

# New Approach for Controlling Broomrape Plants in Faba Bean

Kandil, E. E. E.<sup>1</sup>; A. M. Kordy<sup>1</sup>; and A. A. Abou Zied<sup>2</sup>

## ABSTRACT

To improve controlling Broomrape (*Orobanche crenata*) grown in faba bean crop via new approaches as planting modern cultivars under different times of spraying Roundup<sup>®</sup> (glyphosate 48% WSC) as herbicide and foliar application of some micronutrients (Fe, Mn and Zn), was conducted in a private farm in El-Horreya village, Abou El-Matamir, El-Behira governorate, Egypt, during two successive winter seasons of 2012/2013 and 2013/2014 in sandy loam soil. The experimental design was a split-split plot design with three replications, where as the treatments were distributed at random as follows; Roundup<sup>®</sup> (glyphosate) spraying times (without, one time, or two times) were applied at main plots, while foliar spray of micronutrients (Fe, Mn and Zn) were allocated in the subplots and field bean cultivars (Nubaria1, Nubaria 2 or Nubaria 3) were located in the sub-sub plot. The main results indicated that spraying Roundup<sup>®</sup> (glyphosate 48% WSC) twice where, the first spraying applied beginning the flowering while the second spraying was applied after two weeks from the first treatment at 180 cm<sup>3</sup> (Roundup)/480 L (water)/ha.), caused significant increase of faba bean yield and reduced number and weight of broomrape as compared with other treatments. "Nubaria 2" cultivar achieved the highest mean values for most of yield characters, while the cultivar Nubaria 3 gave the lowest values. On the other hand, spraying micronutrients 2 times after 45 and 60 days from sowing achieved higher yield and its components of faba bean under the study condition in El-Behira Governorate, Egypt.

**Keywords:** Faba bean cultivars; broomrape; yield components; herbicides; micronutrients; glyphosate; Roundup

## INTRODUCTION

Faba bean (*Vicia faba* L.) is a major food and feed legume because of its high nutritional value of the seeds. It is considered one of the most important legumes in Egypt. It has become one of the strategic crops due to its income to the farmers. Also, its important for soil fertility, human nutrition as a good source of vegetarian protein animal feeding and industry purposes (Sharaan *et al.*, 2004). Increasing faba bean production and improving yield quality is the major target to meet the demand of the increasing population, since faba bean constitutes a major part of the diet of people (Zeidan, 2002).

Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is an achlorophyllous, phanerogamic holoparasite that attacks the roots of many dicotyledonous crops. It obtains carbon, nutrients, and water through haustoria which connect the parasite to the host vascular system. Broomrape infestations cause extensive reduction in crop yield, adversely affect crop quality, and result in loss of cropping alternatives in infested fields.

*Orobanche* spp. which belong to the family Orobanchaceae are obligate parasitic flowering plants. The main center of distribution is the Mediterranean basin, where large areas are heavily infested. Yield losses due to *Orobanche* range from 5 to 100 % depending on the region and the crop. *Orobanche* species infesting faba bean in the eastern Mediterranean region of Egypt are: *O. crenata* and *O. aegyptiaca* / *O. ramosa*. Production of several important crops (lentil, faba bean, tomato, potato, sunflower, etc.) is threatened by broomrapes (*Orobanche* spp.). As a result of this threat, the cultivated crop areas infected with broomrapes have been declining steadily in some countries as farmers kept abandoning the production of these crops in heavily infested fields (Bülbül, *et al.*, 2009). In highly infested areas, farmers generally avoid growing faba bean or other susceptible crops, resulting in substantial reductions to both the extent of cultivable areas and to food legume production (Besufikad *et al.* 1999). However, Broomrapes are weedy root parasites that represent a major constraint for faba bean (*Vicia faba*) cultivation in the Mediterranean area. Control methods are being developed that comprise techniques ranging from agronomical practices to precision farming, including chemical and biological control, genetic and induced resistance, modeling, and probably nanotechnology in a nearby future. However, the main concern is that to date, no single method of control provides complete protection against these parasites. For that reason, an integrated approach is needed in which a variety of such techniques are combined, in order to maintain parasite populations below threshold levels of damage (Pérez-de-Luque *et al.*, 2010). Based upon number of strategies for root-parasitic weed control have been developed including cultural practices and biological and chemical control

<sup>1</sup>Plant production Department, The Faculty of Agriculture Saba Basha, Alexandria University, Egypt.

