ISSN 2347-6893

Effects of urea and imazethapyr on Orobanche crenata germination, radicle extension, incidence and faba bean growth and yield

Sawsan, A. Abdel-Daim [1*]; A. G. A. Osman [1]; Rashida, M. Abusin[2]; Amani H. Eltayeb[1] and A. G. T. Babiker [1]

[1]*Sudan University of Science and Technology, College of Agricultural Studies, Shambat, Khartoum. North, Sudan.*

[2]*University of Bahri, Protection, Sudan.*

Corresponding author:

*Sawsan, A. Abdel-Daim.

E- mail: swsan.abd@hotmail.com

Abstract

Orobanche crenata Forsk., a debilitating root parasitic weed, has become an important constraint to production of cool season legumes in Sudan. The present investigation was undertaken to determine the effects of urea, the herbicide imazethapyr and their combinations on the parasite germination, radicle extension, incidence and faba bean growth and yield. The results showed that urea at 20-400 mM reduced *O. crenata* seeds germination and radicle extension in a concentration dependent manner. Imazethapyr did not reduce germination and effected inconsistent reductions in radicle length. Unrestricted parasite growth had no significant adverse effects on number of faba beans leaves and plant height. However, it reduced the number of pods by 20.2 and 38.8% and grain yield by 35.3 and 46.9%. Imazethapyr at 47.6 g a.i. ha^{-1} applied 45, 30, 15 days, subsequent to and at sowing, suppressed *O. crenata* emergence by 22.7, 94.3, 94.8 and 67.7% early in the season. However, late in the season only poor to moderate control was achieved. The corresponding figures for the high rate (71.4 g a.i. ha^{-1}) were 72.4, 97.6, 97.4 and 96.8%. However, late in the season only poor (39%) to satisfactory (69%) control was achieved. Urea, alone, effected poor control (20-43.5%) and when applied to the herbicide treated sub-plots did not inflict further reductions. however, capsules production was reduced by 43-85%. The herbicide alone increased grain yield by 24-79%. The combination herbicide and urea at sowing increased yield by 87.5-111.4%. Contrary to the general belief that *O. crenata* inflicts most of its damage during the subterranean phase the results suggest that in irrigated faba beans most of the damage is synchronized with the parasite emergence.

Key words: *Orobanche crenata*; Ffaba bean; Germination; Radicle extension; Control;

Council for Innovative Research

Peer Review Research Publishing System

Journal of Advances in Biology

Vol. 7, No. 2

www.cirjab.com

editorsjab@gmail.com , editor@cirjab.com

ISSN 2347-6893

Introduction

Faba bean (*Vicia faba L.)*, a Fabaceae, is planted mainly in the Mediterranean regions, the Nile Valley, Ethiopia, Central Asia, and Northern Europe (Bond *et al*., 1985). 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 constituting the alluvial soils of the Nile valley extending north, on both banks of the Nile, from Khartoum to Wadi Halfa on the Egyptian borders (Babiker *et al*., 2007). Faba bean productivity and yield stability are constrained by the root parasitic weed, *Orobanche crenata* Forsk. *O. crenata* is a haloparasite that depends entirely on its hosts for nutrients, carbohydrates and possibly hormones (Parker and Riches, 1993). In the Sudan *O. crenata* was first reported in 2000/2001at Ed Debiba in Merowe governorate in an area of about 2 ha (Babiker *et al*., 2007). Since then the parasite has spread rapidly and becomes a national problem. The parasite, pending infestation level, was reported to inflict an average yield loss ranging from 6-90% (Babiker *et al*., 2007). The infection process followed an orderly sequence of events starting with germination in response to stimulant(s) from the host roots. The radicle elongates forms a haustorium that penetrates the host roots, establishes connection with the host phloem and a tubercle that remains relatively quiescent is formed (Joel *et al*., 1995). Concurrently with host flowering and pod bearing stage a shoot bud develops, elongates and the parasite emerges above the ground (Muller and Distler, 1989). Development of the infection and pathogenesis processes takes place underground prior to the emergence of the parasite and thus early diagnosis of infection and control of the parasite are difficult to achieve by conventional means. Several potential control measures were developed however, any approach applied alone is often only partially effective and the results are sometimes inconsistent due to variable environmental conditions. Therefore, the only effective way to combat the parasite to date is through an integrated approach, combining a variety of measures in a concerted manner (Babiker *et al*., 2007; Rubiales and Fernández-Aparicio, 2012).

Several reports showed that the use of nitrogen fertilizers effectively reduced parasitism and enhanced faba bean growth. Fertilization of faba bean with high rates of nitrogen reduced broomrape infestation and increased faba bean grain yield (Zahran, 1973). Nitrogen in ammonium and urea forms affect negatively root parasitic weed germination (Pieterse, 1991; Van Hezewijk and Verkleij, 1996) and/or elongation of seedling radicle (Pieterse, 1991; Westwood and Foy, 1999).

