JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

UNDERGROUND DEVELOPMENT OF *OROBANCHE CRENATA* ON FABA BEAN UNDER POT EXPERIMENT AND FIELD CONDITION

[1]Mekonnen Misganaw Desta, [2]Wassu Mohammed Ali, [3]Seid Ahmed Kemal and [4]Fouad Maalouf
[1]Researcher in Agriculture (MSc), [2]University Lecturer (Prof), [3]Researcher in Agriculture (PhD) and [4]Researcher in Agriculture (PhD)
[1]Department of Crop Improvement
[1]Amhara Regional Agricultural Research Institute, Sirinka Agricultural Research Center, Bahir Dar, Ethiopia

ABSTRACT

In Ethiopia, faba bean is a food security crop for mid and highlands. It has high importance as a source of food, feed and cash crop, sustain the cropping systems and soil fertility. Even though faba bean plays an important role, its yield below the world average due to biotic factors mainly *Orobanche crenata*. *Orobanche crenata* weed development and damage of its host are underground. The objectives of the study were: identified underground weed development (tubercles development) under field and pot experiments. Pot experiment was conducted in June to November, 2020 on Dessie in green house using 4 faba bean cultivars and 3 rates of weed seeds (2, 4 and 6 mg per pot) on CRD factorial with three replications. Field experiment was conducted in the same season and same faba bean cultivars to pot experiment on Kutaber *woreda* Addisalem locality naturally infected soils on RCBD. Nine parameters on both experiments were recorded. The different tubercle development stages' days on both experiments, showed shorter for susceptible than resistant cultivar (*Ashenge*). *Orobanche* capsule flowering and capsule maturity days showed shorter for susceptible than resistant cultivar. *Orobanche* dry weight had great weight in resistant cultivar than susceptible because resistant cultivar can support the weed for longer time than susceptible cultivar. As recommendation, chemical application will be applied by counting the days according to the cultivars' response starting from wet sowing of faba bean. For future works, researches should be verified the exact application time based on the results of the experiment.

Keywords: Cultivar, Haustorium, Resistant, Susceptible, Tubercle

Introduction

Faba bean has great potential to alleviate malnutrition for the resource-poor farmers. In Ethiopia, it is the principal legume for food, sustain the cropping systems and soil fertility in the mid and highlands of the country CSA (2022).

Faba bean is a temperate grain legume of high importance as human food and animal feed and it is one of the most important cool season crops in the highlands of Ethiopia and the country is considered as the secondary center of diversity. In Ethiopia, faba bean is a food security crop that is predominantly grown in the mid-altitude and highland areas as a multi-purpose crop and leads the pulse category in terms of area and production CSA

(2022). Besides its contribution to food and nutrition security in the households, it plays an important role in management of soil fertility through crop rotation in cereal production hence contributing to agricultural sustainability Agegnehu and Fessehaie (2006). It is also a foreign currency earner for the national economy. Ethiopia is the fourth largest exporting country of faba bean next to France, Australia and United Kingdom FAO (2022).

Even though faba bean plays an important role for Ethiopian farmers, its yield below the world average due to several factors such as poor crop management practices, biotic and abiotic factors, lack of high yielding cultivars and poor soil fertility are some of the causes of low yield CSA (2022).

Orobanche crenata Forskal is considered indigenous in the Mediterranean basin Rubiales *et al*. (2006). *Orobanche* is a parasitic plant found in the genus *Orobanche* and family *Orobanchaceae,* which have about 140 species of *Orobanche*, which attack cultivated crops, wild plant species, and weeds. Most species of *Orobanche* have multiple hosts, and one crop host can be infected by multiple species of *Orobanche* and crop yield loss varies from crop to crop depending on the severity of *Orobanche* infestation Das *et al*. (2020).

Orobanche genus is native to the Mediterranean region (North Africa, the Middle East, and southern Europe) and western Asia where large areas ranging from 5-100% infestation have been recorded Besufekad *et al.* and Nickrent and Musselman (1999; 2016). Their range extends to similar climates in Asia, Africa, Australia, and North-South/America where they also cause significant crop damage. The most important *Orobanche* species that highly invasive for many crop species is *Orobanche crenata* (bean broomrape) which attacks faba bean, field pea, lentil, chickpea, vetch, clover, carrot, parsley, celery, cumin, safflower, Carthamus, eggplant, tomato, lettuce, geranium and verbena. The crop damage reaches up to 100% in the highly infested environments (Sharawy and Karakish, Besufekad *et al.* and Mekonnen (2015; 1999; 2016).

The interaction between host and parasite begins with the secretion of secondary metabolites from the roots of the host plant that induce the germination of the parasite seeds Fernandez-Aparicio *et al*. (2014). The first stage of the infection process is the germination and chemical guidance of the seedling (chemotropism) towards the host root because *Orobanche* species require the presence of germination stimulants exuded from the host root to germinate and locate the host root Fernandez-Aparicio *et al*. (2016). *Orobanche* spend most of their life cycle underground, where they go through a lot of processes such as; germination, development of radicle, haustorial differentiation from the radicle, haustorial penetration of the host, formation of vascular connection with the host (establishment of a xylem connection), acquisition of host nutrients, and storage of resources in a parasite organ called the tubercle or nodule Fernandez-Aparicio *et al*. (2011). After these stages it emerges above ground and rapidly flowers and then produces seeds Das *et al*. (2020).

Germination of *Orobanche* seeds accompanied by exudates of chemical induction from the host-root and leads to the development of a root-like organ, known as the germ tube or procaulome. *Orobanche* seed develops a small seedling that attaches to the host root and differentiates in the attachment organ-the appressorium. On contact with the host root, the radical adheres to the surface by sticky papillae and penetration is facilitated by separation of the host cells, caused by enzymatic activity. Subsequently a connective organ, the haustorium, develops between host and parasite, with cells from each species playing part in the junction Fernandez-Aparicio *et al*. (2011). Once the haustorium has reached the host stele, its' cells penetrate the host vessel members through their pits. These cells then open at their tips and lose their cytoplasm Das *et al*. (2020). Adjacent cortical cells progressively differentiate into xylem elements until a continuous water conducting system is established linking the host and parasite vascular systems Das *et al*. (2020).

The connecting organ, so called haustorium, serves as a bridge for water and nutrient uptakes from the host to the parasite. The development of the Xylem Bridge or haustorium is absolutely dependent upon direct contact with the host stele Fernandez-Aparicio *et al*. (2011). After the attachment to the host root, it invades the host root and intimately connects the vascular tissues of the host root. Once attached to the host root, the outer part in the connection zone develops into a tubercle which gives rise to a spike under favorable conditions Abbes *et al*. (2007b). Subsequently the parasite has developed a haustorium and established vascular connections with the host, it becomes one with the plant, acting as a sink for water and nutrients. This connective structure swells and forms a nodule that after one to two weeks differentiates into a tubercle with shoot bud, and finally flowering shoot.