<sup>2</sup>Legumes Crops Department, Field Crops Institute, Agric. Res. Center (ARC), Egypt.

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(Joel *et al.* 2007; Rubiales *et al.* 2009; Fernández-Aparicio *et al.*, 2011).

It is well-known that Mn, Fe, and Zn are known to be required for all higher plants (Welch, 1995). These elements have been well documented to be involved in photosynthesis, N-fixation, respiration and other biochemical pathways (Marschner, 1986; Marschner *et al.*, 2005). Most of micronutrients, for example Fe and Mn are readily fixed in soil having alkaline pH. Plant roots are unable to absorb these nutrients adequately from dry topsoil (Graham *et al.*, 1992 and Foth and Ellis, 1996). Magnesium and Mn are not easily translocated to leaves within the plant system (Foth and Ellis, 1996). Thus, the application of macro- and micronutrients in the cultivation zone may not be meeting the crop requirement for root growth and nutrient use. The alternative approach is to apply these micronutrients as foliar sprays. Foliar spray of different micronutrients has been equally or more effective as soil application by different researchers and foliar spray could be used effectively to overcome the problem of micronutrients deficiency in subsoil (Grewal *et al.*, 1997).

The management of broomrape is often difficult due to its close association with the host for all of its life cycle. Despite many management strategies tried against broomrape (reviewed by Foy *et al.* 1989; Parker and Riches 1993; Sauerborn 1991), few methods have been reliable and economical, except for use in high-value crops.

The evaluation of tolerant materials under *Orobanche* -infested and *Orobanche*-free fields was investigated by Darwish *et al.* (1999) Abdalla and Darwish (2002), Morsy and Attia, (2002), Darwish *et al.* (2007) and Abdalla and Darwish (2008). The above mentioned authors concluded that there were significant differences among genotypes for most studied traits.

Genotypes differed in the level of resistance and seed yield. The average yield for most of the resistant genotypes was two to three folds higher than the local and susceptible. Thus, the highest mean yield and relatively lowest *Orobanche* infestation was obtained from entries ILB4358, Sel.F5/3053/2003-3, and Sel.F5/3382/2003-4 as compared with others (local and susceptible ones) with highest broomrape infestation. The resistant genotypes seemed to show late *Orobanche* establishment which gave advantage over the parasite. Using these resistant genotypes the dietary requirement of the population and the nutrient depletion of the area could be fulfilled as they are using cereal-based cropping system due to the invasiveness of the weed. Meanwhile, these entries should be promoted for production and utilization of their desirable gene(s) for

further research program and can form appropriate material for an integrated control package (Teferi *et al.*, 2013).

The objectives of the present investigation were; (a) to select a proper faba bean cultivar from the tested modern three cultivars, (b) to determine the adequate number of times of foliar spray of micronutrients and (c) to select appropriate number of times of glyphosate (Roundup) spray and (d) the interaction among these factors, to achieve high yield and controlling broomrape (*Orobanche crenata*).

## MATERIALS AND METHODS

Two field experiments were carried out to test and achieve the above mentioned objectives using the three modern cultivars of faba bean (Nubaria1, Nubaria 2 or Nubaria 3) in a private farm at El-Horreya village, Abou El-Matamir, El-Behira governorate, Egypt, during two successive winter seasons of 2012/2013 and 2013/2014. The mechanical and chemical analyses of the experimental site are presented in (Table, 1) according to Page *et al.*, (1982).