Several systemic herbicides have so far been proposed for broomrape control in vegetables and field crops. These herbicides include the branched-chain amino acid synthesis inhibitors imidazolinones and sulfonylureas which target the enzyme acetolactate synthase (ALS) (Schloss, 1990). Garcia-Torres *et al.* (1999) and Jurado-Expósito *et al.*(1997) reported that imazethapyr and imazapyr, effectively and selectively control broomrape on faba bean. Hence, the present work was undertaken to assess the effects of nitrogen and the herbicide imazethapyr on *O. crenata* germination and radicle extension and the role of urea fertilizer and imazethapyr, each alone and/or in combinations, on *O. crenata* incidence and faba bean growth and yield.

Materials and Methods

A series of laboratory and field experiments were conducted at the *Striga* research laboratory and the parasitic weed enclosure at the College of Agricultural Studies, Sudan University of Science and Technology at Shambat with the objectives of studying the effects of urea and imazethapyr on *O. crenata* germination and radicle extension in response to GR24 and the effects of urea and imazethapyr, each alone and in combinations, on *Orobanche* incidence and faba bean growth and yield.

GR24, a synthetic germination stimulant, was provided by Prof B. Zwanenberg, the University of Nimijhen, the Netherlands, imazethapyr and urea were purchased from the local market, The faba bean cultivar Hudieba 93 was obtained from the Agricultural Research Corporation (ARC), Hudieba Research Station. Seeds of *O. crenata*, collected from under faba bean at Koboshia in the River Nile State, were supplied by Dr Naser Eldeen Abdalla Khiry ARC Shendi Research Station. 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.

O. crenata seeds sterilization and conditioning:

O. crenata seeds were surface sterilized by immersion, for 3 min, in sodium hypochlorite (NaOCl) 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, plotted dry on Whatman No. 1 filter papers, were air-dried in a limanr flow and stored at ambient temperature till used.

For conditioning *Orobanche* seeds (25-30 seeds/disc) were sprinkled on 8 mm glass fiber filter papers (GFA) discs, placed on moist glass fiber filter papers in Petri dishes, sealed with parafilm, wrapped in aluminum foil and incubated in the dark at 20 °C for 14 days.

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

Orobanche seeds, conditioned in distilled water (DW) or urea (20-400 mM) for 14 days, were treated with aliquots (20µl each) of GR24 at 10 ppm or DW. The treated seeds, in Petri-dishes, sealed with parafilm and wrapped in aluminum foil,

ISSN 2347-6893

were incubated in the dark at 20 ºC for 7 days prior to measurement of germination and radicle extension using a stereomicroscope.

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

Orobanche seeds, conditioned in DW or imazethapyr (10-100 µM) for 14days, were treated with aliquots (20µl each) of DW or GR24 at 0.1, 1 and 10 ppm. The treated seeds, in Petri-dishes, sealed with parafilm and wrapped in aluminum foil, were incubated in the dark at 20 ºC for 7 days prior to measurement of germination and radicle extension.

Effects of imazethapyr, urea, and their combinations on *Orobanche* incidence and faba bean growth and yield

The field experiments were conducted in two consecutive seasons (2009/10, and 2010/11) to study the effects of imazethapyr, urea 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). Faba bean (cv: Hudieba 93), was used in the two experiments. Fertilizater, urea at 1N (95.2 kg ha^{-1}) was applied by broadcasting. Uninfested (*Orobanche* free control) and infested control were included as controls for comparison. All sub-plots, excluding those used for the uninfested control, were artificially inoculated with *Orobanche* seeds each season. The inoculums were prepared by adding 1g of clean *Orobanche* seeds to1kg of soil, previously sieved through a 2 mm mesh metal screen, followed by thorough mixing. The inoculums were applied to the soil before faba bean sowing (5 mg *Orobanche* seeds $hole^{-1}$). 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) added and thoroughly mixed by hand. The treated seeds were sown early December. In the first season imazethapyr at 47.6 and 71.4 g a,i, ha^{-1}, was applied 45, 30, 15 days subsequent to and at sowing. In the second season imazethapyr at 47.6 and 71.4 g a,i, ha^{-1} was applied at sowing. Superimposed on the herbicide treatments was urea at 95.2 kg ha^{-1}, (1N) applied , 30, 15 days subsequent to and at sowing. *Orobanche* free, *Orobanche* infested and *Orobanche* infested urea treated sub-plots were included as controls for comparison. Weeds other than *O. crenata* were removed 3 times at biweekly intervals, starting 2 weeks after crop emergence, using a hand hoe. 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, iii) number of pods and v) grain yield (kg ha^{-1}). Data on *O. crenata* included, i) number of *Orobanche* shoots per m^{-2} and ii) number of capsules per plant.

Statistical analysis

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

Results

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. 1). The response 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 mM and similarly treated with GR24, displayed 58.74 % germination. Increasing urea concentration to 30-60 mM decreased germination to 37.32-13.71%. Further increase in urea concentration to 70 mM or more resulted in negligible germination (0 - 4.6%) (Fig. 1).

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 (Fig. 2). 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. 2). Conditioning in urea at 20-70 mM reduced radicle extension by 11.8 to 90.6%. A further increase in urea, in the conditioning medium, to 80 mM or more reduced radicle extension by 85 to 100%.