Breeding techniques for *Orbanche* resistance are conventional and molecular breeding approaches. Faba bean is an important legume crop since its high-yielding potential, nutritional purpose and soil improvement.

There are significant achievements in faba bean improvement in the last four decades, which led to the doubling of the global yield average Maalouf *et al*. (2018). Yield increasing can be obtained through the varietal development in open pollinated situation of high yielding varieties. In the past, many faba bean varieties that are tolerant to biotic stresses like *Oobanche* and abiotic stresses like water logging were released worldwide and the average yield gains varied from 1.65% per year in Syria to 4.17% per year in Ethiopia Maalouf *et al*. (2018).

Conventional plant breeding plays a great role in developing resistant and /or tolerant varieties for abiotic and biotic stresses in different crops including faba bean, but due to the selection process takes very long time and close host-parasitic relationship, developing *Orobanche* resistance genotypes is very difficult Farooq and Azam (2002). Similarly, in quantitatively inherited traits which are controlled by many genes with small effects and influencing by environment factors, selection using conventional methods has slow progress Gebisa and Gressel (2007). Molecular marker-based breeding on DNA analysis is a modern approach based on proper assessment of closely related plant species could be determined. Restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), micro-satellite or simple sequence repeat (SSR) and random amplified polymorphic DNAs (RAPDs) are the most commonly used markers Tanee *et al*. (2009).

There are also many applicable management practices to manage *Orobanche* weed such as chemical control (using herbicides), physical control (soil solarization), growing environment maintain (Maintain of soil fertility) and integrated *Orobanche* managementpractices Das *et al*., Kumar *et al*. and Mekonnen (2020; 2012; 2016).

The most important *Orobanche* species and their hosts are as follows:

Orobanche aegyptiaca (Egyptian broomrape): Tomato, potato, tobacco, eggplant, bell-pepper, pea, vetch, faba bean, carrot, celery, parsley, cumin, cabbage, cauliflower, rape, mustard, turnip, hemp, sunflower, spinach. In some areas, e.g. southern Russia, sweet melon water melon and ornamental plants like Chrysanthemum and Gazania are its hosts.

Orobanche ramosa (branched broomrape or hemp broomrape): Hemp, potato, tobacco and tomato; also on groundnut, cowpea and chilli pepper. O. ramosa was also recorded on aniseed, basil, fennel, and a range of ornamental species.

Orobanche crenata (bean broomrape): An important pest of legumes: faba bean, pea, lentil, chickpea, vetch and clover. Also attacks carrot, parsley, celery, cumin, safflower, eggplant, tomato, lettuce, geranium and verbena.

Orobanche cernua (Nodding broomrape): Attacking solanaceous: tomato, potato, tobacco and eggplant.

Orobanche cumana (sunflower broomrape): An important pest of sunflower, also parasitizing safflower. It may in certain cases parasitize tomato, when growing in soil previously cropped with sunflower.

Orobanche minor (Common broomrape): It attacks red and white clover, Lucerne, tobacco, carrot, lettuce, sunflower and many other crops and ornamentals on a local basis (Sharawy and Karakish, 2015).

Orobanche are holoparasites, devoid of chlorophyll and totally dependent on the host for organic carbon, water and nitrogen Daniel *et al*. (2011). The infection and pathogenesis processes take place underground, damage to the crop occurs prior to the diagnosis of infection and emergence of the shoot. The particular characteristics of this pathogenic weed such as underground development and attachment to the host roots, hamper the development of effective control strategies Joel *et al*. (2007). As the damaging is underground, any control or management practices can be applied before the weed emerged above ground Mekonnen (2016). The damage depends on the levels of environmental infestations and environmental factors such as soil types, soil fertility, soil water holding capacity, rainfall, etc. The level of crop damaged in Ethiopia in terms of both yield and quality ranged from 5 t*o* 100% Besufekad *et al*. (1999). Tubercles development of *Orobanche* weed was studied using growth chamber Abbes *et al.* (2011), and in laboratory using Petri dish Lemma *et al*. (2018), but there was no research result on tubercle development under natural environment. The natural environmental conditions are different from growth chamber and laboratory, damaging in the field and any management practices can be applied in the field before the weed grow above ground. This research is therefore conducted to determine underground tubercles development and parasitism stages of *Orobanche crenata* on faba bean genotypes with varied weed seed rates under pot and natural infestation at field conditions.

Materials and Methods

Experimental site descriptions

Pot experiment was conducted in June to November, 2020 in Dessie in green house (control environment to support field experiment). Dessie is located 401 km distance North of Addis Ababa on the high way to Mekelle. It is situated in latitude 11^0 06'N, longitude $39^0$38'E and an average altitude is 2475 meter above sea level Wagaye *et al*. (2020). The area has a bimodal rainfall distribution with mean annual rainfall ranged from 851.3 to 1612.6 mm. The mean maximum temperature is 19.3^0C while the mean minimum annual temperature is 14.8^0C and soil texture is sandy loamy to clay (Cambisols) which is neutral to weakly acidic with pH value 5.6-6.0 Bichaye, *et al*. and Wagaye *et al*. (2022; 2020). While field experiment was conducted in the same year to the pot experiment in Kutaber *woreda* Addisalem locality naturally infected soils (hotspot) at farmers' field. Kutaber *woreda* is located in Amhara Regional State in north-central Ethiopia, South Wollo Zone. It is 495 km east from Bahir Dar, 419 km north from Addis Ababa, and 18 km north from Dessie. It is surrounded by Dessie zuria *woreda* in the South, Tenta *woreda* in the West, Ambassel *woreda* in the North and Tehulederie woreda in the East. The geographic location extends from 39°18′ E to 39°38′ E Latitude and 11°8′ N to 11°29′ N Longitude and an elevation is ranged from 1400 to 2850 meter above sea level. The woreda has a mean annual rainfall 1110.57 mm, the mean maximum temperature 23.13 ^{0}C, while the mean minimum annual temperature is 6.56^0C and the soil type is Cambisols Bichaye, *et al*. and Nuru, [6, 25).

Experimental Materials for the two Experiments

The experimental materials included three improved faba bean cultivars namely; *Ashenge*, *Dida'a* and *Obse* reported as resistant, moderately resistant and moderately susceptible, respectively Lemma (2018) and highly susceptible farmers' cultivar as check (Table 1).

Table 1. List of experimental materials used for pot and field experiments in 2020 in Dessie town and Kutaber *woreda* northeast Ethiopia.

no	Varieties	Pedigree	Breeding Center	Yield at research (t/h)	Year of released	Altitude (m.a.s.l)
1	*Ashenge*	(ILB 4358)	AARC	1.4-3.5	2014	2400-2446
2	*Did'a*	ICB2717-1Xr-878-3	HARC	2.3-5	2014	1900-2800
3	*Obse*	(EH95073-1)	HARC	2.-6	2007	1900-2800
4	Farmers' cultivar	-	-	-	-	

Note; AARC = Alemata Agricultural Research Center, HARC = Holeta Agricultural Research Center and m.a.s.l. = meter above sea level.