The experimental design was split-split plot design with three replications and the treatments were distributed at random as follows; Roundup® (glyphosate 48% WSC) (*N*-(phosphonomethyl)glycine) spraying times were applied at main plot (without, once and twice time(s) ) beginning the flowering broomrape and after 2 weeks from the first application at 180 cm<sup>3</sup> (Roundup)/480 L (water)/ha., while foliar spray of three micronutrients (Fe, Mn and Zn) as once and twice time(s) spray at rate 480 g/ha., for each spray were in subplot, and faba bean cultivars (Nubaria1, Nubaria 2 or Nubaria 3) were located in sub-sub plot. The sub plots consisted of 5 ridges 3.5 meters in length, 60 cm in width and 20 cm between hills. Faba bean seed treated with *Rhizobium* inoculation (*R. leguminosum* cv. *Viciae bacterium*) suspension containing 10 cell bacterium per one gram. However, Nitrogen fertilizer was applied as urea fertilizer (46% N) at the rates 48 kg N/ha., as one dose. Phosphorus fertilizer was applied before planting as single Calcium- Super Phosphate (15.5 P<sub>2</sub>O<sub>5</sub>) at the rate of 480 kg/ha. Potassium sulphate (48 % K<sub>2</sub>O), was added before the second irrigation at rate of 120 kg/ha. Sowing was done on 18<sup>th</sup> and 20<sup>th</sup> of October in 2013 and 2014 seasons, respectively. The preceding crop was maize (*Zea mays*, L.) during both seasons of the study.

Glyphosate [N-(phosphonomethyl)glycine] is a systemic, nonselective, and foliar applied herbicide. It is readily translocated to underground parts, immature leaves, and meristems. It has been reported to be very stable in plants (Coupland 1984; Devine and Bandeen 1983; Gottrup *et al.* 1976).

**Table 1. Physical and chemical properties of the experimental soil sites during the two cropping seasons (2012/2013 and 2013/2014)**

Soil characteristics	Seasons	
	2013	2014
Soil texture (%)	Sandy loam	Sandy loam
Sand %	60.10	62.30
Silt %	10.60	11.50
Clay %	29.30	26.20
pH (1: 2.5 water suspension)	8.10	7.99
EC (dSm <sup>-1</sup> )	2.10	1.95
<b>Cations (meq/L.)</b>		
Ca <sup>++</sup>	7.60	9.10
Mg <sup>++</sup>	5.20	4.85
Na <sup>+</sup>	4.10	4.00
K <sup>+</sup>	0.20	0.25
<b>Anions (meq/L.)</b>		
HCO <sub>3</sub> <sup>-</sup>	2.00	1.95
Cl <sup>-</sup>	3.85	3.77
SO <sub>4</sub> <sup>-</sup>	10.50	12.20
O.M. (%)	1.85	1.90
CaCO <sub>3</sub> (%)	22.50	23.70
Available Mineral N(mg/kg)	92.40	85.60
Available P (mg/kg)	23.12	25.50

Its mode of action is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that leads to the production of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan.

#### CHARACTERS STUDIED

- Total chlorophyll content (SPAD value) of faba bean leaves was determined by Minolta Chlorophyll Meter 502. Chlorophyll a was calculated by transforming the SPAD units to mg/m<sup>2</sup> using the following equation: Chlorophyll = 1.034 + 0.308 x [SPAD] + 0.110 \* [SPAD]<sup>2</sup> according to Monje and Bugbee (1992).
- At harvest in both seasons, broomrape samples from one square meter area were randomly collected from each plot. Number and dry weight of broomrape plants/m<sup>2</sup> were determined after drying in a forced draft oven at 70 C<sup>0</sup> to constant weight.
- At harvest stage, the following data were recorded: Plant height (cm), Pod length (cm), Number of pods/plant, Number of seeds/plant, 100- seed weight (g), Seed yield (tons/ha), straw yield (tons/ha), biological yield (tons/ha) and harvest index (H.I. %) was calculated from the weight of seeds obtained from each plot.
- Faba bean seed samples were dried, grinded, weighted (0.50 g), burned, made ash solution,

diluted and then measured by using Spectrophotometer and Flam photometer for N, Fe, Mn, and Zn determination, according to Gericke and Kurmies (1952). N- values were multiplied by 6.25 to calculate protein content.

