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PRELIMINARY STUDY FOR TESTING THE SIGNIFICANCE OF BUTYL–ISO–BUTYL PHTHALATE IN CONTROLLING FABA BEAN BROOMRAPE

[115]

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ABSTRACT

Due to deleterious impacts of broomrapes against the host plants, its combating is essential for sustaining crop productivity. Thus, two–year field trail was conducted at El Nubaria experimental farm, National Research Centre, Egypt, on two faba bean genotypes (Misr–3 and Sakha–1) to investigate the importance of butyl–iso–butyl phthalate as an activator for broomrape germination in comparison to glyphosate and unweeded. Findings revealed that glyphosate in 2015/16 and 2016/17 seasons in addition to butyl–iso–butyl phthalate in 2016/17 season caused significant reductions in broomrape biomass and numbers of broomrape–infected faba bean plants plot^{-1} compared to the unweeded. Plots of Misr–3 genotype treated with butyl–iso–butyl phthalate in both season as well as plots of Misr–3 or Sakha–1 sprayed by glyphosate in the first season achieved the maximum seed yield of faba bean. Since butyl–iso–butyl phthalate exhibits suicidal germination of broomrape seeds, it can be exploited as an effective and helpful tool in integrated management programs of broomrape in faba bean fields.

Keywords: Broomrape; Faba bean; Genotypes; Parasitic weeds; Suicidal germination

INTRODUCTION

Faba bean (*Vicia faba*, L.) is one of the earliest food legumes in the world. The food value of faba bean is high and it has been regarded as a meat extender or substitute due to its high protein content ranging from 20–41 % (**Crepona et al 2010**). However, broomrape (*Orobanche crenata*, Forsk) is known to be the highly damaging parasite plant affecting faba bean production in Egypt and worldwide. In this respect, yield losses vary and ranged generally from 5 to 100%, and averaged approximately 34% across all broomrape species (**Stewart and Press, 1990**). Various control methods for broomrape have been proposed, however, no single method has provided satisfactory control (**Parker, 1994; Rubiales, 2014**).

Root–parasitic plants, e.g. *Orobanche* spp., sense their host plant through the recognition of secondary metabolites released by its roots **(Yoder, 1999)**. Moreover, root-parasite seeds germinate only when they receive definite chemical signals released from potential host roots **(Joel et al 2007)**. Germination of *Orobanche* spp. seeds are usually stimulated by several compounds related to strigolactones **(Bouwmeester et al 2003).** However, spectroscopic techniques identified *Orobanche crenata* seed germination stimulators secreted by roots of faba bean plants as butyl–iso–butyl phthalate compounds (**Mohammed et al 2013**).

Therefore, the present study aimed to test the efficacy of butyl–iso–butyl phthalate for enhancing broomrape seed germination as field application in the absence of the plant host.

MATERIALS AND METHODS

Study location

At El Nubaria experimental farm, National Research Centre, Egypt, a two–year field trail was undertaken in 2015/16 and 2016/17 winter season**s**. The soil of the trail site was sandy with pH 8.1 and EC 0.32 dS m^{-1}. Maize was the preceding crop in the first and second seasons.

Procedures, treatments and design

The experiments included three practices for broomrape control (butyl–iso–butyl phthalate at 250 ppm; glyphosate–isopropylammonium (a herbicide) and the unweeded) applied with two faba bean genotypes (Misr–3 and Sakha–1). Butyl–iso–butyl phthalate as a stimulator for broomrape germination was synthesized according to the method suggested by **Liu et al (2011)**.

During seedbed preparation, super phosphate (15.5% P_2O_5) at 150 kg fad^{-1} was applied to the experimental soil. Isobutyl isophethalate sprayed, after soil preparation, followed by irrigating the soil and prior 10 days of sowing date.

On 17th and 27th November of the first and second seasons, respectively, *Rhizobium*–inoculated faba bean seeds were sown (3–4 seeds per hill), with 0.25–m space on the two sides of the ridge, followed immediately by irrigation. At 35 days after sowing (DAS), hand hoeing was performed and then plants were thinned to secure two ones per hill. Glyphosate–isopropylammonium, Round up 48% WSC (Isopropylammonium N–(phosphonomethyl) glycinate) was applied thrice (each of 75 cm^3 fad^{-1}) during faba bean flowering stages (60, 75 and 90 DAS). Nitrogen fertilizer as ammonium nitrate (33.5% N), at 40 kg fad^{-1} was added into equal portions, 35 and 50 DAS. During growth stages, plants were irrigated through trickle irrigation system using emitters of 1.5 L h^{-1} capacity.

The trail treatments were arranged through a strip– plot design using completely randomized blocks with six replicates. Genotypes occupied the vertical main plots, whereas broomrape control treatments were distributed in the horizontal ones. The plot size was 11.40 m^2; comprising five ridges each of 3.5 m length and 0.65 m width.

