, applied at sowing and 45 DAS reduced the parasite emergence by 39 and 47%, respectively. However, the treatments made 15 and 30 DAS reduced the parasite emergence significantly and the observed reductions was 60 and 69%, respectively.

4.3.1.1.2. Effects on faba bean

4.3.1.1.2.1. Number of leaves

Number of faba bean leaves in the *O. crenata* free control at 30 and 60 DAS was 37.3 and 54.8 leaves per plant, respectively (Table 4.12). Unrestricted *O. crenata* growth had no effects on number of faba bean leaves . At 30 DAS, imazethapyr irrespective of rate or application time had no significant adverse effects on number of faba bean leaves (Table 4.12). At 60 DAS, imazethapyr at 20 g a.i. fed^{-1}, applied at planting and 15 and 30 DAS resulted in number of leaves comparable to the *Orobanche* free control (Table 4.12). However treatment made 45 DAS resulted in insignificant decrease in number of leaves. The herbicide at 30 g a.i. fed^{-1}, irrespective of application date effected number of leaves comparable to the *Orobanche* free control. Among all treatments, imazethapyr at 30 g a.i. fed^{-1} applied at planting, 30 and 45 DAS effected the highest number of leaves (Table 4.12).

Table 4.12. Effects of imazethapyr, rate and application time, on herbicidal efficacy and selectivity on faba bean (number of leaves) (season 2009/10)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Imazethapyr application time (DAS)	No. of Leaves plant $^{-1}$	
			30 DAS	60 DAS
Imaz.	**20**	**0**	41.3	53.68^{ab}
Imaz.	**20**	**15**	35.80	49.60^{ab}
Imaz.	**20**	**30**	38.85	51.42^{ab}
Imaz.	**20**	**45**	38.15	44.25^{b}
Imaz.	**30**	**0**	36.85	58.97^{a}
Imaz.	**30**	**15**	38.85	49.67^{b}
Imaz.	**30**	**30**	33.97	59.45^{a}
Imaz.	**30**	**45**	36.83	60.40^{a}
Uninfected control	**-**	**-**	37.30	54.75^{ab}
Infested control	**-**	**-**	32.10	51.65^{ab}
P≤ 0.05			n.s	*
SE±			2.2	3.8
CV%			11. 9	12.6

Imaz. = imazethapyr, DAS = days after sowing. Means within a column followed by different letters are significantly different according to DMRT. *= P ≤ 0.05; No. = number; n.s = non-significant.

4.3.1.1.2.2. Plant height

Observations made 30 and 60 DAS showed that *O. crenata* infestation did not reduce faba bean height. Imazethapyr irrespective of rate or application time, had no significant effects on faba bean height at 30 and 60 DAS (Table 4.13).

Table 4.13. Effects of imazethapyr, rate and application time, on herbicidal efficacy and selectivity on faba bean height (season 2009/10)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Imazethapyr application time (DAS)	Faba bean height (cm)	
			30 DAS	60 DAS
Imaz.	**20**	**0**	32.92	60.90
Imaz.	**20**	**15**	29.73	63.55
Imaz.	**20**	**30**	29.92	62.95
Imaz.	**20**	**45**	29.92	62.95
Imaz.	**30**	**0**	33.47	65.70
Imaz.	**30**	**15**	30.35	56.60
Imaz.	**30**	**30**	28.52	62.80
Imaz.	**30**	**45**	28.88	57.40
Uninfested control	-	-	30.10	61.00
Infested control	-	-	29.80	62.35
P≤ 0.05			n.s	n.s
SE±			1.6	2.9
CV%			10.6	9.5

Imaz. = imazethapyr, DAS = days after sowing, n.s = not significant**.**

4.3.1.1.2.3. Number of pods

Unrestricted *O. crenata* infestation reduced number of pods by 28.8 and 20.2% at 60 and 75 DAS, respectively (Table 4.14). At 60 DAS imazethapyr at 20 g a.i. fed^{-1} applied at sowing and 15 DAS increased number of pods over the *O. crenata* infested control, albeit not significantly (Table 4.14). However, treatments made at 30 and 45 DAS resulted in 1% and 39% reduction, respectively. The herbicide at 30 g a.i. fed^{-1} applied at sowing or 15, 30 and 45 DAS increased number of pods over the infested control, albeit not significantly.

At 75 DAS, however, despite the lack of significant differences between treatments, a trend of an increase in number of pods with delayed applications was displayed. Imazethapyr at 20 g a.i. fed^{-1} applied at sowing, 15 and 30 DAS increased pods production by 27.9, 5 and 27.3% over the infected control. The herbicide at 30 g a.i. fed^{-1} however, increased the number of pods when applied at sowing or 15, 30 and 45 DAS by 57.5, 29.6, 44.9 and 55.8%, respectively.

Table 4.14. Effects of imazethapyr, rate and application time, on pod production in faba bean (season 2009/10)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Imazethapyr application time (DAS)	No. of pods $plant^{-1}$	
			60 DAS	75 DAS
Imaz.	**20**	**0**	12.05	19.25
Imaz.	**20**	**15**	13.15	15.80
Imaz.	**20**	**30**	9.73	19.15
Imaz.	**20**	**45**	6.05	11.55
Imaz.	**30**	**0**	16.35	23.70
Imaz.	**30**	**15**	10.20	19.50
Imaz.	**30**	**30**	16.85	21.80
Imaz.	**30**	**45**	13.40	23.45
Uninfested control	**0**	-	13.80	18.85
Infested control	**0**	-	9.83	15.05
P≤ 0.05			n.s	n.s
SE±			2.34	2.98
CV%			37.8	31.5

Imaz. = imazethapyr, DAS = days after sowing, n.s = not significant**.**

4.3.1.1.2.4. Hundred seed weight and grain yield

Unrestricted *O. crenata* growth had negligible effects on faba bean 100 seed weight (Table 4.15). The herbicide treatment, irrespective of rate, had no significant effects on the 100 seed weight. *O. crenata* infestation reduced faba bean yield by 35% in comparison to the *O. crenata* free control (Table 4.15).

Table 4.15. Effects of imazethapyr, rate and application time, on faba bean hundred seed weight and grain yield (season 2009/10)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Imazethapyr application time (DAS)	100 seed weight (g)	Faba bean yield (kg fed^{-1})
Imaz.	**20**	**0**	40.13	880.83 ^{a}
Imaz.	**20**	**15**	37.44	760.70 ^{ab}
Imaz.	**20**	**30**	39.04	770.54^{ab}
Imaz.	**20**	**45**	40.62	633.12^{bc}
Imaz.	**30**	**0**	36.64	913.96^{a}
Imaz.	**30**	**15**	39.37	828.88^{ab}
Imaz.	**30**	**30**	39.45	816.44^{ab}
Imaz.	**30**	**45**	37.25	643.1^{bc}
Uninfested control	**0**	-	39.88	789.89^{ab}
Infested control	**0**	-	37.83	511.04^{c}
P≤ 0.05			n.s	***
SE±			1.3	65.3
CV%			7	17.3

Imaz. = imazethapyr, DAS = days after sowing. Means within a column followed by different letters are significantly different according to DMRT. ***=P≤0.001. n.s = non-significant.

Imazethapyr, applied at sowing or 15 and 30 DAS, irrespective of rate, significantly, outyielded the infested control and gave yield comparable to the *O. crenata* free control (Table 4.15). Treatments made at sowing effected the highest yield. Treatments made at 45 DAS, on the other hand, outyielded the *O. crenata* infested control albeit not significantly. Imazethapyr at 20 g a.i. fed^{-1} applied at sowing, 15, 30 and 45 DAS increased faba bean grain yield by 72.4, 48.9, 50.8 and 23.9%, respectively over the infested control. The corresponding figures for the herbicide at 30 g a.i. fed^{-1} were 78.8, 62.2, 59.8 and 25.8%.

4.3.2. Season 2010/11

4.3.2.1. Effects of imazethapyr, nitrogen and their combinations on *Orobanche* incidence and faba bean growth and yield

4.3.2.1.1. Effects on *Orobanche*

4. 3.2.1.1.1. Emergence:

At 78 DAS nitrogen at 1N, applied at sowing or 15 and 30 days later reduced *O. crenata* emergence, albeit not significantly, in comparison to the infested control (Table 4.16). Imazethapyr at 20 g a.i. fed^{-1}, alone, reduced *Orobanche* emergence by 94%. Nitrogen applied at sowing or 15 and 30 DAS to sub-plots previously treated with imazethapyr at 20 g a.i. fed^{-1} did not cause further reductions in parasite emergence, a slight non-significant increase in parasite emergence was observed. Imazethapyr alone, at 30 g a.i. fed^{-1} reduced *O. crenata* emergence by 99%. Nitrogen applied at sowing or 15 and 30 days later caused no further reductions in *O. crenata* emergence in sub-plots previously treated with the herbicide. At 86 DAS nitrogen applied at sowing and 30 days later reduced *O. crenata* emergence significantly (Table 4.16).

Table 4.16. Effects of imazethapyr, nitrogen and their combinations on *Orobanche* emergence (season 2010/11)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Urea Application time (DAS)	No. of *O. crenata* m^{-2}	
			78 DAS	86 DAS
N	**0**	**0**	8.44(7.12) [a]	10.5(8.08)[bc]
N	**0**	**15**	9(6.98) [a]	14.9(9.14)[ab]
N	**0**	**30**	8.32(7.18) [a]	11.1(8.25)[bc]
Imaz.	**20**	**-**	0.76(2.03) [bc]	1.08(2.56)[d]
Imaz. + N	**20**	**0**	2.52(3.65) [b]	4.8(5.2)[cd]
Imaz. + N	**20**	**15**	1.16(2.31) [bc]	5.9(6.01)[bcd]
Imaz. + N	**20**	**30**	2.36(3.83) [b]	5.6(5.84)[bcd]
Imaz.	**30**	**-**	0.08(0.00) [c]	0.48(1.22)[d]
Imaz. + N	**30**	**0**	0.24(1.22) [c]	0.84(1.96)[d]
Imaz.+ N	**30**	**15**	0.12(0.43) [c]	0.6(1.5)[d]
Imaz. + N	**30**	**30**	0.12(0.75)[c]	0.56(1.37)[d]
Infested control	-	-	11.7(8.54) [a]	18.6(10.53)[a]
P ≤ 0.05			***	***
SE±			1.3	2.3
CV%			36.5	33.0

Imaz. = imazethapyr, N = nitrogen as urea 40 kg fed^{-1}, DAS = days after sowing. Data in parentheses are square root transformed means. Means within a column followed by different letters are significantly different according to DMRT. ***=P ≤ 0.001

The observed reductions were 44 and 40%, respectively in comparison to the infested control. However, treatment made 15 DAS resulted in a considerable albeit, not significant reduction (20%) (Table 4.16). Imazethapyr at 20 g a.i. fed^{-1}, alone, reduced *Orobanche* emergence by 94%. Nitrogen applied at sowing or 15 and 30 DAS to sub-plots previously treated with imazethapyr at 20 g a.i. fed^{-1} did not cause further reductions in the parasite emergence. In contrast the parasite emergence tended to increase, albeit not significantly, on supplementation of the herbicide treatment with nitrogen. Imazethapyr at 30 g a. i. fed^{-1}, alone, reduced *O. crenata* emergence by 97.4%. Nitrogen applied, at sowing or 15 and 30 days later to sub-plots previously treated with herbicide at 30 g a.i. fed^{-1} did not cause further reductions.

4. 3.2.1.1.2. *O. crenata* capsules production

Nitrogen alone, irrespective of application time, had no significant effect on *Orobanche* capsules production (Table 4.17). Imazethapyr alone, at 20 g a.i. fed^{-1} resulted in non-significant reduction. The observed reduction was 28% (Table 4.17). Imazethapyr at 20 g a.i. fed^{-1} applied simultaneously with nitrogen at planting or followed by nitrogen, 15 and 30 days later, reduced *Orobanche* capsules production by 50, 45 and 43%, respectively. The herbicide alone, at 30 g a.i. fed^{-1} reduced capsules production by 68% (Table 4.17). Imazethapyr at 30 g a.i. fed^{-1} applied simultaneously with nitrogen at planting or followed by nitrogen 15 and 30 days later reduced capsules production by 85, 72 and 54%, respectively (Table 4.17). Delayed application of nitrogen, depressed the suppressive effects of the combination on capsules production, albeit not significantly.