Orobanche crenata seeds used for pot experiment were collected in 2019 cropping season fully matured weed seeds from Kutaber *woreda* Addisalem locality of naturally weed infected farm (hotspot). The soil free from *Orobanche crenata* weed were collected around Dessie farm (no history of faba bean cultivation and it was expected the absence of the parasite weed in the soil) which has similar soil texture with that of *O. crenata* infested soil in Kutaber *woreda*.

Treatments and Experimental Design

The pot experiment consisted of four faba bean cultivars and three rates of *Orobanche.crenata* weed seeds (2, 4 and 6 mg per pot) and a total of 12 treatments in factorial arrangement. The rates of *Orobanche crenata* seeds were determined as suggested by Zouhaier *et al.* (2018) and Trabelsi *et al.* (2016). It was laid out in completely randomized design (CRD) with three replications. The capacity of the pot was 40 cm x 40 cm x 40 cm size (20 liters volume) and assigned for one treatment in each replication where 8 faba bean seeds were placed at 10 cm spacing. The field experiment was consisted of the same number and similar cultivars to that of pot experiment. It was laid out in randomized completely block design (RCBD) with four replications. The plot size was 3 m x 2.40 m = 7.20 m^2 (6 rows and 3 m length). Spacing were 0.4 m between rows, 0.1 m between plants, 0.6 m between plots and 0.8 m between replications.

Experimental Procedures and Managements

The experimental plant materials were collected from Alemata Agricultural Research Center (*Ashenge*), from Holeta Agricultural Research Center (*Dida'a* and *Obse*) and susceptible faba bean from farmers. The *Orobanche* weed seeds were collected by pullout the weed stem holding vertically then put in to a paper bag and bring to Dessie then dried on the sun. After dried the seed were separated from the capsule inside the paper bag then weighing the seeds using sensitive balance according to the rate proposed (sensitive balance sometimes referred to as a top-loading balance and can weighing to the nearest 0.001-1 g). *Orobanche crenata* weed free soils were collected from farmer's field around Dessie which has similar soil texture with that of *Orobanche crenata* infested soils in Kutaber *woreda* Addisalem locality then the soil were filled on the pots up to 5 cm below the doorway of the pots.

Treatment combination and randomizations were arranged on the paper then the pots were labeled with treatments' name and randomly placed in a distance of 0.5 m between pots. *Orobanche* weed seeds were placed on each pot according to treatments' labeled and mixed thoroughly with stick downward up to 5 cm depth then faba bean seeds were planted in eight holes marked at 10 cm distance between holes and two seeds were placed per hole at 5 cm depth to maintain eight plants per pot. One well established seedling at each hole was maintained after the emergence of seeds and the seedlings produce 2 leaves, thus the eight seedlings in each pot were used to collect the data. Water was applied at field capacity for each pot every 5 days throughout the experimental period by hand using a hose. Other inputs like fertilizer were not applied and weeds other than *Orobanche* crenata were removed by hand as they appeared. The field experimental plant materials were the same as pot experiment and it was conducted at Kutaber woreda Addisalem locality naturally infected soils (hotspot) at farmer's field which was well prepared and sowing was applied at effective rainfall. Fertilizer was not applied and non-parasitic weeds were removed three times using hand.

Data Collection

Disturbance sampling (randomly up rooted) method was used three times at flower starting, pod setting and maturity stages of faba bean. Two plants per pot were up rooted from the pot and recorded the days of the tubercle development stage according to Abbes *et al*. (2007a) stated. The pot data collecting were supported by field data, because in the field there were extra plants used for uprooting in three days interval, therefore, after field data recording the pot was also checked without disturbing the seedlings within the same date (only soils disturbed around the host).

Nine characters were recorded such as:

- Haustrium attachment days: these data were indirectly recorded in such that after germination of the weed within 2-3 days haustrium should be attached then 3-4 days small tubercles develop therefore, to get haustrium attachment days back counting of about 3-4 days from tubercle development days.
- Stage1-tubercle development days: these days were recorded when small tubercles without root development were appeared.
- Stage2-tubercle development days: these days were recorded when tubercles with crown roots without shoot formation were appeared.
- Stage3-tubercle development days: these days were recorded when tubercles with root and shoot formation were appeared.
- *Orobanche* spikes emergence days: these days were recorded by counting the days from wet planting of faba bean to *Orobanche* emerging above ground.
- *Orobanche* capsule flowering days: Days from sowing to *Orobanche* capsules 50% flowered.
- *Orobanche* capsule maturity days: Days from sowing to *Orobanche* capsules 90% matured.

- *Orobanche* numbers per host plant: the number of *Orobanche* grown per pot or per plot counted and divided by the number of plants on the pot or plot.
- *Orobanche* dry weight per *Orobanche* plant (ODWtPP) in (g): five *Orobanche* plants taken and recorded the average after 80^{o}c oven dried for 48 hours.

For the field experiment, the data were similar to pot experiment data, but in the field the disturbance sampling was applied randomly up rooted (seedlings together with surrounding mud in 10 cm diameter with the help of pointed knife) five plants per plot per sampling date with three days' interval starting 40 days after wet sowing of the faba bean up to the weed spikes emergence date, then recorded the days, number of *Orobanche* were recorded by counted five host plants' *Orobanche* from the plot and taken the mean, *Orobanche* dry weight were recorded by taken five plants from the plot randomly and recorded the mean after 80^{o}c oven dried for 48 hours, capsule flowering and maturity were similar recording system to the pot experiment.

Data Analysis

The analysis of variance (ANOVA) was carried out to dissect the variation of the cultivars and traits into sources attributable to genotype and error using Gen Stat 18th Edition Paul (2001) with the model developed by Yinglu and Ji-Qian Fang (2003). Means and coefficient of variation was used in order to compare variation between varieties or traits. Mean comparisons were made using Duncan's Multiple Range Test (DMRT) at $P<0.05$ probability level Steel and Torrie (1980).

Table 2. An outline of analysis of variance for complete randomized design for pot experiment.

Source of variation	Degree of freedom	Mean square	Expected mean variance
Treatment	t-1 = 11	MSt	σ^2g
Error	(r - l) x (ab - 1) = 22	MSe	σ^2e
Total	rab – I = 35		

Note, Replication is not considered as source of variation in CRD

The statistical model for the randomized complete block design Federer (1956) was as indicated below;

$Y_{ij} = \mu + g_j + cj + \beta j + \epsilon_{ij}$

Where; Y_{ij} = observation of treatment i in the i^{th} genotype and the j^{th} block,

μ= general mean,

g_j = effect of test treatment,

cj = effect of control treatments in a i^{th} block,

βj = block effects and

ϵij = error

Table 3. An outline of analysis of variance in randomized complete block design for field experiment.