Measurements

Broomrape

The obtained broomrape shoots at harvest were air dried for 10 days and oven–dried for 24 hours at 80°C to a constant weight for estimating the total biomass expressed in dry weight $plot^{-1}$. Moreover, numbers of broomrape–infected faba bean plants $plot^{-1}$ were recorded.

Faba bean traits

At 105 DAS, 6 faba bean plants were randomly chosen from each plot to estimate total dry weight $plant^{-1}$. At harvest (on 24th and 27th April of the 1^{st} and 2^{nd} seasons, respectively), whole plants of the experimental unit were harvested to determine seed yield fad^{-1}.

Statistical analysis

Data of the two seasons were subjected to analysis of variance (ANOVA) according to **Gomez and Gomez (1984)**, using Costat software program, Version 6.303, 2004. The differences among means were compared using LSD test at 0.05 probability level.

RESULTS AND DISCUSSION

ANOVA revealed that both broomrape biomass and numbers of broomrape–infected faba bean plants $plot^{-1}$ markedly responded to broomrape control treatments and faba bean genotypes as well as their interaction in both seasons, except numbers of broomrape–infected plants of faba bean genotypes $plot^{-1}$ in 2016/17 season (Table 1). In this respect, each of glyphosate and butyl–iso–butyl phthalate treatments in both seasons caused significant reductions in the forenamed traits (with the superiority of glyphosate), except the effect of butyl–iso–butyl phthalate on the latter trait (i.e. numbers of broomrape–infected faba bean plants $plot^{-1}$) in the second season. Glyphosate inhibits the enzyme 5–enolpyruvyl shikimate–3–phosphate (EPSP) synthase (**Steinrücken and Amrhein, 1980**), a key enzyme in the shikimate pathway, the biosynthesis pathway of the aromatic amino acids (phenylalanine, tryptophan, and tyrosine). So, the inhibition of EPSP synthase results in limited production of such aromatic amino acids. Furthermore, accumulation of shikimate has been recognized in *broomrape* as early as 10 h after application with glyphosate (**Shilo et al 2016**), referring to the existence of the herbicide in the root–parasite cells. Besides its movement in the plant, several experiments proved that glyphosate is secreted from the roots of treated plants and also acts in the rhizosphere (**Kremer et al 2005; Laitinen et al 2007**). Accordingly, application of glyphosate showed effective control for broomrape as expressed in lowering broomrape biomass and infected faba bean plants $plot^{-1}$ **(Table 1)**. Moreover, application of butyl–iso–butyl phthalate showed marked control for broomrape through enhancing its seed germination in the absence of faba bean plant and consequently exposure to suicidal germination.

On the other hand, lower values of broomrape biomass (in both seasons) and numbers of broomrape–infected faba bean plants plot^{-1} (in the first season only) were obtained with Misr–3 genotype than Sakha–1 one **(Table 1)**. Since root–parasitic weeds need host signals to promote the starting of the parasitic accompanying, breeding for low– motivator cultivars of those modes are explicit purpose for resistance (**Yoder and Scholes, 2010**). Sources of natural resistance based on low exudation of germination–inducing factors exist in legumes and are highly effective in inhibiting broomrape weed parasitism (**Sillero et al 2005; Fernández**–**Aparicio et al 2014**)

In plots of Misr–3 or Sakha–1 genotypes, foliar application of glyphosate was the potent practice for reducing broomrape biomass and numbers of broomrape–infected faba bean plants plot^{-1} in 2015/16 and 2016/17 seasons, with no significant differences with butyl–iso–butyl phthalate application in the second season **(Table 1)**.

Table 1. Broomrape biomass and numbers of broomrape–infected faba bean plants plot^{-1} as influenced by genotypes (C) and broomrape control treatments (M) in 2015/16 and 2016817 seasons

Treatment		2015/16			2016/17		
		Misr–3	**Sakha–1**	**Mean**	**Misr–3**	**Sakha–1**	**Mean**
Broomrape biomass (g)							
Butyl–iso–butyl phthalate		35.3	179.3	107.3	10.0	10.0	10.0
Glyphosate		9.7	24.7	17.2	8.3	10.1	9.2
Unweeded		66.7	195.7	131.2	20.0	31.7	25.8
Mean		**37.2**	**133.2**		**12.7**	**17.2**	
$LSD_{0.05}$	C	47.1			2.3		
	M	21.5			2.3		
	CxM	16.2			9.0		
Numbers of broomrape–infected faba bean plants							
Butyl–iso–butyl phthalate		9.3	41.7	25.5	7.7	9.7	8.7
Glyphosate		1.7	7.0	4.3	4.0	5.3	4.7
Unweeded		18.3	65.0	41.7	10.7	19.0	14.8
Mean		**9.8**	**37.9**		**7.4**	**11.3**	
$LSD_{0.05}$	C	18.9			ns		
	M	5.0			6.3		
	CxM	11.8			6.4		

There were no noticeable variations among weeded practices, genotypes, and their interaction on total dry weight of faba bean plants estimated at 105 DAS, in both seasons, except with glyphosate x Misr–3 interaction in the first season only **(Table 2)**. Such combination showed higher value in this respect. Generally, disappearing differences in dry weight of faba bean plants among weeded practices may refer to that broomrape weed may not cause distinctive negative effect against host plants early at 105 DAS yet. Furthermore, no significant differences were detected between faba bean genotypes regarding total dry weight of faba bean plants **(Table 2)**.