ISSN 2347-6893

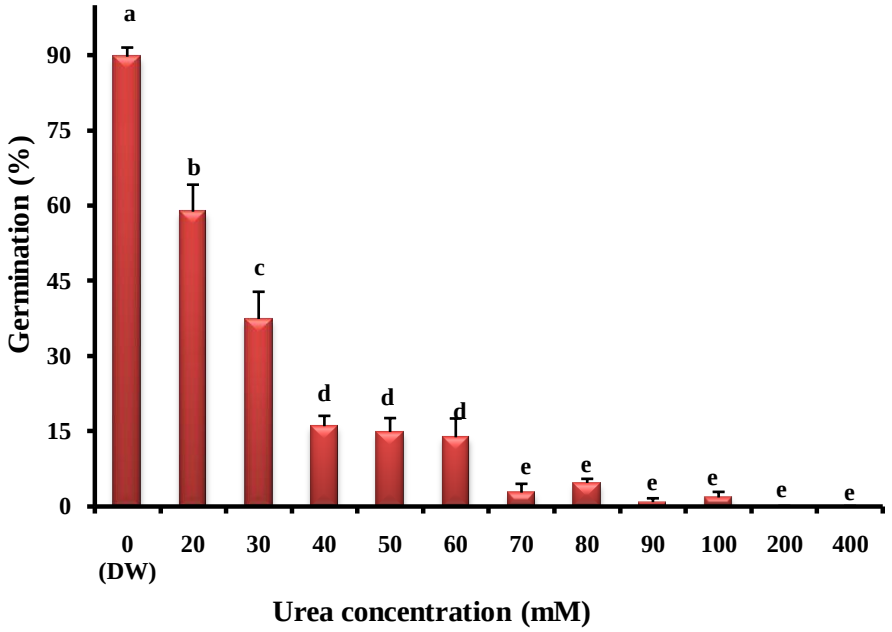

Fig. 1. Effects of urea, applied during conditioning, on *O. crenata* seed germination. Bars are means ± standard errors according to DMRT; P ≤ 0.05. Bars marked with different letters are significantly different at P<0.05 (DMRT).

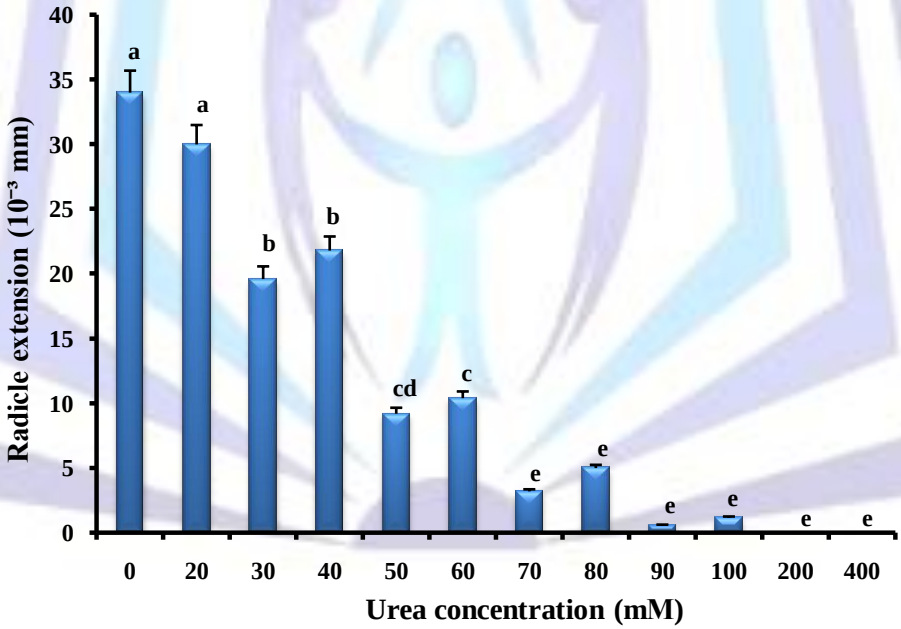

Fig. 2. Effects of urea applied during conditioning on radicle extention of *O. crenata.* Bars are means ± standard errors according to DMRT; P ≤ 0.05. Bars marked with different letters are significantly different at P<0.05 (DMRT).

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

GR24, irrespective of the concentration of imazethapyr during conditioning, induced germination of *O. crenata* seeds in a concentration dependent manner (Table 1). Seeds conditioned in DW displayed 5.8, 40 and 76.6% germination in response to GR24 at 0.1, 1 and 10 ppm, respectively. Seeds conditioned in imazethapyr at 10 µM and subsequently treated with GR24 at 0.1-10 ppm displayed insignificant increase in germination. Imazethapyr at 20 µM had no significant effect on germination in response to GR24 at 0.1 and 1 ppm. However, germination in response to GR24 at 10 ppm was significantly repressed. A further increase in imazethapyr concentration to 40-80 µM did not further reduced germination. However, at 100 µM a significant reduction at the lowest GR24 concentration (0.1 ppm) was evident.

ISSN 2347-6893

Table 1. Effects of imazethapyr applied during conditioning on *O. crenata* seeds germination

Imazethapyr (µM)	Germination% GR24 concentration 0.1	1	10
Aqueous control	5.8 ^{gh}	40.0 ^{cd}	76.6 ^{ab}
10	12.5 ^{fg}	44.7 ^{c}	82.9 ^{a}
20	3.8 ^{hi}	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}	25.0 ^{de}	66.2 ^{b}
100	0.77 ^{i}	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 ≤ 0.001. n.s = not significant.