Table 4.17. Effects of imazethapyr, nitrogen and their combinations on capsules production (season 2010/11)

Treatment	Imazethapyr rate (g a.i.fed^{-})	Urea Application time (DAS)	No. of *O. crenata* Capsules plant^{-1}
N	**0**	**0**	44.68 (6.6)^{ab}
N	**0**	**15**	38.95 (6.2)^{bc}
N	**0**	**30**	59.85 (7.7)^{a}
Imaz.	**20**	-	33.55 (5.7)^{bcd}
Imaz. + N	**20**	**0**	23.3 (4.8)^{cde}
Imaz. + N	**20**	**15**	25.85 (5.1)^{cde}
Imaz. + N	**20**	**30**	26.55 (5.1)^{cde}
Imaz.	**30**	-	14.9 (3.3)^{ef}
Imaz. + N	**30**	**0**	6.95 (1.9)^{f}
Imaz.+ N	**30**	**15**	13.35 (3.4)^{ef}
Imaz. + N	**30**	**30**	21.6 (4)^{def}
Infested control	-	-	46.8 (6.8)^{ab}
P ≤ 0.05			***
SE±			0.8
CV%			29.5

Imaz. = imazethapyr, N=nitrogen as urea 40 kg fed^{-1}, DAS = days after sowing. Means within a column followed by different letters are significantly different according to DMRT. No=number. ***= P ≤ 0.001.

4.3.2.1.2. Effects on faba bean

4.3.2.1.2.1. Number of leaves

Unrestricted *O. crenata* infestation reduced number of faba bean leaves by 21.2%, albeit not significantly (Table 4.18). Nitrogen, alone, applied at sowing, 15 and 30 DAS increased the number of leaves over the infested control, albeit not significantly.

Table 4.18. Effects of imazethapyr, nitrogen and their combinations on number of faba bean leaves (season 2010/11)

Treatment	Imazethapyr rate (g a.i.fed^{-})	Urea Application time (DAS)	No. of leaves Plant^{-1} 45 (DAS)
N	**0**	**0**	44.13^{abcd}
N	**0**	**15**	43.25^{bcd}
N	**0**	**30**	39.8^{bcd}
Imaz.	**20**	**-**	35.10^{d}
Imaz. + N	**20**	**0**	56.70^{a}
Imaz. + N	**20**	**15**	49.10^{abc}
Imaz. + N	**20**	**30**	46.25^{abcd}
Imaz.	**30**	**-**	39.70^{bcd}
Imaz. + N	**30**	**0**	36.93^{cd}
Imaz .+ N	**30**	**15**	39.25^{bcd}
Imaz. + N	**30**	**30**	51.70^{ab}
Uninfested control	-	-	45.80^{abcd}
Infested control	-	-	36.1^{cd}
P ≤ 0.05			*
SE±			4.6
CV%			24.3

Imaz. = imazethapyr, N=nitrogen as urea 40 kg fed^{-1}, DAS = days after sowing. No. = number. n.s = not significant.

The number of leaves progressively declined with delayed nitrogen application, albeit not significantly. Faba bean treated with imazethapyr alone at 20 g a.i. fed^{-1} showed number of leaves comparable to the infested control. Imazethapyr at 20 g $a.i.fed^{-1}$ supplemented with nitrogen at planting increased the number of leaves significantly in comparison to the infested control and the resulting number of leaves was higher than that of the *Orobanche* free control, albeit not significantly (Table 4.18). Delaying, the supplementary nitrogen treatment to 15 and 30 DAS reduced the number of leaves in comparison to treatment made at planting, albeit not significantly (Table 4.18). Imazethapyr at 30 g $a.i.fed^{-1}$, alone, reduced the number of leaves in comparison to the *Orobanche* free control, albeit not significantly (Table 4.18). The herbicide at 30 g $a.i.fed^{-1}$ supplemented with nitrogen at planting or 15 days later had no further effects. However, when followed by nitrogen at 30 DAS the herbicide resulted in number of leaves significantly higher than the infected control.

4.3.2.1.2.2. Plant height

In general none of the treatment had significant effect on faba bean height (Table 4.19). However, certain trends were obvious. Observation made at 45 and 75 DAS showed that unrestricted *O. crenata* parasitism reduced faba bean height by12 and11.7%, respectively (Table 4.19). Nitrogen alone, applied at sowing or 15 and 30 days later, increased faba bean height by 11.8, 23.9 and 5.2%, respectively, in comparison to the *Orobanche* infected control. Imazethapyr at 20 g $a.i.fed^{-1}$, alone, did not increase crop height over the respective infested control. Imazethapyr at 30 g $a.i.fed^{-1}$, alone, increased faba bean height albeit, not significantly. The herbicide at all rates, when supplemented with nitrogen, irrespective of application time, increased plant height over the herbicide alone, albeit not significantly.

Table 4.19. Effects of imazethapyr , nirtrogen and their combinations on faba bean height (season 2010/11)

Treatment	Imazethapyr rate (g a.i. fed^{-1})	Urea application time (DAS)	Plant height (cm)	
			45 DAS	75 DAS
N	**0**	**0**	49.2	97.60
N	**0**	**15**	54.5	92.55
N	**0**	**30**	46.3	88.43
Imaz.	**20**	**-**	45	84.30
Imaz. + N	**20**	**0**	56.75	92.13
Imaz. + N	**20**	**15**	45.35	87.85
Imaz. + N	**20**	**30**	53.85	100.8
Imaz.	**30**	**-**	50.5	97.55
Imaz. + N	**30**	**0**	50.6	94.98
Imaz.+ N	**30**	**15**	47.55	97.75
Imaz. + N	**30**	**30**	51.45	95.95
Uninfested control	-	-	50	94.7
Infested control	-	-	44	83.65
P ≤ 0.05			n.s	n.s
SE±			3.8	4.7
CV%			15.4	10

Imaz. = imazethapyr, N=nitrogen as urea 40 kg fed^{-1}, DAS=days after sowing. n.s = not significant.

4.3.2.1.2.3. Number of flowers and pods

Despite the lack of statistical significances with regard to number of flowers certain trends were apparent (Table 4.20). Unrestricted *O. crenata* infestation inflicted considerable reduction (33.3%) in number of flowers (Table 4.20). Nitrogen alone, increased the number of flowers over the infected control. Furthermore, the number of flowers progressively decreased with delayed

fertilizer application. Imazethapyr, alone, at 20 and 30 g a.i.fed^{-1}, showed 22.4 and 26.4% reduction in number of flowers in comparison to the *Orobanche* free controls, respectively (Table 4.20). The herbicide at 20 g a.i.fed^{-1}, when supplemented with urea at sowing, increased the number of flowers by 111 and 40% in comparison to the parasite infested and free controls, respectively. Delaying supplementation with nitrogen to 15 and 30 DAS reduced the number of flowers per plant considerably, albeit not significantly in comparison to the *Orobanche* free control. However, the number of flowers was slightly higher than that of the parasite infested control. Plant treated with imazethapyr at 30 g a.i.fed^{-1} alone, effected a 10.4% increase in number of flowers in comparison to the *Orobanche* infested control and 26% reduction in comparison to the uninfested control (Table 4.20). The herbicide at 30 g a.i.fed^{-1} when supplemented with urea at planting or 15 and 30 days later increased the number of flowers by 40, 9 and 79% in comparison to the infested control, respectively. However, in comparison to the *Orobanche* free control the number of flowers was reduced when urea was applied at planting and 15 days later. However, when the later was applied at 30 DAS a considerable, but not significant, increase in number of flowers was observed (Table 4.20).

Unrestricted *O. crenata* infestation resulted in 38.8% reduction in number of faba bean pods (Table 4. 20). Nitrogen alone, resulted in number of pods comparable to that achieved by *Orobanche* free control. However treatment made 15 DAS resulted in significant increment (68%) in comparison with the infested control. Imazethaypr at 20 g a.i. fed^{-1} alone or in combination with nitrogen, irrespective of application time, resulted in number of faba bean pods comparable to *Orobanche* free control (Table 4. 20).

Table 4.20. Effects of imazethapyr, nitrogen and their combinations on number of faba bean flowers and pods (season 2010/11)

Treatments	Imazethapyr rate (g a.i.fed^{-1})	Urea application time (DAS)	No.of flowers plant^{-1}	No.of pods plant^{-1}	No.of Flowes +Pods
N	-	**0**	9.43	23.88^{bcd}	33.3
N	-	**15**	9.40	24.63^{abc}	34.03
N	-	**30**	7.25	19.40^{cd}	26.65
Imaz.	**20**	-	7.8	26.35^{abc}	31.9
Imaz. + N	**20**	**0**	14.10	22.65^{bcd}	36.75
Imaz. + N	**20**	**15**	7.55	22.90^{bcd}	30.45
Imaz. + N	**20**	**30**	10	25.10^{abc}	35.1
Imaz.	**30**	-	7.40	27.05^{abc}	34.45
Imaz. + N	**30**	**0**	9.40	27.33^{abc}	36.13
Imaz.+ N	**30**	**15**	7.30	29.60^{ab}	36.2
Imaz. + N	**30**	**30**	12.00	32.85^{a}	44.85
Uninfested control	-	-	10.05	23.95^{bcd}	34
Infested control	-	-	6.70	14.67^{d}	25
P ≤ 0.05			n.s	*	n.s
SE±			2.1	2.67	1.25
CV%			27.1	23.1	25.9

Imaz. = imazethapyr, N=nitrogen as urea 40 kg fed^{-1}, DAS = days after sowing. Means within a column followed by different letters are significantly different according to DMRT. * = P ≤ 0.05. n.s = not significant. No. = number.

However, imazethaypr at 20 g a.i. fed^{-1} alone and when followed by nitrogen 30 DAS resulted in significant increments (80 and 71%) in number of faba bean pods over infested control, respectively. The herbicide when followed by nitrogen at planting and 15 DAS resulted in insignificant increments (Table 4. 20). Imazethaypr at 30 g a.i. fed^{-1} alone or in combinations with nitrogen irrespective of application time, resulted in significant increments in comparison with the infested control. Imazethaypr at 30 g a.i. fed^{-1} alone and in combinations with nitrogen at sowing, 15 and 30 DAS showed significant increments (12.9, 14.1, 23.6 and 37.2%), respectively, in faba bean pods in comparison to the uninfested control (Table 4.20). Imazethaypr 30 g a.i. fed^{-1} followed by nitrogen at 30 DAS, resulted in a significantly higher than the uninfested control.

Unrestricted *O. crenata* parasitism reduced total number of flowers and pods by 27% in comparison to the parasite free control (Table 4.20). However, differences between treatments were not significant. Nitrogen applied at sowing or 15 days later, resulted in total number of flowers and pods identical to the parasite free control (Table 4.20). Nitrogen applied at 30 DAS, resulted in less total number of flowers and pods than the *Orobanche* free control. However, the differences were not significant. Imazethaypr at 20 g a.i. fed^{-1} alone and when supplemented with nitrogen, irrespective of application time, resulted in total number of flowers and pods comparable to the parasite free control. The herbicide at 30 g a.i. fed^{-1}, irrespective of nitrogen supplementation and application time of the later, resulted in total number of flowers and pods comparable to the parasite free control.

4.3.2.1.2.4. Grain yield and hundred seed weight

Unrestricted *O. crenata* infestation had no significant effect on faba bean 100 seeds weight (Table 4.21). Nitrogen and imazethapyr at 20 g a.i. fed^{-1}, each alone

and in combinations when nitrogen supplemented at planting and 15 days later, resulted in non significant decrease in 100 seeds weight. Imazethapyr at 30 g a.i. fed^{-1} alone and in combinations with nitrogen, at 15 and 30 DAS, showed faba bean 100 seeds weight comparable to the controls (Table 4.21). The herbicide at 30 g a.i. fed^{-1} when supplemented with nitrogen at sowing effected the highest 100 seeds weight.