Concerning the seed yield fad^{-1}, butyl–iso–butyl phthalate was as similar as glyphosate for recording higher values exceeding the unweeded in both seasons. Such increases in seed yield obtained from the aforementioned treatments may be attributed to successful control of broomrape weed reducing its parasitic impact **(Table 1)** which reduced competition and consequently favored growth of faba bean plants, then improving seed yield. Similar trend was obtained by **Ghalwash et al (2008) and Ghalwash et al (2012**). In addition, differences between yields of faba bean genotypes were not significant, in both seasons **(Table 2)**, although Misr–3 genotype, which appeared less susceptible to broomrape infection **(Table 1)**, produced yield slightly more than that of Sakha–1.

Plots of Misr–3 genotype treated by butyl–iso–butyl phthalate in both season as well as plots of each planted genotype sprayed by glyphosate in the first season achieved the maximum seed yield

(Table 2). Such finding confirms the integrative effect between faba bean genotype and broomrape control practice.

Eventually, it could be notice that butyl–iso–butyl phthalate has well potentiality against broomrape weed through enhancing its seed germination in the absence of the host (causing suicidal germination) and consequently reducing soil seed bank of broomrape. Thus, butyl–iso–butyl phthalate could be used as an effective and useful tool in broomrape integrative management programs. However, more investigations are required for identifying the significance of butyl–iso–butyl phthalate using various application rates.

Table 2. Total dry weight plant^{-1} (at 105 DAS) and seed yield fad^{-1} of faba bean as influenced by genotypes (C) and broomrape control treatments (M) in 2015/16 and 2016817 seasons

Treatment		**2015/16**			**2016/17**		
		Misr–3	**Sakha–1**	**Mean**	**Misr–3**	**Sakha–1**	**Mean**
Total dry weight plant^{-1} (g)							
Butyl–iso–butyl phthalate		160.6	152.1	156.4	130.8	126.8	128.8
Glyphosate		204.8	143.6	174.2	132.8	109.6	121.2
Unweeded		117.7	92.4	105.0	105.2	74.8	90.0
Mean		**161.0**	**129.4**		**122.9**	**103.7**	
$LSD_{0.05}$	C	ns			ns		
	M	ns			ns		
	CxM	95.6			ns		
Seed yield (ton fad^{-1})							
Butyl–iso–butyl phthalate		0.86	0.63	0.75	1.39	1.10	1.24
Glyphosate		0.74	0.78	0.76	1.16	1.09	1.12
Unweeded		0.54	0.34	0.44	0.74	0.63	0.68
Mean		**0.71**	**0.58**		**1.09**	**0.94**	
$LSD_{0.05}$	C	ns			ns		
	M	0.09			0.35		
	CxM	0.19			0.09		

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دراسة أولية لاختبار أهمية الأيزوبيوتيل فيثالات فى مقاومة هالوك الفول

[115]

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الموجـــــز

نتيجة التأثيرات الضارة للهالوك ضد عوائله النباتية، فـإن الأمـر يتطلـب مكافحتـه لاسـتدامة إنتاجيـة المحصـول. لـذلك فقـد أُجريـت تجربـة حقليـة خـلال موسمين بمزرعة النوبارية التجريبية – المركز القومى للبحـوث – مصـر، علـى صـنفين مـن الفـول البلـدى (مصـر-3، سـخا-1) لدراسة أهمية مركب الأيزوبيوتيل فيثالات كمُنشط لإنبات بذور الهالوك ومقارنته مع مبيد الجليفوسيت وبدون مُعاملة. أظهرت النتائج أن كل من مُعـاملتى الجليفوسـيت فـى موسـمى 16/2015 و 17/2016 والأيزوبيوتيـل فيثـالات فـى موسـم 17/2016 قد سببا نقصاً معنوياً مُقارنة بغير المُعاملة فى الكتلة الحيوية للهالوك وعدد نباتات الفول المُصابة. حققت القطع التجريبية للصنف مصر-3 والتى عُوملت بالأيزوبيوتيل فيثالات فى كلا الموسمين، وكذلك القطع التجريبية للصنف مصـر-3 أوالصـنف سـخا-1 والتـى عُوملـت بالجليفوسـيت فـى الموسـم الأول أعلـى كميـة لبذور الفول البلدى. وحيث أن الأيزوبيوتيل فيثالات قد سـبب إنباتـاً إنتحاريـاً لبـذور الهـالوك فمـن الممكـن إسـتغلاله كـأداة فعالـة ومُسـاعدة فـى بـرامج مكافحـة الهالوك فى حقول الفول البلدى.

الكلمات الدالة: الفول البلدى، أصناف، الهالوك، الحشائش المتطفلة، الإنبات الانتحارى

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