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}, 19.2 x 10^{-3} and 40.2 x 10^{-3}mm, respectively (Table 2). *Orobanch*e germilings from seeds conditioned in imazethapyr, displayed differential radicle extension, the magnitude of which showed dependence on concentration of the herbicide and GR24 (Table 2). Conditioning in imazethapyr, irrespective of concentration, showed inconsistent effects 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, a concentration dependent, significant reductions.

Table 2. Effects of imazethapyr applied during conditioning on *O. crenata* radicle extension

Imazethapyr (µM)	Radicle length (mm 10^{-3}) GR24 concentration 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 ≤ 0.001.

ISSN 2347-6893

Effects of imazethapyr, rate and application time on *Orobanche* incidence and faba bean growth and yield (Season 2009/10)

Effects on *Orobanche* emergence

Imazethapyr, irrespective of rate and application time, significantly reduced *O. crenata* emergence early in the season (Table 3). An observation made 67 DAS showed that imazethapyr, at 47.6 g a,i, ha^{-1}, applied at sowing, 15, 30 and 45 days later reduced the parasite emergence by 67.7, 94.8, 94.3 and 22.7%, respectively (Table 3). The corresponding reductions inflected by the higher rate (71.4 g a.i. ha^{-1}) were 96.8, 97.4, 97.6 and 72.4%, respectively. However, late in the season (82 DAS) imazethapyr at the low rate (47.6 g a,i, ha^{-1}), applied at sowing did not reduce emergence of the parasite. Treatments made 15, 30 and 45 DAS reduced emergence of the parasite by 63, 54 and 27%, respectively. The herbicide at the higher rate (71.4 g a.i. ha^{-1}), applied at sowing, 15, 30 and 45 later reduced emergence by 39, 60, 69 and 47%, respectively.

Table 3. Effects of imazethapyr, rate and application time, on *O. crenata* emergence (season 2009/10)

Treatment	Hebicide rate (g a.i. ha^{-1})	Herbicide application time (DAS)	*O. crenata* plant m^{-2}	
			67 DAS	82 DAS
Imaz.	47.6	0	5 (2.43) [b]	21.34 (4.7)[a]
Imaz.	47.6	15	0.78 (1.3) [c]	7.58 (2.7)[b]
Imaz.	47.6	30	0.88 (1.4) [c]	9.40 (3.04)[ab]
Imaz.	47.6	45	11.98 (3.6) [a]	14.91 (3.9)[ab]
Imaz.	71.4	0	0.5 (1.2) [c]	12.53 (3.6)[ab]
Imaz.	71.4	15	0.41(1.2) [c]	8.18 (2.8)[b]
Imaz.	71.4	30	0.38 (1.2) [c]	6.42 (2.6)[b]
Imaz.	71.4	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.

Effects on faba bean

Number of leaves

Unrestricted *O. crenata* growth had no significant effects on number of faba bean leaves. Imazethapyr at 47.6 g a.i. ha^{-1}, applied at sowing and 15 and 30 DAS resulted in number of leaves comparable to the *Orobanche* free control (Table 4). However treatment made 45 DAS resulted in insignificant decrease in number of leaves. The herbicide at 71.4 g a.i. ha^{-1}, irrespective of application date effected number of leaves higher than the *Orobanche* free control, albeit not significantly (Table 4). Faba bean height was however, not significantly affected by any of the treatments (data not shown).

Number of pods

Unrestricted *O. crenata* infestation reduced number of pods by 20.2% (Table 4). Despite the lack of significant differences between treatments, a trend of an increase in number of pods with delayed applications up to 30 DAS was displayed. Imazethapyr at 47.6 g a.i. ha^{-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 71.4 g a.i. ha^{-1} applied at sowing or 15, 30 and 45 DAS increased the number of pods by 57.5, 29.6, 44.9 and 55.8%, respectively (Table 4).

ISSN 2347-6893

Table 4. Effects of imazethapyr, rate and application time, on herbicidal efficacy and selectivity on faba bean (season 2009/10)

Treatment	Herbicide rate (g a.i. ha^{-1})	Herbicide application time (DAS)	Number of Leaves plant^{-1}	Number of pods plant^{-1}	Faba bean yield
			60 DAS	75 DAS	(kg ha^{-1})
Imaz.	**47.6**	**0**	53.68^{ab}	19.25	2096.38 ^{a}
Imaz.	**47.6**	**15**	49.60^{ab}	15.80	1810. 46^{ab}
Imaz.	**47.6**	**30**	51.42^{ab}	19.15	1833.89^{ab}
Imaz.	**47.6**	**45**	44.25^{b}	11.55	1702.57^{bc}
Imaz.	**71.4**	**0**	58.97^{a}	23.70	2175.22^{a}
Imaz.	**71.4**	**15**	49.67^{b}	19.50	1972.74^{ab}
Imaz.	**71.4**	**30**	59.45^{a}	21.80	1943.13^{ab}
Imaz.	**71.4**	**45**	60.40^{a}	23.45	1530.61^{bc}
Uninfected control	**-**	**-**	54.75^{ab}	18.85	1879.94^{ab}
Infested control	**-**	**-**	51.65^{ab}	15.05	1216.26^{c}
P≤ 0.05			*	n.s	***
SE±			3.8	2.98	60.73
CV%			14.6	32	16.6

Imaz. = imazethapyr, DAS = days after sowing. Means within a column followed by different letters are significantly different according to DMRT. *= P ≤ 0.05, ***=P ≤ 0.001; n.s = non-significant.