Unrestricted *O. crenata* parasitism reduced faba bean grain yield significantly. The observed reduction was 46.9% in comparison to the *O. crenata* free control (Table 4.21). Nitrogen alone, irrespective of application time, had no significant effects on faba bean grain yield. Sub-plot treated with nitrogen at sowing, 15 and 30 DAS displayed 37.6, 35 and 43 % less grain yield than the *Orobanche* free control. However, the yield was higher than that of the infested control, albeit not significantly. Imazethapyr at 20 and 30 g a.i. fed^{-1} each applied alone outyielded the *O. crenata* infested control significantly. However, the yield attained was significantly lower than that of the *O. crenata* free control. The herbicide at 20 g a.i. fed^{-1} applied simultaneously with nitrogen at sowing effected yield comparable to the *Orobanche* free control. However, delayed supplementation of the herbicide treatment with nitrogen to 15 and 30 DAS resulted in yield comparable to that of the herbicide alone. Supplementation of imazethapyr at 30 g a.i. fed^{-1} with nitrogen applied simultaneously with imazethapyr at sowing or 15 days later resulted in grain yield comparable to the *Orobanche* free control. Furthermore, the herbicide when simultaneously applied with nitrogen at sowing resulted in the highest yield. Supplementation of the herbicide treatment with nitrogen at 30 DAS effected yield comparable to the herbicide treatment alone.

Table 4.21. Effects of imazethapyr, nitrogen and their combinations on faba bean 100 seed weight and grain yield (season 2010/11)

Treatment	Imazethapyr rate (g a.i.fed^{-1})	Urea Application time (DAS)	100 seed Weight (g)	Grain Yield (Kg fed^{-1})
N	**0**	**0**	42.22^{cd}	391.28^{fg}
N	**0**	**15**	42.56^{cd}	407.2^{fg}
N	**0**	**30**	42.84^{cd}	356.78^{g}
Imaz.	**20**	**-**	42.90^{cd}	495.40 ^{e}
Imaz. + N	**20**	**0**	42.89^{cd}	623.47^{abc}
Imaz. + N	**20**	**15**	42.76^{cd}	470.40^{ef}
Imaz. + N	**20**	**30**	41.73^{d}	533.18^{de}
Imaz.	**30**	**-**	44.73^{ab}	528.18^{de}
Imaz. + N	**30**	**0**	45.02^{a}	703.20 ^{a}
Imaz.+ N	**30**	**15**	43.72^{abc}	604.94 ^{bcd}
Imaz. + N	**30**	**30**	43.00^{bcd}	541.56^{bcd}
Uninfested control	**0**	**-**	43.69^{abc}	626.86 ^{ab}
Infested control	**0**	**-**	43.40^{abc}	332.59^{g}
P ≤ 0.05			**	***
SE±			0.58	27.9
CV%			2.7	11.0

Imaz.=imazethapyr, N=nitrogen as urea 40 kg fed^{-1}, DAS = days after sowing. Means within a column followed by different letters are significantly different according to LSD test. *** = P ≤ 0.001.

CHAPTER FIVE

DISCUSSION

Production of faba bean, the most important cool season leguminous crop in the Northern and River Nile States of the Sudan, is threatened by the broomrape *O. crenata*. The parasite seeds were voluntary introduced as seeds contaminants in the 1990s when increased urbanization and market demand led to importation of faba bean from neighbouring countries (Babiker *et al.,* 2007). Since then the parasite has spread in the fertile strip of the alluvial soils of the Nile Valley from Khartoum to Wadi Halfa on the Egyptian border (Babiker *et al.,*2007).

The field survey undertaken in west Berber area (Fig. 3.1), showed a wide distribution of the parasite as over 70% of the fields were infested. Of the infested fields, very heavy incidence within farm was noticed by 64% of the participating farmers, while moderate incidence, restricted to the borders, was reported in 36% of the surveyed farms. The wide spread of *O. crenata* is consistent with its invasive nature and the ease of seed distribution together with lack of awareness about the parasite, its biology, reproduction, methods of spread, the nature of its association with its host and lack of natural enemies together with a series of malpractices (Babiker *et al.,* 2007).

The results obtained during the survey indicated that most of the farmers (64%) got their seeds from their own farms and only 8% got their seeds from neighboring farms, while the rest (28%) got their seeds from the market. The use of seeds from the farms is likely to be a major cause of distribution within a farm. Obtaining seeds from the market could be a reason for wide spread of the parasite. Berner *et al.,* (1995) showed that contaminated Sorghum grains are main vehicles for long distance transport of the closely related parasite. The use of fresh manure (uncomposted animal manures) containing seeds excreted in a

viable condition on fields is believed to play a major role in long distance transport of *O. crenata* seeds (Cooke, 2002). However, the results obtained indicated that a small proportion (12%) of farmers use organic manure and no strict crop rotation was adopted (Table 4.2). High infestations prohibit profitable faba bean production. Most of the participating farmers (78%) abandoned planting of faba bean and shifted to wheat and vegetables. Abandonment of faba bean production is expected to increase unless some measures are taken to increase farmers awareness about the parasite and means of its spread. Most of the farmers (54%) received primary education and only 4% were illiterate (Figs. 4.1).

Hand-pulling is a main method of control and was practiced by 46% of the responding farmers, while chemical control of the parasite was only practiced by 6%. The rest of the farmers were adopting no control measures (Appendix VI). Subsequent to hand-pulling the majority of the farmers, collect *O. crenata* spikes and burn them. The rest of the farmers pull the spikes, leave them on the farm or threw them into adjacent roads. Following harvest most of the farms are grazed by animals. Such malpractices may suggest an important role in spreading of the parasite over long distances. Hand-pulling, collection and burning are essential measures of control, however, the success of these practices depends on the stage of growth of the parasite. Considerable damage to the host is normally inflicted by the parasite prior to emergence (Song *et al*., 2006). However, hand-pulling limits the build-up of the seed bank.

Data on *O. crenata* (Tables 4.2) indicated that several factors are responsible for the observed spread of the parasite. The most important factors are i) lack of well planned crop rotation, ii) planting seeds of unknown source, iii) animal grazing following harvest, iv) movement of farm equipment and v) throwing *Orobanche* spike onto adjoing roads and/or piling them in the field. Despite the considerable

proportion of the farmers (26 and 86.4%) are adopting summer and fodder cropping, but the implication of this practice on *O. crenata* seed reserves is doubtful. Annual cycles of seed dormancy in *O. crenata*, showed that with high temperature in summer as reported by Kebreab & Murdoch (1999) leads to secondary dormancy and thus lessen the efficacy of the respective fodders as trap crops. High seeds germination was realized at 15°C while poor germination was achieved following seed conditioning and germination at 20°C (Eltayb, 2010). Thus the need for a properly designed crop rotation involving winter crops is imperative. Source of crop seeds may be a major factor in long distance seed dispersal of the parasite seeds, where over 36% of the farmers obtain their seeds from foreign sources. Berner *et al*., (1995) reported that seed traders on local markets were the most important factor for witchweed seeds transport as over 60% of the sorghum seeds purchased from the market were contaminated with *S. hermonthica* seeds. Animal grazing has been shown to be an important element in long distance transport of noxious weeds including *Orobanche* and *Striga* species (Parker and Riches, 1993). Several workers have shown that *Orobanche* seeds could pass, undamaged, through animal's digestive system (Jacobsohn *et al*., 1987; Berner *et al.*, 1994; Goldwasser *et al.,* 2011). Parasitic weed seeds are transported to other fields through contaminated soil and water (by run-off) and because parasitic weed seeds adhere to the fur of grazing animals, farming implements like ploughs or boots and clothing, further distribution of the parasite seeds within the farm will occur. Throwing of *O. crenata* spikes in the farm helps maintenance of the parasite within the field. It has been shown that *O. crenata* can produce viable seeds after hand-pulling (Linke, 1999). Throwing *Orobanche* spikes onto adjoining roads may further spread the parasite between farms within a locality.

Germination of *O. crenata* and radicle extension increased with increasing GR24 concentration (Tables 4.7, 4.8, 4.9 and 4.10). GR24 at low concentration

(0.1 ppm) did not elicit germination of *O. crenata* and radicle extension. Increasing GR24 concentration to 10 ppm increased germination and radicle extension (Tables 4.7, 4.8, 4.9, 4.10). This finding is in line with previous reports on *Orobanche* germination (Saghir, 1986; Eltayb, 2010). The need for high concentration of the stimulant to induce germination of *O. crenata* seeds suggests that the low stimulant host plants could be an avenue for development of integrated management for the parasite. At the same time higher stimulant producers non-host crops may be used in rotation with faba bean to deplete the parasite seed bank. However, it has to be noted that several reports have indicated involvement of gibberellins in *O. crenata* germination (Parker and Riches, 1993; Joel, 1995) and that the nodulation in faba bean could enhance germination and/or penetration of the host root. Nodules have been reported to be rich in gibberellic acids (Mamaril *et al.,* 1988) and have also been reported to be weak points that facilitates penetration of the parasite into the host roots.

Conditioning in urea at 20-400 mM reduced germination in response to GR24 at 10 ppm significantly. *O. crenata* seed germination decreased with increasing urea concentration (Fig. 4.3). Urea applied at concentrations 20-60 mM, decreased germination significantly. Further increase in urea concentration to 70 mM or more resulted in negligible germination (0 – 4.6%) (Fig. 4. 3). Radicle extension followed the same trend of seed germination (Fig. 4.4). Urea at 20-60 mM reduced radicle extension by 11.8-69%. A further increase in urea concentration to 70 mM or more resulted in further reductions 85-100%. This finding is in line with that of Pieterse (1991) who reported that germination and growth of *O. crenata* were severely decreased when the seeds were exposed to urea during conditioning. Jain and Foy (1992) reported similar results with *P. aegyptiaca* when nitrogen was applied during the preconditioning period. Nitrogen in ammonium form affects negatively root parasitic weed germination (van-Hezewijk and Verkleij 1996) and/or elongation of the seedling radicle

(Westwood and Foy 1999). van-Hezewijk and Verkleij (1996) reported that ammonium sulphate (8 mM) applied during conditioning, reduced germination of seeds from 46% to 26%. A lower concentration of 4 mM ammonium sulphate did not inhibit germination. Urea (8 mM) reduced germination to only a limited extent (from 58% to 40%). It is hypothesized that the inhibition by ammonia of *O. crenata* germination is connected with a reduced ability to detoxify ammonia. The inhibitory effect of urea may be indirect, occurring through conversion to ammonium. This is in agreement with the report by Bremner and Krogmeier (1989) that the adverse effect of urea fertilizer on seed germination is due to ammonia formed through hydrolysis of urea and is not due to urea itself. Urea had no effect at 2 mg l^{-1} (based on N) but was promotive at higher concentrations. These results provide a basis for the inhibitory effects of nitrogen fertilizer on infestation by root parasitic weeds, broomrapes and witchweeds, and may explain why these parasites prevail in areas where there is lower phosphorus and nitrogen availability in soils.

Urea when applied, irrespective of concentration, subsequent to GR24, irrespective of application time, had no adverse effect on *O. crenata* germination (Table 4. 3).

Germilings from seeds treated with GR24 and subsequently treated with urea, irrespective of concentration and timing of urea treatment, showed inconsistent radicle extension (Table 4.4).

Orobanche seeds conditioned in DW and subsequently treated with GR24 alone 1, 4, 8 and 24 h later, showed decreased germination with increasing time lapse (Table 4.5). *Orobanche* seeds conditioned in DW and treated with GR24 1, 4, 8 and 24 h subsequent to urea treatment, showed inconsistent germination (Table 4.5).

Radicle extension of *Orobanche* germilings from seeds treated with GR24 1, 4, 8 and 24 h subsequent to urea , followed the same trend of seed germination (Table4.6). Germilings from seeds treated with urea irrespective of concentration 1 and 4 h prior to GR24 treatment, showed reduced radicle extension. However, those treated with urea 8h prior to GR24 displayed radicle extension comparable to the GR24 treated control and those treated with urea 24 h prior to GR24 showed increased radicle extension (Table 4.6). These findings need to be clarified by further research.

Seeds treated with mixtures of GR24 and urea, showed significantly less germination, compared with seeds treated with GR24 alone (Table 4.7). The effect of urea was more pronounced at the lower concentrations of GR24.

Orobanche germilings from seeds treated with GR24 in mixtures with urea, irrespective of concentration, displayed significantly less radicle extension compared with those treated with GR24 alone (Table 4.8). These findings are in line with that of Pieterse (1991) who reported that germination and growth of *O. crenata* were severely decreased when the seeds were exposed to urea at 4 mM during conditioning and/ or when urea was applied simultaneously with the germination stimulant. Furthermore, this results suggest that urea will be more effective when host resistance is associated with low stimulant production.