Source of variation	Degree of freedom	Mean square	Expected mean variance
Treatment	t-1	MSg	$\sigma^2 e + g\sigma^2 r$
Replication	r-1	MSr	$\sigma^2 e + r\sigma^2 g$
Error	(t-1)(r-1)	MSe	$\sigma^2 e$
Total	tr-1		

Where; $\sigma^2 e$ = error mean square, r = number of replication, g = number of genotypes, MSr = mean square of replication, MSg = mean square of genotypes, MSe = mean square of error, $\sigma^2 g$ = genotypic variance, $\sigma^2 r$ = variance of replication and $\sigma^2 e$ = variance of error (genotype mean square - error mean square)/r.

Results and Discussions

Analysis of Variance

The analysis of variance for growth and development of different *Orobanche* weed parameters in the pot revealed non-significant differences between seed rate, significant (P<0.05) differences between cultivars and highly significant (P<0.01) differences for weed seed rate and cultivar interactions and the results were presented in (Table 4).

Table 4. Mean squares from analysis of variance for growth and development of *Oroanche* weed characteristics as influenced by interaction of cultivars and seed rates of weed for pot experiment conducted in 2020 in Dessie.

***Oroanche* weed characteristics**	**Source of variation**					
	Treatment (11)	**Cultivar (A) (3)**	**Seed rate (B) (2)**	**A x B (6)**	**Error (22)**	**CV (%)**
Days to haustrium attachment (HAD)	56.88*	59.21*	7.86ns	72.05**	19	8.4
Stage1- small tubercles without root development days (S1TD)	77.87**	69.52*	8.11ns	105.30**	20.25	8.1
Stage2-tubercles with crown roots but not shoot development days (S2TD)	103.29**	86.22*	5.53ns	144.42**	20.61	7.6
Stage3- tubercles with root and shoot formation days (S3TD)	138.63**	119.58**	4.86ns	192.75**	21.69	7.4
Days to weed spikes emergence (OSED)	171.36**	130.19*	0.75ns	248.82**	34.33	8.3
Days to *Orobanche* weed capsule flower (OCFD)	190.88**	149.21*	7.69ns	272.77**	35.31	7.6
Days to *Orobanche* weed capsule maturity (OCMD)	319.97**	217.51**	1.68ns	477.23**	21.08	5.1
Number of *Orobanche* weeds per plant (ONPP)	21.66**	48.99**	0.36ns	15.10**	0.81	9.6
Dry weight of *Oroanche* weeds per plant (g) {(ODwtPP (g)}	23.16**	24.18**	0.58*	30.18**	0.58	8.3

Note: ns, *and **, non-significant, significant at P<0.05 and P<0.01, respectively, number in parenthesis represented degree of freedom, Seed rate = *Oroanche* weed seeds in mg incorporated in pots and CV (%) = percentage of coefficient of variation.

In the mean square analysis of variance for the treatment effect, only haustrium attachment days (HAD) revealed significant (P< 0.05) difference, all others such as stage1-small tubercles without root development days (S1TD), stage2-tubercles with crown roots but not shoot formation days (S2TD), stage3-tubercles with root and shoot formation days (S3TD), *Orobanche* weed spikes emergence days (OSED), *Orobanche* capsule flowering days (OCFD), *Orobanche* capsule maturity days (OCMD), *Orobanche* number per host plant (ONPP) and *Orobanche* dry weight per weed plant showed highly significant (P<0.01) difference (Table 4).

For the cultivar effect, HAD, S1TD, S2TD, OSED and OCFD revealed significant (P< 0.05) difference, S3TD, OCMD, ONPP and ODwtPP revealed highly significant (P<0.01) difference (Table 4). For the seed rate effect, only ODwtPP showed significant (P< 0.05) difference, but all others *Orobanche* weed growth and development parameters showed non- significant difference (Table 4) this is due to germination, growth and development of *Orobanche* weed were influenced by hormones produced by the host plant instead the seed quantity of the weed. For interaction effect (A x B), all the recorded characters showed highly significant (P<0.01) differences (Table 4) this revealed the development of different weed parameters (tubercles) were determine by the degree of resistance or susceptibility of the cultivars rather than the weed seed rates and the result indicated that there were genetically differences between the tested cultivars for *Orobanche* resistances. This result was in agreement with the finding of Lemma *et al.* (2020) who did on faba bean with *Orobanche* using Petri dish experiment and the result of Trabelsi *et al.* (2016) who did on faba bean with *Orobanche* using pot experiment.

Analysis of Interaction Effect of Cultivar and Seed Rate for Days to Weed Appearance:

The interaction of cultivar and seed rate of *Orobanche* weed on days to weed appearance of *Orobanche* growth characters on pot experiment revealed highly significant (P<0.01) differences and results were presented in (Table 5).

Table 5. Analysis of interaction effect of cultivar and seed rate of weed on days to appearance of *Orobanche* growth characters for pot experiment conducted in 2020 in Dessie.

Treatment		Weed characteristics						
Cltivar	Seed rate (mg)	HAD	S1TD	S2TD	S3TD	OSED	OCFD	OCMD
Ashenge	2	61.33a	66.33a	72.33a	80.33a	87.33a	98.00a	113.33a
Dida’a	2	46.33c	49.33c	52.33c	55.33c	63.33b	72.67b	83.33b
Obse	2	53.33ab	57.33ab	61.33ab	65.67ab	64.00b	74.33b	84.00b
Farmers’ cultivar	2	46.67c	49.67c	52.67c	55.67c	63.33b	68.67c	78.33c
Ashenge	4	59.33a	63.33a	68.67a	73.00a	84.67a	95.00a	108.67a
Dida’a	4	55.33ab	58.33ab	63.00ab	66.67ab	72.67ab	76.33b	85.67b
Obse	4	50.33b	53.33b	56.33b	59.33bc	64.67b	72.67b	82.67b
Farmers’ cultivar	4	52.00b	55.67ab	58.00b	64.67ab	67.00b	71.67b	79.00c
Ashenge	6	60.00a	63.67a	68.33a	74.00a	84.33a	96.33a	110.33a
Dida’a	6	53.00ab	56.67ab	59.67b	62.33b	66.00b	74.67b	81.00b
Obse	6	52.67b	55.33ab	58.33b	62.33b	65.33b	74.00b	81.33b
Farmers’ cultivar	6	45.33c	48.33c	51.33c	54.33c	58.33c	64.00c	74.33c
LSD (5%)		8.4	7.58	7.65	7.85	9.87	10.01	9.87
Overall mean		52.97	56.44	60.19	64.47	70.08	78.20	88.50

Note: LSD (5%) = Least significant difference at P≤0.05 probability level, means in each column with similar letter(s) are not significantly different each other, HAD = haustrium attachment days, S1TD = stage1-small tubercle development days, S2TD = stage2-tubercles with crown roots without shoot development days, S3TD = stage3-tubercles with root and shoot formation days, OSED = *Orobanche* spikes emergence days, OCFD = *Orobanche* capsule flowering days and OCMD = *Orobanche* capsule maturity days.