Grain yield

Unrestricted *O. crenata* infestation reduced faba bean grain yield by 35% (Table 4). 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). Imazethapyr at 47.6 g a.i. ha^{-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 71.4 g a.i. ha^{-1} were 78.8, 62.2, 59.8 and 25.8%.

Effects of imazethapyr, urea and their combinations on *Orobanche* incidence and faba bean growth and yield (season 2010/11)

Effects on *Orobanch* emergence and capsules production

The number of emerged *Orobanche* spikes, in the infested untreated control, was 11.7 and 18.6 m^{-2} 78 and 86 DAS, respectively. At 78 DAS urea at 1N, applied at sowing or 15 and 30 days later reduced *O. crenata* emergence, albeit not significantly (Table 5). However, at 86 DAS urea applied at sowing and 30 days later reduced *O. crenata* emergence significantly and the observed reductions were 44 and 40%, respectively. However, treatment made 15 DAS resulted in a considerable, albeit not a significant reduction (20%). Imazethapyr at 47.4 g a.i. ha^{-1}, alone, reduced *Orobanche* emergence by 94% at 78 DAS with no further change in percent suppression up to 86 DAS. The herbicide at 71.4 g a.i. ha^{-1}, reduced *O. crenata* emergence by 99 and 97.4 % 78 and 86 DAS, respectively. Urea applied, irrespective of application time or date of observation, to sub-plots previously treated with the herbicide did not cause further reductions.

O. crenata capsules production

Urea alone, irrespective of application time, had no significant effects on *Orobanche* capsules production (Table 5). Imazethapyr, alone, at 47.6 g a.i. ha^{-1} resulted in non-significant reduction (28%) (Table 5). Imazethapyr at 47.6 g a.i. ha^{-1} applied together with urea at sowing or followed by urea, 15 and 30 days later, reduced *Orobanche* capsules production by 50, 45 and 43%, respectively. The herbicide alone, at 71.4 g a.i. ha^{-1} reduced capsules production by 68% (Table 4.17). Imazethapyr at 71.4 g a.i. ha^{-1} applied together with urea at sowing or followed by urea 15 and 30 days later reduced capsules production by 85, 72 and 54%, respectively. Delayed application of urea, depressed the suppressive

ISSN 2347-6893

effects of the combination on capsules production, albeit not significantly.

Table 5. Effects of imazethapyr, urea and their combinations on *Orobanche* emergence and capsules production (season 2010/11)

Treatment	Herbicide rate (g a.i. ha^{-1})	Urea application time (DAS)	No. of *O. crenata* capsules plant^{-1}	No. of *O. crenata* m^{-2}	
				78 DAS	86 DAS
N	**0**	**0**	44.68^{ab}	8.44(7.12)^{a}	10.5(8.08)^{bc}
N	**0**	**15**	38.95^{bc}	9(6.98)^{a}	14.9(9.14)^{ab}
N	**0**	**30**	59.85^{a}	8.32(7.18)^{a}	11.1(8.25)^{bc}
Imaz.	**47.6**	**-**	33.55^{bcd}	0.76(2.03)^{b}	1.08(2.56)^{d}
Imaz. + N	**47.6**	**0**	23.3^{cde}	2.52(3.65)^{b}	4.8(5.2)^{cd}
Imaz. + N	**47.6**	**15**	25.85^{cde}	1.16(2.31)^{b}	5.92(6.01)^{cd}
Imaz. + N	**47.6**	**30**	26.55^{cde}	2.36(3.83)^{b}	5.6(5.84)^{cd}
Imaz.	**71.4**	-	14.9^{ef}	0.08(0.00)^{b}	0.48(1.22)^{d}
Imaz. + N	**71.4**	**0**	6.95^{f}	0.24(1.22)^{b}	0.84(1.96)^{d}
Imaz.+ N	**71.4**	**15**	13.35^{ef}	0.12(0.43)^{b}	0.6(1.5)^{d}
Imaz. + N	**71.4**	**30**	21.6^{def}	0.12(0.75)^{b}	0.56(1.37)^{d}
Infested control	-	-	46.8^{ab}	11.7(8.54)^{a}	18.6(10.53)^{a}
P ≤ 0.05			***	***	***
SE±			5.55	1.3	2.3
CV%			29.5	36.5	33.0

Imaz. = imazethapyr, N = nitrogen as urea 95.2 kg ha^{-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. No. = number.