Seeds conditioned in imazethapyr at 10 µM and subsequently treated with GR24 at 0.1, 1 and 10 ppm, showed increased germination, albeit not significantly (Table 4.9). However seeds conditioned in imazethapyr at 20-80 µM despite lack of consistency showed decreased germination which was more pronounced at the highest GR24 concentration. The results indicate that imazethapyr may not consistently affect germination in practice.

Imazethapyr, irrespective of concentration showed inconsistent effect on radicles of germilings from seeds induced to germinate with GR24 at 0.I and 1 ppm.

However, germilings from seeds induced to germinate with GR24 at 10 ppm showed significant reduction in radicle extension. Reduction in radicle extension may reduced contact between the parasite and the host roots and thus reduced parasitism.

The herbicide imazethapyr , irrespective of rates and application time, effected considerable to significant reductions in *Orobanche crenata* infestation (Tables 4.11 and 4.16). The herbicide at 30 g a.i. fed^{-1} irrespective of application time, was the most effective and more suppressive to the parasite than at 20 g a.i. fed^{-1} (Tables 4.11 and 4.16). Nitrogen at 40 kg fed^{-1} (1N) as urea, irrespective of application time showed considerable suppression of the parasite late in the season. The herbicide imazethapyr alone was more suppressive to the parasite than when followed by nitrogen (Table 4.16).

In the first season *Orobanche* displayed delayed emergence up to 67 DAS (Table 4.11). At 67 DAS the parasite displayed maximum emergence (15.5 plants m^{-2}) in the untreated control sub-plots, followed by sub-plots treated with imazethapyr at 20 g a.i. fed^{-1} at 45 DAS and at planting and imazethapyr at 30 g a.i. fed^{-1} when applied at 45 DAS (Table 4.11). Imazethapyr at 30 g a.i. fed^{-1} when applied at planting or at 15 and 30 DAS and imazethapyr at 20 g a.i. fed^{-1} applied at 15 and 30 DAS effected excellent suppression of the parasite (over 90%) (Table 4.11). At 82 DAS, *Orobanche* emergence was maximal in the untreated control (20.4 plants m^{-2}), and in sub-plots treated with imazethapyr at 20 g a.i. fed^{-1} at planting (21.25 plants m^{-2}), followed in descending order by imazethapyr at 20 g a.i. fed^{-1} applied at 45 DAS and imazethapyr at 30 g a.i. fed^{-1} applied at planting and 45 DAS. However, the herbicide tend to maintain its excellent suppressive effect when applied at 15 and 30 DAS (Table 4.11). The observations that, irrespective of rate, the herbicide was less effective when applied at planting or 45 days later may be attributed to a multitude of variables

associated with the herbicide, the parasite and the host plant. Early application may lead to dissipation of the herbicide through leaching and/or break down. The former entails a soil dilution effect. However, considering reports on persistence of imazethapyr (Geisel, 2007) dissipation through leaching is the likely possibility. Leaching could decrease activity of the product taking into account the low rate of the herbicide used. This is substantiated by the observation that the loss in activity at the higher rate (30 g a.i. fed^{-1}) was comparatively less affected than the lower rate (Tables 4.11 and 4.16).

The results of the present study revealed that the herbicide has no effects on *O. crenata* seeds per se and the germination process (Table 4.9). It is noteworthy that *O. crenata* seeds germination is complex. The seeds need a pre-treatment under moist relatively cool conditions (20°C) to be physiologically receptive. However, response of *O. crenata* seed to germination stimulant is claimed to be promoted by gibberllins (GAs). Joel *et al*. (1995), based on inhibition of *O. crenata* seeds germination by the GA inhibitor pacloptrozol, claimed that *O. crenata* produces GAs during conditioning. However, a survey of the literature showed that nodules are a rich source of GAs (Mamaril *et al.,* 1988) and that *O. crenata* infestation is highest in nodulated roots (Parker and Riches, 1993). Nodulation in faba bean is reported to occur at flowering. Enhanced germination subsequent to nodulation together with herbicide dissipation may, at least in part, explain the decrease in efficacy of treatment made at sowing. The lower efficacy of the herbicide when applied at 45 DAS may be related to a shallow soil layer permeated by the herbicide and rapid emergence of the parasite leading to less contact with the herbicide permeated soil and consequently less uptake. Although late application (45 DAS) did not curtail emergence of the parasite, but the emerged spikes were malformed, displayed stunted growth and many died soon after emergence (Appendix II).

Orobanche infestation had no significant effects on number of faba bean leaves or faba bean height (Tables 4.12 and 4.13). These findings are consistent with that obtained by Eltayb, (2010) who reported that *O. crenata* infection had no significant effect in number of faba bean leaves and height at 30-60 DAS. Similar results were reported by Hibberd *et al.* (1998).The results are in line with the quiescent nature of infestation at the early stage of the development. *Orobanche* is induced to germinate by host-derived stimulants. Following germination and attachment a tubercle develops and the parasite stays quiescent (Plate 6). Bud formation of broomrapes is simulated to 10 days after the start of seedfillings in faba bean (Kropff and Schippers, 1986). Contrary to the general belief that *Orobanche* species are most damaging during the subterranean stage. The result suggest that *Orobanche* may exert its harmful effects on the crop during emergence, the bulk of which coincides with late flowering, bud formation and seedfilling. However the possibility of differential behavior of the system (host and parasite) due to variation in abiotic and biotic factors can not be ruled out. As indicated by ter Borg, (1986) both biotic and a biotic factors influence the relationship between *Orobanche* and its hosts. At 30 and 60 DAS *O. crenata* did not reduced number of leaves and plant height, however, it reduced the number of faba bean pods, albeit not significantly (Table 4.14). At 60 DAS imazethapyr at 20 g a.i. fed^{-1} applied at planting or 15 and 30 DAS, showed an increase in number of pods over the infested control, albeit not significantly (Table 4.14). However, treatment made 45 DAS, showed a considerable reduction but non-significant. The herbicide at 30 g a.i. fed^{-1} irrespective of application time increased the number of pods over the infested control, albeit not significantly. At 75 DAS imazethapyr, irrespective of rate and application time, tend to maintain its effects on number of pods.

O. crenata infestation reduced faba bean 100 seed wieght, albeit not significantly (Table 4.15). None of the treatments of imazethapyr, had adverse

effect on faba bean 100 seed weight, however, they showed inconsistent effects on grain yield (Table 4.15). Imazethapyr, irrespective of rate and application time, outyielded the infested control. Of all treatments imazethapyr at 30 g a.i. fed^{-1} when applied at planting yielded significantly the highest grain yield and realized 78.8% increase in grain yield over the infested control followed by imazethapyr at 20 g a.i. fed^{-1} when applied at planting which resulted in 72.4% increase in grain yield. Treatments made 15 and 30 DAS realized comparable increase in grain yield (48.9-62.2%) over infested control. Furthermore, the yield obtained was comparable with that of the *Orobanche* free control. However, treatments made 45 DAS showed decreased grain yield in comparison to the *Orobanche* free control (Table 4.15). The decrease in yield despite the lack of statistical significance, may indicate phytotoxicity and flower shedding. Of interest is the highest increase in yield obtained from sub-plots treated with the imazethapyr despite the decreased efficacy against the parasite. The high increase in yield attained with treatments made at planting may be attributed, at least in part, to the attained good control of weeds other than *Orobanche*.

In the second season, nitrogen alone irrespective of application time, reduced *Orobanche* emergence at 78 DAS, albeit not significantly (Table 4.16). However, at 86 DAS significant reductions were observed. These findings are in line with notion that nitrogen delays *Orobanche* emergence (Kasasian,1973b; Kukula and Masri, 1984; Eltayb, 2010). Furthermore, the results are consistent with reports by Jain and Foy (1987); van-Hezewij *et al.* (1991) that *Orobanche* species are more detrimental to crops on soils of low fertility. Improving soil fertility decreases *Orobanche* infestation and its suppressive effects on host growth. The reasons for the reduction in parasitism of crop growing in well fertilized soils, however, are not clearly understood (Pieterse, 1991). Several reports showed that fertilizers, mainly phosphorus and nitrogen, lead to significant reductions in infestation of host crops by *Striga*, *Orobanche* and

Phelipanche and the reduced infestation is attributed to reduction in strigolactone production (Cechin and Press 1993; Lendzemo, *et al.,* 2007; Abu-Ramailah, 2008; Hassan, *et al.*, 2009). Furthermore, the results are in line with the hypothesis that nodulation plays a crucial role in *Orobanche* infestation in faba bean due to their high contents of gibberellins and/or that they provide easy routs for haustorium penetration (Parker and Riches, 1993). However, no concrete conclusion could be reached as nodulation was not measured in this study.

Imazethapyr alone, irrespective of rate, or when followed by nitrogen, irrespective of application time, at 78 and 86 DAS suppressed *Orobanche* emergence significantly (Table 4.16). At 78 DAS, *Orobanche* emergence was maximal in the untreated control and in sub-plots treated with nitrogen at planting, 15 and 30 DAS. Imazethapyr at 20 g a.i. fed^{-1}, alone effected excellent suppression (94%) of the parasite. However, it showed slight decrease in efficacy when followed by nitrogen (Table 4.16). Imazethapyr at 30 g a.i. fed^{-1}, alone or when followed by nitrogen effected excellent suppression (over 98%) of the parasite. Furthermore, the herbicide tended to maintain its excellent suppressive effect at 86 DAS. This is could be attributed to prolonged persistence of imazethapyr at the higher rate (Geisel, 2007).

Nitrogen alone had no effect on number of *Orobanche* capsules (Table 4.17). Imazethapyr at 20 g a.i. fed^{-1}, alone showed considerable reductions (28.3%) on number of capsules, albeit not significantly. However, when followed by nitrogen the number of capsules was reduced significantly (43.3-50.2%). Imazethapyr at 30 g a.i. fed^{-1}, alone or when followed by nitrogen, irrespective of application time reduced number of *Orobanche* capsules significantly. However, the highest reduction (85%) was attained when nitrogen was applied at planting (Table 4.17). Reduction in capsules production suggests reduction in seed production.

O. crenata infestation resulted in considerable reduction (21.2%) in number of faba bean leaves, albeit not significantly (Table 4.18). Nitrogen alone increased the number of leaves in comparison to the infested control, albeit not significantly (Table 4.18). This findings are consistent with those obtained in the first season (Table 4.12) and those of Mesa-Garcia and Garcia-Torres (1986) and Eltayb (2010). Imazethapyr at 20 g a.i. fed^{-1}, alone, resulted in number of leaves comparable to the *Orobanche* infested control. However, when followed by nitrogen an increase in number of leaves was observed. The increment was higher when nitrogen was applied at planting and decreased with delayed application time. Imazethapyr at 30 g a.i. fed^{-1}, irrespective of nitrogen treatment, had no adverse effect on faba bean number of leaves. This finding is in agreement with those reported by Jurdo-Exposito *et al.*(1997) and Garcia-Torres *et al.*(1999) that imazethapyr did not affect seed germination and crop growth . Imazethapyr at 30 g a.i. fed^{-1} alone, or followed by nitrogen irrespective of application time showed increase in number of faba bean leaves over infested control, albeit not significantly.

Non of the treatment including, had adverse effect on faba bean height (Table 4.19). The lack of adverse effects of *O. crenata* parasitism which is in line with those obtained by Jain and Foy (1992) validate the results of the first season and substantiate the hypothesis that most of *O. crenata* damage to faba bean during emergence of the parasite. Imazethapyr at 20 g a.i. fed^{-1} alone slightly decreased faba bean height at 45 DAS. However, at 75DAS a non-significant increase in faba bean height over the infested control was attained (Table 4.19). The observed increment in faba bean height, indicates recovery of the treated crop with time. Imazethapyr at 20 g a.i. fed^{-1} when followed by nitrogen, irrespective of application time, had no adverse effects on faba bean height at 45 and 75 DAS. Imazethapyr at 30 g a.i. fed^{-1}, alone, and in combinations with nitrogen had no adverse effect on faba bean height at 45 and 75 DAS (Table 4.19).