The cultivar and *Orobanche* weed seed rate interaction analysis, farmers ’cultivar shown shorter days for the successive weed developing appearances than the rest cultivars (45.33 days obtained combined with 6 mg weed seed rate for HAD, 48.33 days obtained combined with 6 mg weed seed rate for S1TD, 51.33 days obtained combined with 6 mg weed seed rate for S2TD, 54.33 days obtained combined with 6 mg weed seed rate for S3TD, 58.33 days obtained combined with 6 mg weed seed rate for OSED, 64 days obtained combined with 6 mg weed seed rate for OCFD and 74.33 days obtained combined with 6 mg weed seed rate for OCMD (Table 5). The intermediate resistant (*Dida’a*) and less susceptible (*Obse*) cultivars had in between of the resistant and farmers’ cultivars (Table 5). The successive weed developing appearances were shorter in 6 mg than 2 mg and 4 mg weed seeds rate, this is because the number of weed seeds increases in the same host and same volume of soil, there is a great chance to contact with the stimulants produced by the host then can be germinate in a short period of time this result is similar with finding of Lemma *et al*. (2018). On the resistant cultivar (*Ashenge*) the different weed growth parameters recorded long days as compared to the other cultivars, this is due to in the resistant host stimulants produced at later growth stage therefore, the weed seeds receiving the stimulants in late time therefore, weed characters development delayed, it indicated the growth and development of parasite weed was cultivar dependent or determined by resistance/susceptible ability of the host or tested cultivars, this result is in accordance to the finding of (Abbes, *et al*., Abbes *et al*., Trabelsi *et al.* and Lemma *et al.* (2007a; 2007b; 2016; 2020).

Analysis of Interaction Effect of Cultivar and Seed Rate for Number and Dry Weight of *Orobanche*

The analysis of interaction effect of cultivar and seed rate of weed on *Orobanche* number per host plant and dry weight of *Orobanche* per weed plant in the pot revealed highly significant (p<0.01) differences and the results were presented in (Table 6).

Table 6. Analysis of interaction effect of cultivar and seed rate of weed on number and dry weight of *Orobanche* for pot experiment conducted in 2020 in Dessie.

Treatment		Weed characteristics	
Cultivar	Seed rate (mg)	ONPP	ODWtPP (g)
Ashenge	2	5.00f	14.67^{a}
Dida'a	2	9.00de	8.00^{cd}
Obse	2	10.67c	8.00^{cd}
Farmers' cultivar	2	12.33ab	7.00^{d}
Ashenge	4	5.67f	12.67^{ab}
Dida'a	4	8.67de	8.00^{cd}
Obse	4	11.00bc	7.67^{cd}
Farmers' cultivar	4	12.67a	7.00^{d}
Ashenge	6	6.33f	12.67^{b}
Dida'a	6	9.67de	8.67^{c}
Obse	6	8.00e	8.00^{cd}
Farmers' cultivar	6	12.61a	6.67^{d}
LSD (5%)		1.51	1.29
Overall mean		9.31	9.09S

Note: letters same indicated there is no difference between the cultivars, LSD (5%) = Least significant difference at P≤0.05 probability level, ODWtpp (g) = *Orobanche* dry weight per weed plant in (g), ONPP = *Orobanche* number per host plant, Seed rate (mg) = *Oroanche* weed seed rate in mg incorporated in the pot.

Orobanche numbers per host plant and dry weight per weed plant as presented in (Table 6), indicated that less *Orobanche* numbers per host plant for resistant cultivar *(Ashenge*) ranged 5.00 to 8.67 obtained from combination of 2 mg and 4 mg, respectively, for farmers' cultivar more *Orobanche* numbers per host plant ranged 12.33 to 12.67 obtained from combination of 2 mg and 4 mg, respectively. For the intermediate cultivar (*Dida'a*) and less susceptible cultivar (*Obse*) had *Orobanche* numbers in between *Ashenge* and farmers' cultivar (Table 6). *Ashenge* had less *Orobanche* numbers per host plant indicated the cultivar is less stimulant producer, but farmers' cultivar had more *Orobanche* numbers per host plant indicated the cultivar can produces excess amount of stimulant per host plant. *Dida'a* and *Obse* had *Orobanche* numbers per host plant in between the two extreme cultivars, shown they are less resistant and less susceptible, this result is in accordance with the results of Abbes, *et al.,* Lemma *et al*. and Trabelsia *et al*. (2011; 2018; 2016).

Orobanche dry weight per weed plant (ODWtPP) for resistant cultivar (*Ashenge*) recorded relatively great weight ranged 8.00 (g) achieved combined with 6 mg weed seeds to 14.67 (g) achieved combined with 2 mg weed seeds, for farmers' cultivar ranged 6.67 (g) achieved combined with 6 mg weed seeds to 7.00 (g) achieved combined with 2 mg weed seeds. For *Dida'a* ranged 8.00 (g) achieved combined with 2 mg weed seeds to 12.67 (g) achieved combined with 4 mg weed seeds and for *Obse* ranged 7.67 (g) achieved combined with 4 mg weed seeds to 12.67 (g) achieved combined with 6 mg weed seeds (Table 3.6). For the resistant cultivar great *Orobanche* dry weight per weed plant recorded indicated, the resistant cultivar is alive up to normal physiological maturity then *Orobanche* exists throughout the growing stage of the cultivar then *Orobanche* gain adequate amount of food and the farmers' cultivar had less *Orobanche* dry weight per weed plant showed that, the host is shortly survived after the attachment of the parasitic weed (Table 6).

The intermediate resistant and less susceptible cultivars recorded *Orobanche* dry weight per parasitic plant in between the resistant and the susceptible cultivars (*Ashenge* and farmers' cultivar) this is because, they were alive a small percentage longer time than farmers' cultivar but smaller than *Ashenge,* this result is dissimilar with the result of Abbes, *et al.* (2011) in that, their results indicated, *Orobanche* dry weight (g) per weed plant was greater in susceptible genotype than for resistant genotype (Table 6).

Analysis of Variance and Mean Comparison

Analysis of variance and mean comparison of *Orobanche* weed growth and development characteristics for field experiment results were presented in (Tables 7 and 8), respectively. The analysis of variance for growth and development of different *Orobanche* weed characteristics in the field revealed highly significant (p<0.01) differences between cultivars.

Table 7. Analysis of mean squares for growth and development of *Orobanche* weed characteristics on faba bean cultivars for field experiment conducted in 2020 at Kutaber *woreda* northeast Ethiopia.

	Source of variation			
Oroanche weed characteristics	Treatment (15)	Replication (3)	Error (45)	CV (%)
Days to haustrium attachment (HAD)	169.25**	2.25	0.86	6.3
Stage1- small tubercles without root development days (S1TD)	170.90**	1.23	1.34	6.9
Stage2-tubercles with crown roots but not shoot development days (S2TD)	170.90**	0.40	1.67	6.4
Stage3- tubercles with root and shoot formation days (S3TD)	181.33**	3.17	4.17	7.3
Days to weed spikes emergence (OSED)	188.42**	8.25	6.64	6.8
Days to *Orobanche* weed capsule flower (OCFD)	156.92**	42.75	9.19	7.3
Days to *Orobanche* weed capsule maturity (OCMD)	183.75**	63.42	10.92	6.3
Number of *Orobanche* weeds per plant (ONPP)	59.68**	1.72	0.45	7.7
Dry weight of *Oroanche* weeds per plant (g) {(ODwtPP (g)}	51768.6**	163.4	200.3	10.7

Note: ** significant difference at P<0.01, number in parenthesis represented degree of freedom and CV (%) = percentage of coefficient of variation.