Effects on faba bean

Number of leaves

Unrestricted *O. crenata* infestation reduced number of faba bean leaves by 22.4% (Table 6). Urea, alone, applied at sowing and 30 DAS increased the number of leaves over the infested control, albeit not significantly (Table 6). Faba bean treated with imazethapyr alone at 47.6 g a.i. ha^{-1} showed number of leaves comparable to the infested control. Imazethapyr at 47.6 g a.i. ha^{-1} supplemented with urea, at all application dates, increased the number of leaves over the *Orobanche* infested control, albeit not significantly (Table 6). Imazethapyr at 71.4 g a.i. ha^{-1}, alone, and when supplemented with urea at sowing reduced the number of leaves in comparison to the *Orobanche* infested control, albeit not significantly. The herbicide at 71.4 g a.i. ha^{-1} supplemented with urea 15 and 30 DAS increased the number of leaves substantially over the herbicide alone, but not significantly. Faba bean height was not significantly affected by any of the treatments (data not shown).

Table 6. Effects of imazethapyr, urea and their combinations on number of faba bean leaves, pods and grain yield (season 2010/11)

Treatments	Imazethapyr rate (g a.i.ha^{-1})	Urea application time (DAS)	No. of leaves plant^{-1} 45 DAS	No. of pods plant^{-1} 75 DAS	Grain yield (Kg ha^{-1})

ISSN 2347-6893

N	-	**0**	45.58	23.88^{abc}	931.25^{fg}
N	**-**	**15**	41.39	24.63^{abc}	969.11^{fg}
N	**-**	**30**	45.2	19.40^{bc}	849.14^{g}
Imaz.	**47.6**	-	40.3	26.35^{ab}	1179.04^{e}
Imaz. + N	**47.6**	**0**	54.1	22.65^{bc}	1483.86^{abc}
Imaz. + N	**47.6**	**15**	53.6	22.90^{bc}	1119.55^{ef}
Imaz. + N	**47.6**	**30**	48.47	25.10^{abc}	1268.97^{de}
Imaz.	**71.4**	**-**	38.07	27.05^{ab}	1257.07^{de}
Imaz. + N	**71.4**	**0**	36.17	27.33^{ab}	1673.61^{a}
Imaz.+ N	**71.4**	**15**	45.47	29.60^{ab}	1439.76 ^{bcd}
Imaz. + N	**71.4**	**30**	51.73	32.85^{a}	1288.92^{bcd}
Uninfested control	**-**	-	53.7	23.95^{abc}	1491.93 ^{ab}
Infested control	**-**	-	41.67	14.67^{c}	791.56^{g}
P ≤ 0.05			n.s	*	***
SE±			4.08	2.67	39.77
CV%			16.67	23.1	10.73

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.

Number of pods

Unrestricted *O. crenata* infestation resulted in 38.8% reduction in number of faba bean pods (Table 6). Urea and imazethaypr at 47.6 g a.i. ha^{-1}, each alone or in combinations, irrespective of the former application time, increased the number of pods considerably, albeit not significantly. Imazethaypr at 71.4 g a.i. ha^{-1} alone and in combinations with urea at sowing, 15 and 30 DAS increased the number of pods by 12.9, 14.1, 23.6 and 37.2%), respectively, in comparison to the uninfested control (Table 6).

Grain yield

Unrestricted *O. crenata* parasitism reduced faba bean grain yield significantly and the observed reduction was 46.9% (Table 6). Urea alone, irrespective of application time, increased grain yield over the infested untreated control considerably, but not significantly. Imazethapyr at 47.6 and 71.4 g a.i. ha^{-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 47.6 g a.i. ha^{-1} applied together with urea at sowing effected yield comparable to the *Orobanche* free control. However, delaying the urea treatment to 15 and 30 DAS decreased yield significantly. Imazethapyr at 71.4 g a.i. ha^{-1} supplemented with urea at sowing outyielded the *Orobanche* free control, albeit not significantly. Delaying the urea treatment to 15 or 30 DAS decreased yield significantly. Nevertheless, the yield attained was comparable to that of the *Orobanche* free control (Table 6).

Discussion

Urea at 30-60 mM, applied during conditioning, reduced germination and radicle extension significantly. Increasing urea to 70 mM or more resulted in negligible germination (0-.6%) and radicle extension (0-5x10^{-3} mm) (Figs.1 and 2). These findings are in line with those of Pieterse (1991) who reported that germination and radicle extension in *O. crenata* were severely decreased when the seeds were exposed to urea during conditioning. The inhibitory effects of urea on seed germination, as proposed by Bremner and Krogmeier (1989), may be indirect, occurring through conversion to ammonia. This suggestion is consistent with a report on inhibition of root parasitic weed, including *O. crenata*, germination and radicle elongation by nitrogen in ammonium form (Van Hezewijk and Verkleij, 1996) and is attributed to a reduced ability to detoxify ammonia (Van Hezewijk and Verkleij, 1996; Westwood and Foy; 1999).

Germination of *O. crenata* and radicle extension increased with increasing GR24 concentration (Tables 1 and 2). 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, as for *Striga* species (Ejeta *et al.,* 1993) low stimulant production could be an avenue for development of integrated management for the parasite. At the same time high stimulant producers among non-host crops may be used in rotation with faba bean to deplete the parasite seed bank. However, it has to be noted, as enunciated by several reports, that induction of germination of *O. crenata* seeds is enhanced by gibberellins (GAs) (Parker and Riches, 1993; Joel *et al.,* 1995). Enhancement of germination of *O. crenata* by GAs is consistent with the recent findings that the natural *Orobanche* germination stimulants, strigolactones,

enhanced germination of the closely related parasitic weeds, *Striga* species, by decreasing Abscisic acid (ABA)/GA ratio in seeds (Toh *et al.*, 2012). It is noteworthy that nodules in faba bean are renowned for their high GAs contents (Mamaril *et al.,* 1988). Production of GAs in faba bean nodules and their potentials for interactions with stimulants should be taken into account in breeding programms focusing on resistance based on low stimulants production.