Non of the treatments had a significant adverse effect on flowering of faba bean. However, the data showed obvious trends (Table 4.20). *O. crenata* infestation reduced flowering by 33.3%. Nitrogen alone, increased the number of flowers over the infected control, albeit not significantly. Imazethapyr at 20 g a.i.fed^{-}1 alone, increased the number of flowers by 16% over infested control. However, it yielded 22.4% less flowers than the *Orobanche* free control (Table 4.20). The herbicide when followed by nitrogen at sowing, increased the number of flowers by 110 and 40% in comparison to parasite infested and free controls, respectively. Delaying application of nitrogen to 15 and 30 DAS decreased the treatment efficacy, albeit not significantly (Table 4.20). Imazethapyr at 30 g a.i.fed^{-1} alone, effected a slight increase (10%) in number of flowers compared to the infested control, but sustained less number of flowers than the *Orobanche* free control (Table 4.20). However when followed by nitrogen at planting or 15 and 30 days it showed 10-79% increase in number of flowers in comparison to the infested control (Table 4.20).

O. crenata infestation resulted in significant reduction (38.8%) in number of faba bean pods (Table 4. 20). Nitrogen alone, when applied at planting, 15 and 30 DAS increased the number of pods by 63, 68 and 32% over the infested control. Imazethaypr at 20 g a.i. fed^{-1} alone, increased pod number by 79.6% over the infested control. However, the herbicide treatment when supplemented with nitrogen at planting, 15 and 30 DAS resulted in 54, 56 and 71% increase in pod yield in comparison to the infested control. Imazethaypr at 30 g a.i. fed^{-1} alone, increased the number of pods by 84%. However, when supplemented with nitrogen at planting, 15 and 30 DAS the increase in pod number was 86, 102 and 124%, respectively.

O. crenata parasitism showed considerable reduction (27%) in total number of flowers and pods in comparison to the uninfested control (Table 4. 20). Nitrogen

applied at sowing or 15 days later, resulted in total number of flowers and pods comparable to the parasite free control. However, nitrogen applied at 30 DAS, resulted in less total number of flowers and pods than the *Orobanche* free control. Imazethaypr at 20 g a.i. fed^{-1} alone or when followed by nitrogen at planting, 15 and 30 DAS increased the number of pods by 28, 47, 22 and 40%, respectively. The corresponding figures for the herbicide at higher rate were 38, 44.5, 44.8 and 79%, respectively (Table 4.20).

O. crenata infestation had no adverse effect on faba bean 100 seeds weight (Table 4.21). Nitrogen and imazethapyr at 20 g a.i. fed^{-1}, each alone showed non-significant decrease in 100 seeds weight. However, the herbicide when followed by nitrogen 30 DAS showed significant decrease in 100 seeds weight. Imazethapyr at 30 g a.i. fed^{-1} alone and in combinations with nitrogen, at sowing and 15 days later, increased faba bean 100 seeds weight, albeit not significantly (Table 4.21). However, when followed by nitrogen 30 DAS showed insignificant decrease in100 seeds weight.

O. crenata parasitism resulted in significant reduction (46.9%) in faba bean grain yield in comparison to the *O. crenata* free control (Table 4.21). Nitrogen, alone increased faba bean yield in comparison to infested control, albeit not significantly (Table 4.21). Similar results were reported by Kukula and Masri (1984). Zahran (1973) showed that fertilizing faba bean with a high rate of nitrogen (92.5 kg/ha) increased seed yield in comparison to a lower rate (47.6 kg/ha). Imazethapyr at 20 g a.i. fed^{-1} alone or followed by nitrogen increased faba bean yield significantly in comparison to the *Orobanche* infected control. However, Imazethapyr at 20 g a.i. fed^{-1} followed by nitrogen applied at planting effected yield comparable to the *Orobanche* free control. Imazethapyr at 30 g a.i. fed^{-1} alone or followed by nitrogen increased faba bean yield significantly in comparison to the *Orobanche* infected control. However, imazethapyr at 30 g a.i.

fed^{-1} followed by nitrogen applied at planting, outyielded the *Orobanche* free control. These results indicate that imazethapyr has no toxic effect on faba bean (Table 4.21). However, an effect done to early and lasting weed control cannot be ruled out.

These results, (Tables 4.18, 4.19, 4.20 and 4.21) indicate clearly the consistency of performance of the combinations of the herbicide and nitrogen. Furthermore, the results indicate that, in addition to improved performance, nitrogen allows the use of low herbicide rate. Low herbicide rate in addition to economic benefits, safeguard against phytotoxicity and prolonged persistence which may be expected from high rates.

CONCLUSIONS

1. *O. crenata* is widely distributed in the west Berber. Its wide spread could be attributed to its invasive nature, lack of awareness, malpractices and lack of crop rotation and phytosanitary measures.
2. Nitrogen as urea, applied during conditioning or simultaneously with the germination stimulants reduced germination and radicle extension. On the other hand, imazethapyr had inconsistent effects.
3. Nitrogen had no significant effects on early *O. crenata* emergence, but, significantly, suppressed late emergence. The fertilizer increased, slightly, most of the crop growth and yield parameters. Imazethapyr provided good to excellent (68-99%) suppression of the parasite throughout the season. The herbicide increased crop growth attributes. However, its effects were more pronounced and significant on post-flowering growth stages.
4. Contrary to the general belief, that parasitic weeds including *O. crenata* are more damaging to their hosts during the subterranean phase of growth, the results are consistent with a model in which *O. crenata* damage is synchronized with the emergence phase.

REFERENCES

Abang, M. M.; Bayaa, B.; Abu-Irmaileh, B. E. and Yahyaoui, A. (2007). A participatory farming system approach for sustainable broomrape (*Orobanche* spp.) management in the Near East and North Africa. *Crop Protection*, **26**(12): 1723-173.

Abouzeid, M. A. (2009). Fusaric acids content in relation to mycoherbicidal activity of *Fusarium oxysporum* isolates against broorape "*Orobanche crenata*". *Egyptian Journal of Natural Toxins*, **6**: 94-114.

Abu-Irmaielh, B. E. (2008). Integrated *Orobanche* management. In: Labrada, R (ed.). *Progress on Farmer Training in Parasitic Weed Management. Plant Production and Protection Division, Rome*, FAO. Pp. 17- 29.

Abu-Irmaileh, B. E. (1994). Nitrogen reduces branched broomrape (*Orobanche ramosa*) seed germination. *Weed Science,* **42**: 57- 60.

Al-Menoufi, A. O. (1991). Crop rotation as a control measure of *Orobanche crenata* in *Vicia faba* fields. In: Wegmann, K. and Musselman, L. J.(eds.). *Progress in Orobanche Research. Proceedings of the International Workshop on Orobanche Research*. Eberhard-Karls University, Tubingen. Pp 241-247.

Amsellem, Z.; Barghouthi, S.; Cohen, B.; Goldwasser, Y.; Gressel, J.; Hornok, L.; Kerenyi, Z.; Kleifeld, Y.; Kroschel, J.; Sauerborn, J.; Muller-Stover, D.; Thomas, H., Vurro, M., and Zonno, M. (2001). Recent advances in the biocontrol of *Orobanche* (broomrape) species. *Biocontrol,* **46**: 211-228.

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. *Agricultural Technology,* **5**: 201-212.

Babiker, A. G. T. and Hamdoun, A. M.(1983). Factors affecting the activity of ethephon in stimulating seed germination of *Striga hermonthica* (Del.) Benth. *Weed Research*, **23**: 125–131.

Babiker, A. G. T.; Mohamed, E. S. and El Mana, M. E. (1994). *Orobanche* problem and management in the Sudan. In: Pieterse, A. H.; Verkleij, J. A. C. and ter Borg ,S. J. (eds.). *Proceedings of the 3rd international workshop on Orobanche and Related Striga Research*. Royal Tropical Institute, Amsterdam, Pp 672–676.

Babiker, A. G. T.; Ahmed, E. A.; Dawoud, D. A. and Abdella, N. K. (2007). *Orobanche* species in Sudan: History, distribution and management. *Sudan Journal of Agricultural Research*, **10**:107-114.

Bandaranayake, P. C. G. and Yoder, J. I. (2013). Haustorium Initiation and Early Development. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds.). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 61-74.

Berner, D. K.; Cardwell, K. F.; Faturoti, B. O.; Ikie, F. O. and Williams, O. A. (1994). Relative roles of wind, crop seeds, and cattle in dispersal of *Striga* spp. *Plant Diseases,* **78**: 402–406.

Berner, D. K.; King, J. G. and Singh, B. B. (1995). *Striga* research and control a perspective from Africa. *Plant Diseases*, **79**: 652-660.

Bond, D. A.; laws, D. A.; Hawtin, G. C.; Saxena, M. C. and Stephen, J. S. (1985). Faba bean (*Vicia faba* L.). In: Summerfield, R. J. and Roberts, E. H. (eds.). *Grain Legume Crops*. William Collins Sons Co. Ltd. London. Pp 199-265.

Bouwmeester, H. J.; Matusova, R.; Zhongkui, S. and Beale, M. H. (2003). Secondary metabolites signalling in host-parasitic plants interactions. *Current Opinion in Plant Biology*, **6**: 358-364.

Bremner, J. and Krogmeier, M.J. (1989). Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease (biuret/phenylphosphorodiamidate). *Acadimic Science*, **86**: 8185-8188.

Brown, R.; Greenwood, A. D.; Johnson, A. W. and Long, A. G. (1951). The stimulant involved in the germination of *Orobanche minor* Sm. 1. Assay technique and bulk preparation of the stimulant. *Biochemistry* , **48**: 559-564.

Brown, R.; Johnson, A.W.; Robinson, E. and Tyler, G. L. (1952). The *Striga* germination factor.II. Chromatographic purification of crude concentrates. *Biochemistry*, **50**: 596-600.

Butler, L. G. (1995). Chemical communication between the parasitic weed *Striga* and its crop host. A new dimension in allelochemistry. In: Inderjit, K. and Einhellig, F. A. (eds.). *Allelopathy, Organism, Processes and Applications.* American Chemical Society. ACS Symposium Series, Washington, DC. Pp 158–168.

Cechin, I and Press, M. C. (1993). Nitrogen relations of the sorghum *Striga hermonthica* host-parasite association, germination, attachment and early growth. *New Phytologist*, **124**: 681-687.

Chaven, J. K.; Kute, L. S. and Kadam, S. S. (1989). Broad bean. In: *Handbook of World Food Legumes: Nutritional, Processing, Technology and Utilization*. Salunkhe, D. D. and Kadam, S.S. (eds.). vol I. CRC Press, Boca Raton, Fl. Pp 223-245.

Christopher, S. (2011). *Evaluation of Bioactivity of Phytotoxins from Pathogenic Fungi of Orobanche spp*. Ph. D thesis, Agricultural University of Athens, Athens. Pp 283.

Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E. and Egley, G. H. (1966). Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* **154**: 1189-1190.

Cooke, D. (2002). Control of Branched Broomrape. A literature review. *Government of South Australia. Department of Water, Land and Biodiversity Conservation*. Pp 1-38.

Cubero, J. I. (1974). On evolution of *Vicia faba* L. *Theoratical and Applied Genetics,* **45**: 47-51.

Cubero, J. I. (1994). Breeding work in Spain for *Orobanche* resistance in faba bean and sunflower. In: Pieterse, A. H.; Verkleij, J. A. C. and ter Borg, S. J. (eds.). *Proceedings of the Third International Workshop on Orobanche and related Striga Research.* Royal Tropical Institute, Amsterdam, The Netherlands. Pp 465-473.

Dube, M.P. and Olivier, A. (2001). *Striga* gesnerioides and its host, cowpea: interaction and methods of control. *Canadian Journal of Botany*,**79** (10) 1225-1240.

Edwards, W.; Hiron, R. and Mallet, A. I. (1976). Aspects of the germination of *Orobanche crenata* seeds. *Orobanche* Research Project. Pp 105-111.

Eizenberg, H.; Colquhoun, J. B. and Mallory-Smith, C. A. (2003). Variation in clover response to small broomrape (*Orobanche minor*). *Weed Science,* **51**: 759-763.

Eizenberg, H.; Colquhoun, J. B. and Mallory-Smith, C. A. (2005). Predictive degree-days model for small broomrape (*Orobanche minor*) parasitism in red clover (*Trifolium pratense*) in Oregon. *Weed Science*, **53**: 37-40.