All the data collected were subjected to statistical analysis of variance according to Gomez and Gomez (1984), using CoStat (1998) program.

#### RESULTS AND DISCUSSION

The results in Tables (2 and 3) show the significant effect of times spray of roundup, foliar spray of micronutrients and cultivars of faba bean and their interactions on plant height (cm), total chlorophyll, pod length (cm), number of pods/plant, seed yield, straw and biological yields, harvest index (H.I.%) and seed protein % during two growing winter seasons. Whereas, the highest mean values from plant height, total chlorophyll (mg/m<sup>2</sup>), yield and its components of faba bean plants were achieved with spray roundup as herbicide, two times (after 4 and 7 weeks from sowing) and Nubaria 2 with no significant differences different on plant height when spray roundup was sprayed once, while the lowest values from plant height and faba bean leaves content from chlorophyll as recorded with without roundup spray. This could be due to low stimulation of broomrape germination and a delay in orobanche formation and development (Abbes *et al.*,

2006), and to a deeper root system of the host (Abbes *et al.*, 2007).

Also results in Table, 2 declared that the longest pod, highest number of pods/plant, heaviest 100- seed weight seed yield, straw and biological yields (tons/ha.), harvest index (H.I.%) and highest concentration of seed protein % of faba bean was observed due to spraying roundup twice as comparison with other treatments (once and without spray). Similar results, were obtained by Zein *et al.* (2004) who reported that the application of glyphosate at 86.4 g a.i./ha., (Two or three times) or Imazethapyer at 72 g a.i./ha., (soil incorporation) were the most effective treatments against broomrape and produced the highest faba bean yield. This reduction was exaggerated when releasing *P. orobanchia* was combined with glyphosate at 86.4 g a.i./ha., or with hand pulling (once). Releasing *Phytomyza* with glyphosate achieved the highest increase in faba bean seed yield.

Foliar sprays twice of micronutrients (Fe, Mn and Zn), led to the highest mean values of plant height (cm), total chlorophyll, pod length (cm), number of pods/plant, seed, straw and biological yields, harvest index (H.I.%) and seed protein % as compared with other treatment (spray once) during both seasons. It is clear the faba bean yield was increased and its components in the productivity of faba bean plants as results of micronutrients might have increased photosynthesis activity and consequently produced more metabolites required for building up the growing pods (Abdel Monem *et al.* 2009).

These results were in agreement with those obtained by Grewal *et al.* (1997) who revealed that dry matter production increase with application of micronutrients over control. Also, Sadana *et al.* (2002) indicated that increase dry matter yield with Mn application.

Regarding the effect of faba bean cultivars, the obtained results that there were significant differences among three cultivars, where, the cultivar Nubaria 2 gave the highest mean values of plant height (cm), total chlorophyll, pod length (cm), number of pods/plant, seed, straw and biological yields, harvest index (H.I.%) and seed protein % . While the “Nubaria 1” cv., recorded the lowest ones during both seasons.

The interactions effect between “Roundup x Micronutrients”, “Roundup x cultivars”, “Micronutrients x cultivars” and “Roundup x Micronutrients x cultivars” on plant height (cm), total chlorophyll, pod length (cm), number of pods/plant, seed, straw and biological yields, harvest index (H.I.%) and seed protein % were mentioned in (Table 2 and 3) during two growing winter seasons.

Data presented in Table (4) indicated that there was significant effect of number of spray times of roundup, foliar spray of micronutrients and three cultivars of faba bean on number and weight of Broomrape/m<sup>2</sup>, seed content of Fe, Mn and Zn (mg/g) during the two seasons. Whereas, number and weight of broomrape/m<sup>2</sup> were reduced with spraying of roundup twice as compared with others treatment (No spray and spraying once from roundup) during both seasons.