The *Orobanche* weed growing and development parameters; haustrium attachment days (HAD), stage1- tubercles without root development days (S1TD), stage2- tubercles with crown roots but not shoot development days (S2TD), stage3-ubercles with root and shoot formation days (S3TD), *Orobanche* weed spikes emergence days (OSED), *Orobanche* capsule flowering days (OCFD), *Orobanche* capsule maturity days (OCMD), *Orobanche* number per host plant (ONPP) and *Orobanche* dry weight per weed plant (g) (ODwtPP) revealed highly significant (P<0.01) difference (Table 7).

Table 8. Mean comparison of *Orobanche* weed growth characteristics on host faba bean cultivars for field experiment conducted in 2020 at Kutaber *woreda* northeast Ethiopia.

Cultivar	HAD	S1TD	S2TD	S3TD	SED	OFD	OSSD	On pp	ODw (g)
Ahsenge	68.25[a]	71.50[a]	74.75[a]	78.75[a]	82.75[a]	90.25[a]	116.75[a]	3.10[c]	15.23[a]
Dida'a	54.75[c]	58.00[c]	61.25[b]	64.75[b]	68.25[b]	77.75[b]	87.25[b]	9.12[b]	9.12[b]
Obse	57.00[b]	60.00[b]	63.25[ab]	66.75[b]	71.00[b]	77.25[b]	86.50[b]	11.47[a]	9.31[b]
Farmer' cultivar	54.50[c]	57.75[c]	61.00[c]	64.75[b]	68.50[b]	75.25[b]	85.00[b]	11.05[a]	7.21[c]
Grand mean	58.62	61.81	65.06	68.75	72.63	80.13	85.88	8.69	10.47
LSD (5%)	1.48	1.85	2.07	3.27	4.12	4.85	5.29	1.08	22.64

Note: Means in each column with similar letter(s) are not significantly different each other, LSD (5%) = Least significant difference at P≤0.05 probability level, HAD = haustrium attachment days, S1TD = stage1-small tubercle development days, S2TD = stage2-tubercles with crown roots without shoot developmet days, S3TD = stage3-tubercles with root and shoot formation days, OSED = *Orobanche* spikes emergence day, OCFD = *Orobanche* capsule flowering days and OCMD = *Orobanche* capsule maturity days.

On the field experiment, the resistant cultivar (*Ashenge*) had late response for development of different *Orobanche* weed growth characters, farmers' cultivar had early response and *Dida'a* and *Obse* had an intermediate response of *Ashenge* and farmers' cultivar (Table 8 and Figure 2). The field experiment results follow similar trends to the pot experiment (Table 8 and Figure 1), this finding was similar to the results of Abbes, *et al*. (2007a; 2007b).

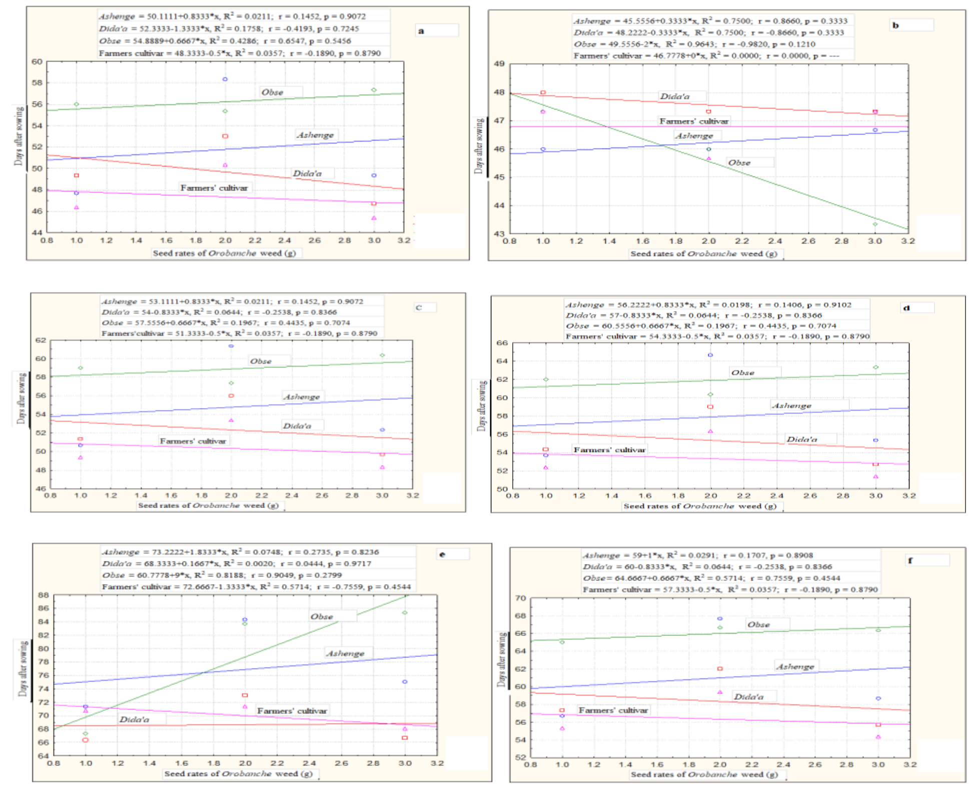

Figure 1. Response of faba bean cultivars to days to haustrium attachment (a), flowering (b), small tubercles without root development (c), spikes emergence (d), tubercles with crown roots & no shoot formation (e) and underground tubercles with root formation (f) as influenced seed rates of Orobanche weed.

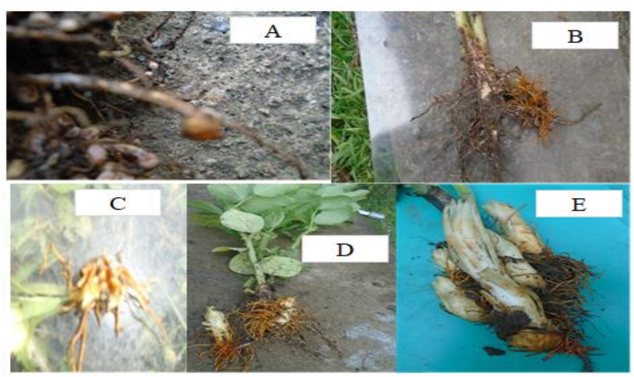

Figure 2.Tubercle development (stage-1) (A), haustrium attachment (B), Tubercle development (stage-2) (C), Tubercle development (stage-3) (D) and Spikes emergence above ground (E) on susceptible farmers cultivar (local check) photo taken at field experiment from 31 August to 13 September 2020.