Seeds conditioned in imazethapyr at 20-80 µM, despite lack of consistency, showed decreased germination which was more pronounced at the highest GR24 concentration (Table 1). The results indicate that imazethapyr may not consistently affect germination in practice. However, germilings from seeds conditioned in imazethapyr and induced to germinate with GR24 at 10 ppm showed significant reductions in radicle extension (Table 2). Reduction in radicle extension may reduce contact between the parasite and the host roots and thus reduces parasitism.

The herbicide imazethapyr , irrespective of rates and application time, effected considerable to significant reductions in *O. crenata* infestation (Tables 3 and 5). The herbicide at 71.4 g a.i. ha^{-1}, irrespective of application time, was the most effective and more suppressive to the parasite than at 47.6 g a.i. ha^{-1} (Tables 3 and 5). Urea at 95.2 kg ha^{-1} (1N), irrespective of application time showed considerable suppression of the parasite late in the season. The herbicide imazethapyre alone was more suppressive to the parasite emergence than when followed by urea (Table 5). The enhancement by urea of the parasite emergence in plots treated with imazethapyr is in line with the observed increase in *O. crenata* emergence with decreased attachment to chickpea (*Cier arietinum* L.) roots reported by Link *et al*., (1991) and could be attributed to a relief by nitrogen of competition between underground attached *Orobanche* seedlings. Furthermore, it is notable (Table 3) that, irrespective of rate, the herbicide was less effective when applied at planting or 45 days later. Such a decline in activity 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 prolonged 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 used. This is substantiated by the observation that the loss in activity at the higher rate (71.4 g a.i. ha^{-1}) was comparatively less than at the lower rate (Tables 3 and 5). The decline in activity of the herbicide on delaying application to 45 DAS may be due to less contact time between the herbicide and the emerging *O. crenata* spikes and/or to a growth dilution effect. Although delaying application to 45 DAS reduced activity, as evident from data on the parasite emergence, but the emerged spikes were malformed, stunted and many died soon after emergence. Such observations indicate translocation of the herbicide from the host to the parasite. Imazethapyr, an imidazolinone, is active through the soil and is easily translocated in plants through both the xylem and phloem (Garcia-Torres *et al.*, 1989). Imazethapyr when followed by urea, irrespective of the latter application date, reduced number of *Orobanche* capsules significantly (Tables 3). Reduction in capsules production entails reduction in seed production. *Orobanche* infestation had inconsistent and often non-significant effects on number of faba bean leaves (Tables 4 and 6). These findings are consistent with those obtained by Hibbere *et al.,* (1998) and Eltayb, (2010) who reported that *O. crenata* infection had no significant effects on number of faba bean leaves at 30-60 DAS. These findings are in line with the relatively quiescent nature of infestation at the early stage of the parasite development. Maximum translocation of assimilates from the host to the parasite and hence the debilitating effects of the parasite occur at and following the shoot bud stage which coincides with advanced flowering and pod stage in faba bean (Muller and Distler, 1989).

O. crenata reduced the number of faba bean pods, albeit not significantly (Tables 4 and 6). In the first season imazethapyr applied at sowing and 15 and 30 DAS, irrespective of rate, increased the number of pods over the infested control, albeit not significantly (Table 4). However, treatment made 45 DAS, showed a considerable reduction, particularly at the low rate (47.6 g a.i. ha^{-1}) and a high increment at the high rate (71.4 g a.i. ha^{-1}) (Table 4). The notable disparity in performance between the two rates may be attributed to the expected rapid uptake of the herbicide at the high rate, rapid translocation from the host to the parasite and a dilution growth effect due to the size of the parasite. In the second season imazethapyr, at 47.6 g a.i. ha^{-1}, irrespective of the supplementary urea treatment increased the number of pods over the *Orobanche* infested control. However, the increase in number of pods was only significant when the herbicide treatment was not supplemented by urea. Imazethaypr at the higher rate, irrespective of the supplementary urea treatment, increased number of pods significantly over the infested control. The herbicide alone increased the number of pods by 84%. However, when supplemented with urea at planting, 15 and 30 DAS the increase in number of pods was 86, 102 and 124%, respectively (Table 6)

In the first season imazethapyr at 47.6 g a.i. ha^{-1} applied at sowing and 15, 30 and 45 DAS increased grain yield by 72, 48.84, 40.12 and 23.9%, respectively over the infested control. The corresponding figures for the high rate were 78.85, 62.2, 59.77 and 25.85% (Table 4). The high yield obtained from treatments made at sowing together with the progressive decline in yield with delayed application suggest that combating early infestations of the parasite is important. However, a substantial contribution, to yield, due to improved control of weeds, other than *O. crenata* cannot be ruled out. The substantial decrease in yield from sub-plots treated 45 DAS compared to those from plots treated at sowing is consistent with the relative decrease in curtailment of *Orobanche* emergence (Table 3).