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**: 923–927.

Eizenberg, H. ; Hershenhorn, J. ; Ephrath, J.H. and Kanampiu, F. (2013). Chemical Control. In: Joel, D. M., Gressel, J. and Musselman, L. J. (eds). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 415-428.

Ejeta, G. (2005). Integrating biotechnology, breeding, and agronomy in the control of the parasitic weed *Striga* spp in sorghum. In: Tuberosa, R.; Phillips, R. L. and Gale, M. (eds.). *In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution*. Avenue Media, Bologna. Pp 239 - 251.

Eltayeb, A. H. (2010). *Integration of cultural practices for containment and control of Orobanche crenata* Forsk. *in Vicia faba* L. Ph.D thesis, Sudan University of Science and Technology, Sudan. Pp 161.

Elzein, A. E. and Kroschel, J. (2003). Progress on management of parasitic weeds. In: Labrada, R. (ed.). *FAO Plant Production and Protection. Weed Management for Developing Countries. Paper 120 Addendum, Food and Agriculture Organization of the United Nations* , Rome. Pp 109-144.

Fernadez - Aparicio, M.; Andolfin, A.; Evidente, A.; Perez - De-Luque, A. and Rubiales, E. D. (2007). Fenugreek root exudates show species specific stimulation of *Orobanche* seed germination. *Weed Research,* **48**: 163-168.

Foy, C. L.; Jain, R. and Jacobsohn, R. (1989). Recent approaches for chemical control of broomrape (*Orobanche* spp.). *Reviews of Weed Science*, **4**: 123-152.

Galindo, J. C. G.; de-Luque, A. P.; Jorrin, J. and Macias, F. A. (2002). Studies of sesquiterpene lactones as *Orobanche cumana* seed germination stimulants. *Journal of Agriculture and Food Chemistry*, **50**: 1911-1917

Galindo, J. C.; Macias, F. A.; Garcia-Diaz, M. D. and Jorrin, J. (2004). Chemistry of host- parasite interactions. In: Macias, F. A.; Galindo, J. G., Molinillo, J. M. G. and Cutler, H. G. (eds.). *Allelopathy: Chemistry and Mode of Action of Allelochemicals.* CRC Press, Boca Raton. Pp 125- 148.

.

Garcia-Torres, L. (1998). Reflection on parasitic weed control: available or needed technology. In Wegmann, K.; Musselman, L.J. and Joel, D.M. (eds.). *Current problems of Orobanche researches. Proc. of the 4th Int. Orobanche Workshop*. Albena, Bulgaria. Pp 323-326.

García-Torres L. and Mesa-García J. (1991). Status of *Orobanche crenata* in faba bean in the Mediterranean region and its control. In : Cubero J.I. (ed.), Saxena M.C. (ed.). *Present status and future prospects of faba bean production and improvement in the Mediterranean countries*. Zaragoza : CIHEAM, 1991. Pp 75-78.

Garcia - Torres, L. and Lopez - Granados, F. (1991). Control of broomrape (*Orobanche crenata* Forsk.) in broad bean (*Vicia faba* L.) with imidazolinones and other herbicides. *Weed Research,* **31**: 227- 235.

Garcia-Torres, L.; Lopez-Granados, F.; Jurado-Exposito, M. and DiazSanchez, J. (1999). Chemical control of *Orobanche* spp. In legumes: Advances in Parasitic Weed Control at On-farm Level: Achievement and constraints. In: Kroschel, J.; Abderahibi, M. and Betz, H. (eds.). *Joint Action to Control Orobanche in the WANA Region*. Margraf Verlag, Weikersheim, Germany. Pp 239-250.

Gasim, S.; Abdelmula, A. and Khalifa, J. (2011). Analysis of the degree of cross-fertilization and auto fertility and their impact on breeding faba bean (*Vicia faba* L.) cultivars grown in Sudan. *African Journal of Agricultural Research,* **6**: 6387-6390.

Gevezova, M., Dekalska, T., Stoyanov, K., Kostov, K., Batchvarova, R. and Denev, I. (2012). Recent advances in Broomrapes research. *BioScience. Biotechnology*. **1**: 91-105.

Geisel, B. G. (2007). *The phytotoxic effect of ALS inhibiting herbicide combinations in prairie soils*. M.Sc thesis. University of Saskatchewan, Canada Pp 77.

Goldwasser, Y.; Kleifeld, Y.; Plakine, D. and Rubin, B. (1997). Variation in vetch (*Vicia* spp.) response to *Orobanche aegyptiaca*. *Weed Science.,* **45**: 756-762.

Goldwasser, Y.; Eizenberg, H., Golan, S.; Hershenhorn , J. and Kleifeld, Y. (2001). *Orobanche aegyptiaca* control in potato. *Crop Protection*, **20**: 403–410.

Goldwasser, Y. and Kleifeld, Y. (2004). Recent approaches to *Orobanche* management: a review. In *Weed Biology and Management (Inderjit ed)*, Pp 439–566.

Goldwasser, Y.; Meron, J.; Sibony, M.; Sorek, P.; Joseph, E. and Rubin, B. (2011). Spread of weed seeds by cattle manure -is there a danger of infesting farmers' fields with weeds? In: *Third International conference on novel and sustainable weed management in arid and semi-arid ecosystems,* Huesca, Spain, p 5.

Goldwasser, Y. and Rodenburg, J. (2013). Integrated Agronomic Management of Parasitic Weed Seed Banks. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies.* Springer: Verlag. Berlin, Heidelberg. Pp 393-413.

Habimana, S.; Nduwumuremyi, A. and Chinama, D. (2014). Management of *Orobanche* in field crops- A review. *Soil Science, Plant Nutrition*, **14**: 43-62.

Hassan, M. M.; Abdelgani, M. E. and Babiker, A.G.T. (2009). Potential of bacteria strains and nitrogen in reduction of *Striga hermonthica* (Del.) Benth. Infesting Sorghum. *Advances in Environmental Biology*, **3**: 1-9.

Hassan, M. M.; Osman, A. G.; Yagoub, S. A.; Sherif, A. M.; Rugheim, A. M., Mohamed, I. S; Abdel Gani, M. E. and Babiker, A. G. T. (2012). Effects of bacterial strains and chicken manure on *Orobanche crenata* infesting faba bean. *International Journal of Applied Science and Technology*, **2**: 122-129.

Hassan, M. M. and Abakeer, R. A. (2013). Effects of arbuscular mycorrhizal fungi (AMF) and bacterial strains on *Orobanche crenata* Forsk., on faba bean. *Universal Journal of Applied Science* **1**: 27-32.

Heide-Jørgensen, H. S. (2013). Introduction: The parasitic syndrome in higher plants. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds). *Parasitic Orobanchaceae: Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 1-18

Hibberd, J. M.; Quick, W. P.; Press, M. C. and Scholes, J. D. (1998). Can source-sink relations explain the response of tobacco to infection by the root holoparasite *Orobanche cernua*. *Plant Cell and Environment* **21**:333 -340.

Idris, K. I.; Khan, Z. R. and Babiker, A. G. T. (2013). Influence of *Desmodium* species root exudates on *Striga hermonthica* (Del.) Benth. and *Orobanche ramosa* L. Germination. *Persian Gulf Crop Protection*, **2**: 7-14.

Jacobsohn, R. A. (1986). Broomrape avoidence and control: Agronomic problems and available methods. In: ter Borgs, S. J. (ed.). *Proceedings of Workshop on Biology and Control of Orobanche ,* Wageningen, The Netherlands. Pp 18- 24.

Jacobsohn, R.; Ben-Ghedalia, D. and Marton, K. (1987). Effect of the animals a digestive system on the infectivity of *Orobanche* seeds. *Weed Research,* **27**: 87-90.

Jain, R. and Foy, C. (1987). Influence of various nutrients and growth regulators on germination and parasitism of *Orobanche aegyptiaca*. In: Weber, H. C. and Forstreuter, W. (eds*.). Proceedings of the 4th International. Symposium. Parasitic Flowering Plants*. Marburg, Germany: Philipps-University. Pp 427-436

Jain, R. and Foy, C. (1992). Nutrient effects on parasitism and germination of broomrape (*Orobanche aegyptiaca*). *Weed Technology*, **6**: 269-275.

Joel, D. M. (2000). The long-term approach to parasitic weeds control: Manipulation of specific developmental mechanisms of the parasite. *Crop Protection*, **19**: 753–758.

Joel, D. J. (2013). The Haustorium and the Life Cycles of Parasitic Orobanchaceae. In: Joel, D. M., Gressel, J. and Musselman, L. J. (eds). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 25-60.

Joel, D. M.; Back, A.; Kleifeld, Y. and Gepstein, S. (1991). Seed conditioning and its role in Orobanche seed germination, inhibition by paclobutrazol. In: Wegmann, K. and Musselman, L. J. (eds.). *Progress in Orobanche Research. Proceedings of the International Workshop on Orobanche Research. Eberhard- Karls University*, Tubingen. Pp 147-156.

Joel, D. M.; Steffens, J. C. and Mathews, D. E. (1995). Germination of weedy root parasites. In: Kigel, J. and Galili, G. (eds.). *Seed Development and Germination*. Marcel Dekker, New York, USA. Pp567- 597.

Joel, D. M.; Hershenhorn, Y.; Eizenberg, R.; Aly, R., Ejeta, G.; Rich, P.; Ransom, J. K.; Sauerborn, J. and Rubiales, D. (2007). Biology and

Management of weedy Root parasites. *Horticultural Reviews*, **33**: 267-349.

Joel, D. M. and Bar, H. (2013). The Seed and the Seedling. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds.). *Parasitic Orobanchaceae: Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 147-194.

Jurado-Expósito, M.; Garcia-Torres, L. and Castejón-Muñoz, M. (1997). Broad bean and lentil seed treatments with imidazolinones for the control of broomrape (*Orobanche crenata*). *Journal of Agricultural Science, Cambridge,* **129**: 307-314.

Kanampiu FK, Ransom JK, Gressel J (2001) Imazapyr seed dressings forStrigacontrol on acetolactate synthase target-site resistant maize. *Crop Prot* **20**:885–895.

Kasasian, L. (1973a). Miscellaneous observation on the biology of *Orobanche crenata* and the *O. aegyptiaca*. In: Proceedings of the European Weed Research Council, Symposium on Parasitic Weeds. Wageningen, The Netherlands: Malta University Press. Pp 68-75.

Kasasian, L. (1973b). The chemical control of *Orobanche crenata* in *Vicia faba* and the susceptibility of 53 cultivars of *Vicia faba* to *Orobanche crenata*. In: *Proceedings of the European Weed Research Council Symposium on Parasitic Weeds*. Wageningen, The Netherlands: Malta University Press. Pp 224.

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**: 655-664.

Kebreab, E. and Murdoch, A. J. (2001). Simulation of integrated control strategies for *Orobanche* spp. based on a life cycle model. *Experimental Agriculture,* **37**: 37-51.

Khalaf, K. A. (1992). Evaluation of the biological activity of flax as a trap crop against *Orobanche* parasitism of *Vicia faba*. *Tropical Agriculture, (Trinidad)* **69**: 35-38.

Kharrat, M.; Halila, M. H.; Linke, K.H. and Haddar T. (1992). First report of *Orobanche foetida* Poiret. on faba bean in Tunisia. *FABIS Newsl*, **30**: 46-47.

Kharrat, M., Abbes, Z. and Amri, M. (2010). A new faba bean small seeded variety Najeh tolerant to *Orobanche* registered in the Tunisian catalogue. *Tunis Journal of Plant Protection,* **5**: 125-130.

Kotoula-Syka, E. and Eleftherohorinos, I. G. (1991). *Orobanche ramosa* L. (broomrape) control in tomato (Lycopersicon esculentum Mill.) with chlorsulfuron, glyphosate, and imazethapyr. *Weed Research*, **31**:19-27.

Kroff, M. J. and Schippers. (1986). Sumulation of growth of faba bean (*Vicia faba* L.) infested with broomrape (*Orobanche crenata* Forsk.) In: ter Borg, S. J. (ed.) *Proceeding of a workshop on biology and control of Orobanche*. LH/VPO Wageningen, The Netherlands. Pp 70-79.