Conclusions

In Ethiopia, faba bean is a food security crop that is predominantly grown in the mid-altitude and highland areas as a multi-purpose crop and leads the pulse category in terms of area and production. Faba bean is a temperate grain legume of high importance as a source of food, feed, and cash crop for foreign currency and it is one of the most important cool season crops in the mid and highlands of Ethiopia and the country is considered as the secondary center of diversity. Faba bean has great potential to alleviate malnutrition for the resource-poor farmers. It is the principal legume for food, sustain the cropping systems and soil fertility. Ethiopia is the fourth largest exporting country of faba bean next to France, Australia and United Kingdom.

Even though faba bean plays an important role for Ethiopian farmers, its yield below the world average due to several factors such as poor crop management practices, biotic and abiotic factors and lack of high yielding cultivars are some of the causes of low yield.

Orobanche crenata Forskal is considered indigenous in the Mediterranean basin and it is a parasitic plant found in the genus *Orobanche* and family *Orobanchaceae*, which have about 140 species which attack cultivated crops, wild plant species, and weeds. Most species of *Orobanche* have multiple hosts, and one crop host can be infected by multiple species of *Orobanche* and crop yield loss varies from crop to crop depending on the severity of *Orobanche* infestation. The particular characteristics of the weed are underground development, underground attachment and underground damage of its host.

The objectives of the study were to determine underground tubercles development and parasitism stages of *Orobanche crenata* on faba bean genotypes with varied weed seed rates under pot and natural infestation at field conditions, and cultivars' response for weed seed rates under control environment.

The cultivar effect revealed significant (P< 0.05) difference, but the seed rate effect of *Orobanche* weed growth and development parameters showed non- significant difference except ODwtPP showed significant (P< 0.05) difference this is due to germination, growth and development of *Orobanche* weed were influenced by hormones produced by the host plant instead the seed quantity of the weed. The interaction effect (A x B) of all the recorded characters showed highly significant (P<0.01) differences this revealed the development of different weed parameters (tubercles) were determine by the degree of resistance or susceptibility of the cultivars rather than the weed seed rates and the result indicated that there were genetically differences between the tested cultivars for *Orobanche* resistance.

The interaction effect (cultivar and *Orobanche* weed seed rate interaction), farmers 'cultivar shown shorter days for the successive weed developing appearances than the rest cultivars. The intermediate resistant (*Dida'a*) and less susceptible (*Obse*) cultivars had in between of the resistant and farmers' cultivars. The successive weed developing appearances were shorter in 6 mg than 2 mg and 4 mg weed seeds rate, this is may be the number of weed seeds increases in the same host and same volume of soil, there is a great chance to contact with the stimulants produced by the host plant then can be germinate in a short period of time.

On the resistant cultivar (*Ashenge*) the different weed growth parameters recorded long days as compared to the other cultivars, this is due to in the resistant host stimulants produced at later growth stage therefore, the weed seeds receiving the stimulants in late time therefore, weed parameters development delayed, it indicated the growth and development of parasite weed was cultivar dependent or determined by resistance/susceptible ability of the host or tested cultivars.

Orobanche numbers per host plant was less for resistant cultivar *(Ashenge*) indicated the cultivar is less stimulant producer but for farmers' cultivar more *Orobanche* numbers per host plant indicated the cultivar can produces excess amount of stimulant per host plant. *Dida'a* and *Obse* had *Orobanche* numbers per host plant in between the two extreme cultivars, shown they are less resistant and less susceptible.

Orobanche dry weight per weed plant (ODWtPP) for resistant cultivar recorded relatively great weight than farmers' cultivar this is because, the resistant cultivar is alive up to normal physiological maturity then *Orobanche* exists throughout the growing stage of the cultivar then *Orobanche* gain adequate amount of food but the farmers' cultivar had less *Orobanche* dry weight per weed plant showed that, the host is shortly survived after the attachment of the parasitic weed. The intermediate resistant and less susceptible cultivars recorded *Orobanche* dry weight per parasitic plant in between the resistant and the susceptible cultivars (*Ashenge* and farmers' cultivar) this is because, they were alive a small percentage longer time than farmers' cultivar but smaller than *Ashenge.*

On the field experiment, the resistant cultivar (*Ashenge*) had late response for development of different *Orobanche* weed growth parameters, farmers' cultivar had early response and *Dida'a* and *Obse* had an intermediate response of *Ashenge* and farmers' this indicated that, the field experiment results follow similar trends to the pot experiment.

As a recommendation, any management practice will be applied based on the cultivar responses for the parasitic weed and chemical application should be applied by counting the days from faba bean wet sowing according to the tubercles development on susceptible and resistant cultivars.

The way forward is that, researches should be confirmed the exact application time based on the results of the experiment, it means application of any chemical or other management practice to control or manage *Orobanche crenata* can be effective on time of tubercle development or before tubercle development or after the tubercle development by counting the days from wet sowing of the host plant.

Funding statement

The research was funded by Amhara Regional Agricultural Research Institute (ARARI) that is the first author organization.

Data Availability Statement

The data will be available on Haramaya University database system.

Author contribution

All authors made a significant, and academic contribution for the work, and accepted it for publication.

Acknowledgements

The authors wish to thank the Amhara Regional Agricultural Research Institute, Sirinka Agricultural Research Center for their financial support, Haramaya University for their technical support to this study and the technical staff for their help.

Declaration of Interest statement

The authors declare no conflict of interest.

References

Abbes, Z., Sellami F., Amri M. & Kharrat M. 2011. Variation in the resistance of some faba bean genotypes to Orobanche crenata. *Pakistan Journal of Botany*, 43: 2017–2021.

Abbes, Z., M. Kharrat, P. Delavault, P. Simier and W. Chaïbi. 2007a. Field evaluation of the resistance of some faba bean (*Vicia faba* L.) cultivars to the parasitic weed *Orobanche foetida* Poiret. *Crop Prot*., 26: 1777-1784.

Abbes, Z., M. Kharrat, P. Simier and W. Chaïbi. 2007b. Characterization of resistance to crenate broomrape (*Orobanche crenata*) in a small-seeded line of Tunisian faba beans. *Phytoprotection*, 88: 83-92.

Agegnehu, G., and R. Fessehaie. 2006. Response of faba bean to phosphate fertilizer and weed control on nitosols of Ethiopian highlands. *Italian Journal of Agronomy* 2:281-290.

Besufekad Tadesse, Legesse Admassu and Rezene Fessehaie. 1999. *Orobanche* Species, Distribution and intensity of infestation in south Wollo.

Bichaye Tesfaye, Monica Lengoiboni, Jaap Zevenbergen and Belay Simane. 2022. Land Preservation Uptakes in the Escarpments of North-eastern Ethiopia: Drivers, Sustainability and Constraints. Food Security Studies, College of Development Studies, Department of Urban and Regional Planning and Geo-Information Management, University of Twente and Environment and Sustainable Development, College of Development Studies, Addis Ababa University, Ethiopia.