In the second season unrestricted *O. crenata* growth reduced grain yield by 46.94% in comparison with the *Orobanche* infested control (Table 6). Urea increased faba bean yield over the respective *Orobanche* infested control, but not significantly. All herbicide treatments, irrespective of the supporting urea treatments, significantly increased yield in comparison to the infested control. The herbicide at the low and high rates increased grain yield by 48.95 and 58.8%, respectively over the infested control. However, on supplementation with urea, at sowing, the corresponding yield increments were 87.5 and 111.4%. Delaying supplementation of the herbicide treatments with urea to 15 and 30 DAS decreased yield, though often not significantly (Table 6). The increase in yield effected by the herbicide treatments is consistent with the first season results and could be due to the effective control of the parasite. The substantial increase in yield effected by the combinations of the herbicide and urea could be due to direct effects on the parasite or indirect

effects of urea on faba bean. Urea has been reported to restrict symbiotic nitrogen fixation, reduce nodulation and nitrogenase activity, nevertheless it was reported to increase intensity of photosynthesis which in turns facilitates nitrogen accumulation and increase faba bean yield (Kocon, 2010).

The results of this study, confirm early work on the inhibitory effects of urea on germination of *O. crenata* germination and radicle extension. Furthermore the results, though do not refute the damaging effects of early developmental stages of *O. crenata* to faba bean, provide evidence, contrary to the general belief, that *O. crenata* damage is synchronized with the parasite emergence, the bulk of which coincides with the productive phase of faba bean.

Conclusions

Urea, applied during conditioning, reduced germination and radicle extension. On the other hand, imazethapyr had inconsistent effects.

Urea had no significant effects on early *O. crenata* emergence, but, significantly, suppressed late emergence. Imazethapyr at the low rate (47.6 g a.i. ha^{-1}) applied at sowing or 15 and 30 DAS consistently effected excellent early season control of the parasite, however late season control showed seasonal variability. The herbicide at the high rate (71.4 g a.i. ha^{-1}), consistently effected excellent and lasing control of the parasite.

Most *O. crenata* damage to faba bean is synchronized with the emergence phase which coincides with the crop productive growth.

To attain and maintain adequate and lasting control of *O. crenata* in faba bean and ensure excellent crop yield research has to focus on determining the appropriate herbicide and urea rates and their time of application.

References

1. 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.
2. 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.
3. 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.
4. Ejeta, G.; L. G. Butler and A. G. T. Babiker (1993). New Approaches to the Control of Striga. Striga Research at Purdue University. Research Bulletin No. 991, Pp. 27.
5. 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.
6. 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.
7. Garcia-Torres, L.; Lopez-Granados, F. and Saavedra, M. (1989) New herbicide for broomrape (*Orobanche crenata*) control in faba bean (*Vicia faba*). In: Wegmann, K. and Musselman, L. J. (eds), *Progress in Orobanche Research*. Eberhard-karl-Universitat, Tubingen Germany. Pp. 200-208.
8. Geisel, B. G. (2007). *The phytotoxic effect of ALS inhibiting herbicide combinations in prairie soils*. M.Sc thesis. University of Saskatchewan, Canada Pp 77.
9. 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.
10. 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. Pp 567- 597.
11. 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.
12. Kocon, A. (2010).The effect of foliar or soil dressing of urea on some physiological processes and seed yield of faba bean. *Polish journal of Agronomy*, **3**:15-19.
13. Link, K. H.; Singh, K. B.; and Saxena, M. C. (1991). Screening technique for resistence to *Orobanche crenata* Forcks. In chickpea. *International Chickpea Newsletter*, **24**:32-34.
14. 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. Pp 465.
15. Muller, F. and Distler, B. (1989). Transloction of glyphosate in the host/parasite system *Vicia faba* and *Orobanche crenata*. In: Wegmann, K. and Musselman, L. J. (eds). *Progress in Orobanche Research, Proceedings of the International Workshop on Orobanche Research*. Eberhard-karl-Universitat, Tubingen Germany. Pp. 226-231.
16. Parker, C. and Riches, C. R. (1993). *Parasitic Weeds of the World: Biology and Control.* CAB International, Wallingford, Oxon, UK. Pp 332.
17. 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.
18. Rubiales, D. and Fernández-Aparicio, M. (2012). Innovations in parasitic weeds management in legume crops: A review. *Agronomy for Sustainable Development,* **32**: 433-449.
19. 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. The Netherland. Pp 25-34.
20. Schloss, J. V. (1990). Acetolactate synthase, mechanism of action and its herbicide binding site. *Pesticide Science*, **29**: 283-292.
21. Toh, S.; Kamiya, Y.; Kawakami, N.; Nambara, E.; McCourt, P. and Tsuchiya, Y. (2012). Thermoinhibition uncovers a role for strigolactones in Arabidopsis seed germination. *Plant Cell Physiology*, **53**:107-117.
22. VanHezewijk, 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.
23. 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.
24. Zahran, M. K. (1973). Control of broomrape in field and vegetable crops in Egypt. *Final Technical Report, Agricultural Research Program.* Pp 4-40.