Kroschel, J. (2001). Atechnical manual for parasitic weed research and extension. Kluwer Academic Publishers, Dordrecht, The Netherlands. Pp256.

Kroschel, J. and Klein, O. (2002). Biological control of *Orobanche* spp. in the Near East and North Africa by inundative releases of the herbivore *Phytomyza orobanchia*. *Biocontrol*, **47**: 245-277.

Kukula, S. and Masri, H. (1984). Integrated cultural practices and chemical control of *Orobanche crenata* in faba bean. In: Parker, C.; Musselman, L. J.; Pohill, R. M. and Wilson, K. A. (eds.). *Proceedings of the Third International Symposium on Parasitic Weeds,* ICARDA, Allepo, Syria. Pp 256-261.

Labrada, R. (2008). Farmer training on parasitic weed management. In: Labrada, R. (ed.). *Progress on Farmer Training in Parasitic Weed*

Management. Plant Production and Protection Division, Rome, FAO. Pp 1- 14.

Labrousse, P.; Arnaud, M. C.; Seryes, H.; Berville, A. and Thalouarn, P. (2001). Several mechanisms are involved in resistance of Helianthus to *Orobanche cumana. Annal Botany*, **88**: 859-868.

Lendzemo, V. W.; Kuyper, T. W.; Matusova, R.; Bouwmeester, H. J. and Ast, A. V. (2007). Colonization by Arbuscular Mycorrhizal Fungi of Sorghum leads to reduced germination and subsequent attachment and emergence of *Striga hermonthica*, *Plant Signalling Behaviour*, **2**: 58–62.

Linke, K. H. (1992). Biology and Control of *Orobanche* in Legumes Crops. PLITS. Magraf Verlag, Weikersheim, Germany. Pp 10- 66.

Linke, K. H. (1999). Status quo of *Orobanche* management: Preventive, cultural, and physical control. In: Kroschel, J. M.; Abderabihi, M. and Betz, H. (eds.). *Advances in Parasitic Weed Control at on-farm Level. Joint action to Control Orobanche in WANA Region*. Margraf Verlag, Weikersheim, Germany. Pp 107-133.

Linke, K. H. and Vogt, W. (1987). A method and its application for observing germination and early development of *Striga* (Scrophulariaceae) and *Orobanche* (Orobanchaceae). In: Weber, H. C. and Fostreuter, W. (eds.). *Proceedings of the 4th International Symposium on Parasitic Flowering Plants*. Marburg, Germany. Pp 501–509.

Linke, K. H.; Sauerborn, J. and Saxena, M. C. (1989). *Orobanche Field Guide*. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, Pp. 42.

Lopez-Granados, F. and Garcia-Torres, L. (1993). Population dynamics of crenate broomrape (*Orobanche crenata*) in faba bean (*Vicia faba*). *Weed Science,* **41**: 563-567.

López-Ráez, J.A.; Matusova, R.; Cardoso, C.;Jamil, M.; Charnikhova, T., Kohlen, W.; Ruyter-Spira, C.; Verstappen, F. and Bouwmeester, H. (2009). Strigolactones: ecological significance and use as a target for parasitic plant control. *Pest Management Science*, **65**: 471-477.

Mamaril, J. C.; Trinidad, L. C. and Palacpac, E. S. (1988). The involvement of plant growth hormones in the nodulation of some tropical legumes. In: Bothe, H.; de Bruijn, F. J. and Newton, W. E. (eds.). *Nitrogen Fixation: Hundred Years After.* VCH publishing, New York. p 465.

Matusova R.; van Mourik, T. and Bouwmeester, H. J. (2004). Changes in the sensitivity of parasitic weed seeds to germination stimulants. *Seed Science Research,* **14**: 335-344.

Matusova, R.; Rani, K.; Verstappen, F.W. A.; Franssen, M. C. R.; Beale, M. H. and Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp are derived from thecarotenoid pathway. *Plant Physiology*, 1**39**: 920-934.

Mauromicale, G.; Restuccia, G. and Marchese, M. (2001). Soil solarization a non-chemical technique for controlling *Orobanche crenata* and improving yield of faba bean. *Agronomy,* **21**: 757- 765.

Mesa-Garcia, J. and Garcia-Torres, L. (1986). Effect of planting date on parasitism of broad bean (*Vicia faba*) by crenate broomrape (*Orobanche crenata*). *Weed Science,* **34:** 544-550.

Miller, P. (1994). The biology and distribution of parasitic plants within the Orobanchaceae: An overview of secondary plant compound involvement. Colorado State University. Fort Collin, Colorado. USA. In: www. Colostate. Edu / Depts / Entomology / coursen / en 570 papers.

Muller- Stover, D.; Thomas, H.; Sauerborn, J. and Kroschel, J. (2004). Tow granular formulations of *Fusarium oxysporum* fsp orthoceras to

mitigate sunflower broomrape (*Orobanche cumana*). *Biological control*, **49**: 595- 602.

Murdoch, A. J. and Kebreab, E. (2013). Germination Ecophysiology. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds.). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 195-219.

Musselman, L. J. (1980). The biology of *Striga*, *Orobanche* and other root parasitic weeds. *Annual Review of Phytopathology*. **18**: 463–489.

Musselman, L. (1986). Taxonomy of *Orobanche.* Proceedings of a workshop on biology and control of *Orobanche,* Wageningen, Netherlands, Pp 2-10.

Musselman, L. J.; Yoder, J. I. and Westwood J. H. (2001). Parasitic plants major problem of food crops, Science 293, 1434.

Nandula, V. K. (1998). *Selective control of Egyptian broomrape (Orobanche aegyptiaca Pers.) by glyphosate and its amino acids status in relation to selected hosts.* Ph. D. Thesis, Virginia Polytechnic Institute and State University. Blacksburg. Pp 121.

Nassib, A. M.; Ibrahim, A. A. and Khalil, S. A. (1982). Breeding for resistance to *Orobanche*. In: Hawtin, G. and Webb, C. (eds.). *Faba bean Improvement. Proceedings of faba bean Conference*, *Cairo, Egypt*. Martinus Nijhoff, The Hague, The Netherlands. Pp 199-206.

Nickrent, D. L. (2002). Plant as parásitas en el mundo. Capitulo. In: J. A. LópezSáez, J. A., Catalán, P. and Sáez, L. (eds.). Plantas Parásitas de la Península Ibérica e Islas Baleares. Mundi-Prensa Libros, S. A., Madrid. pp 7-27.Seen as abstract.

Nickrent, D. L. (2008). Parasitic plants. In: *McGraw-Hill Yearbook of Science and Technology*, Pp 251-253.

Nickrent, D. L. and Musselman, L. J. (2004). Introduction to parasitic flowering

plants. In: The plant health instructor. DOI : 10.1094/ PHI-I-2004-0330-01. Pp 1-7.

Norambuena, H.; Diaz, J.; Kroschel, J.; Klein, O. and Escobar, S. (2001). Rearing and field release of *Phytomyza orobanchia* on *Orobanche ramosa* in Chile. In: Fer, A., Thalouarn, P., Joel, D.M., Musselman, L.J., Parker, C. and Verkleij, J.A.C. (eds.). *Proc. of the 7th Int. Parasitic Weed Symposium,* Nantes, France. Pp 258-261.

Nun, N. B. and Mayer, A. (1993). Preconditioning and germination of *Orobanche* seeds: Respiration and protein synthesis . *Phytochemistry*, **34**: 39-45.

Parker, C. (2009). Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Manaement Science*, **65**: 453–459.

Parker, C. (2013). The Parasitic Weeds of the Orobanchaceae. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds.). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 313-344.

Parker, C. and Riches, C. R. (1993). *Parasitic Weeds of the World: Biology and Control.* CAB International, Wallingford, Oxon, UK. Pp 332.

Pérez-de-Luque, A.; Cubero, J. I.; Rubiales, D. and Joel, D.M. (2001). Histology of incompatible interactions between *Orobanche crenata* and some host legumes. In: Fer, A. et al. (eds.). *Proc. 7th Int. Parasitic Weed Symp.* Pp 174–177. Nantes, France

Pérez-de-Luque, A.; Lozano, M. D.; Cubero, J. I; González-Melendi, P.; Risueño, M. C. and Rubiales, D. (2006). Mucilage production during the incompatible interaction between *Orobanche crenata* and *Vicia sativa*. *Journal of Experimental Botany*, **57**: 931-942.

Pe´rez-Vich, B.; Velasco, L.; Rich, P. J. and Ejeta, G. (2013). Marker-Assisted and Physiology-Based Breeding for Resistance to Root Parasitic

Orobanchaceae. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds.). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 369-390.

Pieterse, A. H. (1991). The effect of nitrogen fertilizers on the germination of seeds of *Striga hermonthica* and *Orobanche crenata*. In: Wegmann, K. and Musselman, L. J. (eds.). *Progress in Orobanche Research, Proceedings of the International Workshop on Orobanche Research.* Eberhard- Karls-University, Tubingen. Pp 115-124.

Plaza, L.; Fernández, I.; Juan, R.; Pastor, J. and Pujadas-Salvà, A. (2004). Micromorphological studies on seeds of *Orobanche* species from the Iberian Peninsula and the Balearic Islands, and their systematic significance. *Annals of Botany,* **94:** 167-178.

Press, M. C. and Graves, J. D. (1995). *Parasitic Plants*. Chapman and Hall, London. Pp 292.

Pujadas-Salvà, A. J. (2002). *Orobanche* L. In: López-Sáez, J. A.; Catalán, P. and Sáez, L. I. (eds.). In: *Medicago tuncatula hand book Root Parasitic Plants and Legumes*. Pp 2 -31.

Ransom, J.K.; Babiker, A.G.T. and Odhiambo, G. D. (2007). Integrating crop management practices for *Striga* control In: Ejeta and Gressel (eds.). Towards Ending the Witch-hunt: *Proceeding of an International Symposium on Integrating new Technologies for Striga Control*. Addis Ababa, Ethiopia, World Scientific Publishing Company, Singapore. Pp 224-239.

Restuccia, A.; Marchese, M.; Mauromicale, G. and Restuccia, G. (2009). Biological Characteristics and Control of *Orobanche Crenata* Forsk., a Review. *Ital. J. Agron. / Riv. Agron*., **1**: 53-68.

Rubiales, D. (2003). Parasitic plants, wild relatives and the nature of resistance. *New Phytol.* **160**:459-461.

Rubiales, D.; Alcántara, C.; Joel, D. M.; Pérez-de-Luque, A. and J. C. Sillero, J. C. (2003). Characterization of the resistance to *Orobanche crenata* in chickpea. *Weed Science*, **51**: 702–707.

Rubiales, D., Sadiki, M. and Román. B. (2005). First Report of *Orobanche foetida* on Common Vetch (*Vicia sativa*) in Morocco. Plant Disease, 89: 528.

Rubiales, D.; Fernández-Aparicio, M.; Pérez-de-Luque, A., Prats, E., Castillejo, M. A., Sillero, J. C., Rispail, N. and Fondevilla, S. (2009b) Breeding approaches for crenate broomrape (*Orobanche crenata* Forsk.) management in pea (*Pisum sativum* L.). *Pest Management Science,* **65**:553–559.

Rubiales, D.; Fernández-Aparicio, M.; Wegmann, K. and Joel, D. (2009a). Revisiting strategies for reducing the seed bank of *Orobanche* and *Phelipanche* spp. *Weed Research*, **49**: 23-33.

Rubiales, D. and Fernández-Aparicio, M. (2012). Innovations in parasitic weeds management in legume crops. A review. *Agronomy for Sustainable Development,* **32**: 433-449.

Runyon, J. B.; Tooker, J. F.; Mescher, M. C. and De Moraes, C. M. (2009). Parasitic Plants in Agriculture: Chemical Ecology of Germination and Host-Plant Location as Targets for Sustainable Control. In: Lichtfouse, E. (ed.). *Organic Farming, Pest Control and Remediation of Soil Pollutants, Sustainable Agriculture Reviews 1*, Springer Science+Business Media B.V. 2009. Pp 123-136.

Saber, H. A.; El-Hady, M.; Khalil, S. A.; El-Sherbeeny, M. H. and Hassan, M. W. (1994). New herbicides for *Orobanche* control in faba bean in Egypt. Biology and Management of *Orobanche*. In: Pieterse, A. H.; Verkleij, J. A. C. and ter Borg, S. J. (eds.). *Proceedings of the Third International*

Workshop on Orobanche and related Striga research. Amsterdam, The Netherlands. (Royal TropicalInstitute). Pp. 572-575.