CSA. 2022. Area and production of major crops (private peasant holdings, Meher Season). V1. Statistical Bulletin No.584. Addis Ababa, Ethiopia.

Daniel M. Joel Swapan K. Chaudhuri, Dina Plakhine, Hammam Ziadna and John C. Steffens. 2011.

Dehydrocostus lactone is exuded from sunflower roots and stimulates germination of the root parasite *Orobanche Cumana.*

Das T. K., Sonaka Ghosh, Kamlika Gupta, Suman Sen, Biswaranjan Behera, Rishi and Raj. 2020. The weed *Orobanche*: species distribution, diversity, biology and management. Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi – 110012, India.

Centre for Environment Science and Climate Resilient Agriculture; ICAR-Indian Agricultural Research Institute, New Delhi -110012*, India.* .*Journal of Research in Weed Science* Volume 3 Issue 2, pp 162-180

Farooq S. and F. Azam. 2002. Molecular Markers in Plant Breeding-lll: Practical Applications and Difficulties Encountered. Nuclear Institute for Agriculture and Biology, P.O. Box 128, Jhang Road, Faisalabad, Pakistan, *Pakistan Journal of Biological Sciences* 5(10): 1148-1154, 2002

Federer WT. 1956. Augmented design, Hawain planters *Record*. **55**: 191-208.

Fernandez-Aparicio M., Xavier Reboud and Stephanie Gibot-Leclerc. 2016. Broomrape Weeds. Underground Mechanisms of Parasitism and Associated Strategies for their Control: A Review, Dijon, France.

Fernandez-Aparicio M., Xiaonan Xie, Takaya Kisugi and Diego Rubiales. 2014. Low Strigolactone Root Exudation: A Novel Mechanism of Broomrape (*Orobanche* and *Phelipanche spp*.) Resistance Available for Faba Bean Breeding. *In Journal of Agricultural and Food Chemistry* 62 (29).

Fernandez-Aparicio, M., Emeran, A. A. and Rubiales, D. 2011. Inter-cropping faba bean with berseem, fenugreek or oat can contribute to broomrape management, Grain Legumes, 56: 31.

Food and Agriculture Organization. 2022. Progress on farmer training in parasitic weed management. Rome-Italy.

Gebisa Ejeta and Jonathan Gressel. 2007. Integrating New Technologies for Striga Control towards Ending the Witch-hunt. World scientific publishing. co. Pte.Ltd. London.

Institute Inc. 2008. "SAS. 9.2. Companion for Windows," SAS Publishing, SAS Institute Inc., Cary.

Joel, D. M., J. Hershenhorn, H. Eizeinberg, R. Aly, G. Ejeta, P. J. Rich, J. K. Ransom, J. Sauerborn, and D. Rubiales. 2007. Biology and management of weedy root parasites. Hortic. Rev. 33: 267-350.

Kumar Mukesh, Das T.K, Yaduraju N.T. 2012. An integrated approach for management of *Cyperusrotundus* (purple nutsedge) in soybean - wheat cropping system. Crop Prot. 33: 74-81.

Lemma Diriba, Firew Mekbib and Mussa Jarso. 2020. Evaluation of Faba Bean (*Vicia faba* L.) Varieties Grown with Crenate Broomrape (*Orobanche crenata*) in Petri dish under Controlled Environment. Ethiopian Institute of Agricultural Research, Department of Plant Sciences, Haramaya University, Ethiopia. Acta Scientific Agriculture (ASAG), Issue 2-

Lemma Diriba. 2018. Performance of Faba Bean (*Vicia faba* L.) Varieties Grown Under Broomrapes (*Orobanche* spp.) Infestation in South Tigray, Ethiopia. MSc Thesis Haramaya University, Haramaya.

Maalouf, F., Jinguo Hu. Donal M. O'Sullivan, Xuxiao Zong, Aladdin Hamwieh, Shiv Kumar and Michael Baum. 2018. Breeding and genomics status in faba bean (*Vicia faba* L.). Review Article.

Mekonnen Misganaw. 2016. Integrated weed (*Orobanche crenata*) management on faba bean. *American Journal of Agricultural Research* (ISSN: 2475-2002).

Nickrent D. L. and Musselman L. J. 2016. Introduction to Parasitic Flowering Plants. Department of Plant Biology, Southern Illinois University and Department of Biological Sciences, Old Dominion University.

Nuru Sherif Seid. 2020. Assessment of Local Climate Change Adaptation Options in Agriculture: a case of Kutaber Woreda, south Wollo zone. MSc thesis submitted to the Center for Environment and Development College of Development studies for Partial Fulfillment of the Requirements for the Degree of Master of Arts in Development studies, Addis Ababa University School of Graduate studies, Addis Ababa.

Paul W. Goedhart and Jac T. N. M. Thissen. 2001. Biomeris GenStat Procedure and Library Manual 17th edition, Wageningen University and Research Center.

Rubiales, D., A. Pérez-de-Luque, M. Fernández-Aparicio, J. C. Sillero, B. Román, M. Kharrat. 2006. Screening techniques and sources of resistance against parasitic weeds in grain legumes. Euphytica 147: 187-199.

Sharawy Sherif and Eman Karakish. 2015. Taxonomic Relationships of some species of Orobanche L. evidence from RAPD-PCR and ISSR markers. Permanent address: Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt.

Steel, R.G.D., Torrie J.H.1980. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York: 633 p.

Tanee, T., A. Chaveerach, R. Sudmoon, A. Tanomtong and P. Mokkamul. 2009. Genetic relationships of cercopithecidae in Thailand as inferred from rDNA ITS regions. *J. Biol. Sci.* 9: 51-56.

Tantawy, A. A., S. Sh. Abdullah, Y. A. M. Hefny and A. R. M. Ridwan. 2022. Performance of Some Faba bean Genotypes for Tolerance to the Infestation of Broomrape (*Orobanche crenata*) Weed. ISSN 2357-0725 https://jsasj.journals.ekb.eg JSAS; 7(2): 138-153.

Trabelsi, I. Zouhaier Abbes, Moez Amri and Mohamed Kharrat. 2016. Study of some resistance mechanisms to *Orobanche* spp. infestation in faba bean (*Vicia faba* L.) breeding lines in Tunisia. Field crop laboratory, national institute for agricultural research of Tunisia (INRAT), Tunisia.

Wagaye Bahiru Abegaz and Endalew Assefa Abera. 2020. Temperature and Rainfall Trends in North Eastern Ethiopia. National Meteorology Agency of Ethiopia, Ethiopian Institute of Agricultural Research, Ethiopia.

Yinglu and Ji-Qian Fang. 2003. Advanced Medical Statistics. World Scintific, New Jersey, London, Singapore, Hong Kong.

Zouhaier Abbes, Mohamed Kharrat, Philippe Simier, and Wided Chaïbi. 2018. Characterization of resistance to *Orobanche crenata* in a new small-seeded line of Tunisian faba beans. *Phytoprotection* 88: 83-92.