Saghir, A. R. (1986). Dormancy and germination of *Orobanche* seeds in relation to control methods. In: ter Borg, S. J. (ed.). *Proceedings of a Workshop on Biology and Control of Orobanche.* Wageningen. TheNetherland. Pp 25-34.

Sauerborn, J. (1991). The economic importance of the phytoparasites *Striga* and *Orobanche* and. In: Ransom, J. K.; Musselman, L. J.; Worsham,A. D. and Parker, C. (eds.). *Proceedings of the Fifth International Symposium on Parasitic Weeds*, Nairobi, Kenya. Pp 137-143.

Sauerborn J.; Saxena, M. C. and Mayer, A. (1989). Broomrape control in faba bean (*Vicia fabaL*.) with glyphosate and imazaquin. *Weed Research,* **29**: 97-102.

Sauerborn, J.; Müller-Stöver, D.; Hershenhorn, J. (2007). The role of biological control in managing parasitic weeds. *Crop Protection*, **26**: 246-254.

Schloss, J. V. (1990). Acetolactate synthase, mechanism of action and it sherbicide binding site. *Pesticide Science*, **29**: 283-292.

Schmitt, U. M.; Schluter, K. and Boorsma, P. A. (1979). Chemical control of *Orobanche crenata* in broad beans. FAO. *Plant Protection Bulletin*, **27**: 88- 91.

Schnell, H.; Linke, K. H. and Saueroborn, J. (1994). Trap cropping and its effect on yield and *Orobanche crenata* Forsk. infestation on following pea (*Pisum sativum* L.) crops. *Tropical Science*, **34**: 306-314.

Song, W. J.; Zhou, W. J. ; Jin, Z. L.; Cao, D. D.; Joel, D. M.; Takeuch, Y. and Yoneyama, K. (2005). Germination response of *Orobanche* seed subjected to conditioning temperature, water potential and growth regulator treatments. *Weed Research,* **45**: 64- 67.

Song, W. J.; Zhou, W. J.; JIN, Z. L.; Zhang, D.; Joel, D. M.; Yoneyama, K. and Takeuch, Y. (2006). Growth regulators restore germination of *Orobanche* seeds that are conditioned under water stress and suboptimal temperature. *Australian Journal of Agricultural Research,* **57**: 1195–1201.

Sun, Z. (2008). *Biosynthesis of Germination Stimulants of Parasitic Weeds Striga and Orobanche.* Ph.D. Thesis Wageningen University. The Netherlands, pp 117.

Tan, S.; Evans. R. R.; Dahmer, M. L.; Singh, B. K. and Shaner, D. (2005). Imidazolinone-tolerant crops: history, current status and future. *Pest Management Science*, **61**: 246-257.

ter Borg, S. J. (1986). Effect of environmental factor on *Orobanche*-host relationships, a review on some recent results. In: ter Borg, S. J. (ed.). Proceedings of a Workshop on Biology and Control of Orobanche. Wageningen. The Netherland. Pp 57-69.

Uematsu, K.; Nakajima, M.; Yamaguchi.; Yoneyama, K. and Fukui, Y. (2007). Role of cAMP in gibberellin promotion of seed germination in *Orobanche minor* Smith. *Plant Growth Regulation*, **26**(3): 245-254.

van Hezewijk, M. J.; Linke, K. H.; Verkleij, J. A. C. and Pieterse, A. H. (1991).The effect of ammonium fertilizer in combination with nitrification inhibitors on *Orobanche crenata* infestation in faba bean. In: Ransom, J. K.; Musselman, L. J.; Worsham, A. D. and Parker, C.(eds.) *Proceedings of the Fifth International Symposium on Parasitic Weeds*. CIMMYT, Nairobi, Kenya. Pp 470-480.

van Hezewijk, M. J.; van Beem, A. P.; Verkleij, J. A. and Pieterse, A. H. (1993). Germination of *Orobanche crenata* seeds, as influenced by conditioning temperature period. *Canadian Journal of Botany*, **71**: 786-792.

van Hezewijk, M. J. and Verkleij, J. A. C. (1996). The effect of nitrogenous

compounds on in vitro germination of O. crenata Forsk. Weed Research, 36: 395- 404.

Westwood, J. H. and Foy, C. L. (1999). Influence of nitrogen on germination and early development of broomrape (*Orobanche* spp.). *Weed Science*, **47**: 2-7.

Westwood, J. H.; Yoder, J. I.; Timko, M. P. and dePamphilis, C. W. (2010). The evolution of parasitism in plants. *Trends Plant Science,* **15**: 227–235.

Wigchert, S. C. M.; Kuiper, E.; Boelhouwer, G. J.; Nefkens, G. H. L.; Verkleij, J. A. and Zwanenburg, B. (1999). Dose-response of seeds of the parasitic weed *Striga* and *Orobanche* towards the synthetic germination stimulants GR 24 and Nijmegen 1. *Journal of Agricultureand Food Chemistry,* **47**: 1705-1710.

Wilhelm, S.; Srokan, R. C. E.; Benson, R. C.; Sagen, J. E. and Carpenter, T. (1959). Large scale soil fumigation against broomrape. *Phytopathology*, **49**: 530–532.

Yokota, T.; Sakai, H.; Okuno, K.; Yoneyama, K. and Takeuchi, Y. (1998). Alectrol and orobanchol, germination stimulants for *Orobanche minor*, from its host red clover. *Phytochemistry,* **49**: 1967–1973.

Yoneyama, K.; Ruyter-Spira, C. and Bouwmeester, H. (2013). Induction of Germination. In: Joel, D. M.; Gressel, J. and Musselman, L. J. (eds.). *Parasitic Orobanchaceae. Parasitic mechanisms and control strategies*. Springer: Verlag. Berlin, Heidelberg. Pp 167-194.

Young, N. D.; Steiner, K. E. and de Pamphilis, C. W. (1999). The evolution of parasitism in Scrophulariaceae/Orobanchaceae: Plastid gene sequences refute an evolutionary transition series. *Ann. Missouri Bot. Garden,* **86**: 876-893.

Zabalza, G.; González, E. M.; Aparicio-Tejo, P. M.; Royuela, M. and Igor, A. (2002). Imazethapyr, an inhibitor of the branched-chain amino acid

biosynthesis, induces aerobic fermentation in pea plants. *Physiologia Plantarum*, **114**: 524-532.

Zehhar, N.; Labrousse, P.; Arnaud, A. C.; Boulet, C.; Bouya, D. and Fer, A. (2003). Study of resistance to *Orobanche ramosa* in host (oilseed rape and carrot) and non-host (maize) plants. *European Journal of Plant Pathology*. **109**: 75-82.

Zahran, M. K. (1973). Control of broomrape in field and vegetable crops in Egypt, Final Technical Report, Agricultural Research Program. Pp 4-40.

Zermane, N.; Souissi, T.; Kroschel, J. and Sikora, R. (2007). Biocontrol of broomrape *Orobanche crenata* Forsk. and *Orobanche foetida* Poir. by *Pseudomonas fluorescens* isolate Bf 7-9 from faba bean rizosphere. *Biocontrol Science and Technology*, **17**: 483- 497.

APPENDICES

Appendix I

Orobanche crenata Survey (Questionnaire for participants)

1. Age……

2. Educational level……

3. Total area of the field……

4. Type of ownership?

a) Owner ( ) b) Partner ( ) c) Lease ( )

5. When did start farming…….

6. Sowing date ……

7. Land flooding? a) Yes ( ) b) No ( )

8. Annual flooding? a) Yes ( ) b) No ( )

9. Crop rotation? a) Yes ( ) b) No ( )

10.Crop grown in the rotation?

a) Summer crops 1)……..2)…….3)

…….

b) Winter crops

1)……..2)…….3)…….

11.When did you first learn about *O. crenata* as a parasite in faba bean?

a) One year ago ( ) b) Two years ago ( ) c) Three years ago ( )

12.When did notice *Orobanche* infestation in your field?

a) One year ago ( ) b) Two years ago ( ) c) Three years ago ( )

13. Time the broomrape emerges in relation to crop growth stage?

a) Before flowering ( ) b) With flowering ( )

c) After flowering ( ) d) during flowering to harvest ( )

14. Where was *O. crenata* first observed?

a) Field border ( ) b) In the form of spots all over the field ( )

15. Assessments of the affected area in the field?

a) All the field ( ) b) Three-quarters of the field ( )

c) Half of the field ( ) d) Quarter of the field ( )

16. Location of the infection in the field?

a) Field border ( ) b) Centre of the field ( ) c) All over the field ( )

17. *O. crenata* impact on land productivity farmer opinion?

a) Has an impact ( ) b) Has no impact ( )

18. Farm productivity before infection? ……

19. Farm productivity after infection? ……

20. Are there changes in cropping system due to *O. crenata* infection?

a) Yes ( ) b) No ( )

21. What were the crops that had been introduced?

a)……b) ……c) ……..

22. Was the land abandoned after infestation by the parasite?

a) Yes ( ) b) No ( )

23. What are the control methods used by the farmer?

a)……b) …….c) ……..

24. How are crop residues in *O. crenata* affected area managed?

a) Grazing ( ) * by farm animals ( )* by animals from other farms ( )

b) Burned ( ) c) Other methods ( )

25. Seed source?

a) Own farm ( ) b) Neighbouring farm ( ) c) Other sources ( )

26. Use of Organic manure?

a) Yes ( ) b) No ( )

27. Manure source?

a) Own farm ( ) b) Neighbour farm ( )

28. Do you use your own farm machinery or do you share these with others?

ii

……

29. How do you get rid of broomrape plants after hand-pulling?

a) Throwing in the river ( ) b) Throwing onto adjoining roads ( )

c) Pile in the farm ( )

30. How does *O. crenata* spread in your opinion?

a) Crop seeds ( ) b) Agricultural machinery ( )

c) Grazing ( ) d) Water ( )

Appendix II

Orobanche crenata spikes showing stunting, malformed growth and eventually death following treatment with imazethapyr at 30 g a.i. fed^{-1} 45 DAS.

Imazethapyr treated *O. crenata* spike

Untreated *O. crenata* spike

Imazethapyr treated *O. crenata* spike

Appendix III

GR24 concentrations calculation

GR24 10ppm (S. Solution)

100 µL (S. Solution) +900 ml (DW) = 1ppm

100µL from (1)+900µL(DW) = 1000 µL = 0.1ppm

Appendix IV

Imazethapyr concentrations calculation

Molecular formula C15H19N3O3

Molecular weight 289.3

289.3 g in 1L =1M

289.3 mg in1L =1mM

28.93 mg in 100ml =1mM

2.89mg in 10ml =1mM

0.29mg in 10ml =0.1mM

Formulation 10% 0.29/0.1 = 2.9 mg

29 ul in 10 ml water =1mM (S. Solution)

1ml from (S. Solution) +9ml (DW) =10 =0.1mM = 100uM

H2O	S. Solution	Concentration
2ml	8ml	80 µM
4ml	6ml	60 µM
6ml	4ml	40 µM
8ml	2ml	20 µM
9ml	1ml	10 µM
DW	Control	

Appendix V

Urea concentrations calculation

Molecular formula CH_4N_2O

Molecular weight 60

60g/L =1M

120g/L =2M

120mg/L =2mM

12mg/100ml =2mM

1.2g/100ml = 200mM

1200mg/100ml =200m M

$1m^2$ = 0.952 X 10^4 mg/m^2

1 petri dish = 60.5 mg = 60mg/5ml water

=12mg/ml water = 1N =200 mM

2N = 2.4g /100ml

1N = (10 (2N) +10ml water) = S. Solution = 200 mM

H2O	S. Solution	Concentration (mM)
1.100 µL	900 µL	90
1.200 µL	800 µL	80
1.300 µL	700 µL	70
1.400 µ	600 µL	60

1.500 µL	500 µL	50
1.600 µL	400 µL	40
1.700 µL	300 µL	30
1.800 µL	200 µL	20

Appendix VI

Control methods adopted by farmers

Method of control	Frequency	%
Hand-pulling	23	46
Chemical	3	6
Non	24	48
Total	50	100