Abebe *et al.* (2013) detected that the genotype ILB1814 (susceptible check) was most strongly affected by *O. crenata*, showing the highest broomrape population and the lowest grain yield, despite its high yield potential when not infested. On the other hand, ILB4358, Sel.F5/3382/2003-4 and ILL4338 were less susceptible and may carry some genes rendering them resistant to *Orobanche*. Also, Abd El-Wahab, 2007 reported that faba bean genotypes differed from each other in their yield and yield components regarding *Orobanche* infestation.

With the respect of Table, 4 spraying micronutrients twice of (Fe, Mn and Zn) gave the lowest number and weight of broomrape/m<sup>2</sup> as compared with other treatment (one spray) during both seasons.

With regard to the faba bean cultivars, “Nubaria 2” cultivar recorded the lowest mean value of number of broomrape/m<sup>2</sup> and lightest weight of broomrape/m<sup>2</sup> in comparison with other field bean cultivars “Nubaria, 3 and 1”.

There were considerable differences among faba bean genotypes in their response to *O. crenata*. Generally, differences in infection and seed yield among the seasons can be attributed to variations in weather conditions, which are known to influence both the extent of *Orobanche* infestation and faba bean growth (Rubiales *et al.*, 2006). Also, they inducted that several criteria have been used by the authors to quantify resistance to *Orobanche* infestation, such as: number of *Orobanche* per host plant; dry matter of parasitic plants per host plant; height of the tallest parasitic shoot; number of *Orobanche* per sown surface unit, etc.

Also, in Table (4) it was observed that the highest mean values of faba bean chemical composition (Fe, Mn and Zn) was observed with spraying roundup twice as compared with other treatments (No spray and once).

Number of foliar sprays times of micronutrients exerted, significantly, on faba bean chemical composition (Fe, Mn and Zn). However, spraying micronutrients twice gave the highest concentrations of Fe, Mn and Zn (mg/g) as compared with other treatment (i.e. spraying once) during both growing seasons.

Table 2. Means of plant height (cm), total chlorophyll (mg/m<sup>2</sup>), pod length (cm), and number of pods/plant of three faba bean cultivars as influenced by Roundup, foliar spray times of micronutrients and their interactions during the two seasons (2012/2013 and 2013/2014).

Treatments	Season											
	2012/2013		2013/2014		2012/2013		2013/2014		012/013		013/014	
	Plant height (cm)	Total Chlorophyll (mg/m <sup>2</sup> )	Pod length (cm)	Number of pods/plant	100- seed weight (g)							
<b>Roundup</b>												
No spray	123.74	126.45	205.17	200.88	8.22	8.44	12.99	12.39	84.84	82.42		
Once	145.94	138.55	210.95	201.88	9.17	9.05	18.12	18.93	101.28	87.26		
Twice	144.36	140.52	226.48	223.42	9.28	9.44	20.08	15.80	94.00	86.15		
L.S.D.at 0.05	8.02	2.22	12.41	12.56	0.577	0.612	2.92	2.23	3.43	1.79		
<b>Micronutrients</b>												
Once	135.41	132.85	210.63	200.68	8.96	8.85	15.35	15.016	91.57	84.29		
Twice	140.62	137.49	217.77	216.78	8.81	9.11	18.78	16.40	95.24	86.26		
L.S.D.at 0.05	4.08	4.17	6.62	10.82	0.594	0.479	1.42	2.15	1.71	1.15		
<b>Bean Cultivars</b>												
Nubaria 1	134.34	128.59	180.99	176.82	8.33	8.33	14.40	12.29	91.89	83.26		
Nubaria 2	141.82	143.17	258.01	255.16	9.89	9.89	21.51	20.96	97.08	90.52		
Nubaria 3	137.89	133.75	203.60	194.21	8.44	8.44	15.28	13.88	91.25	82.04		
L.S.D.at 0.05	4.97	4.03	17.97	15.21	0.525	0.525	2.49	2.067	1.35	2.11		
<b>Interaction</b>												
Roundup	X	*	*	ns	ns	ns	ns	*	*	*	*	*
Micronutrients												
Roundup x cultivars	*	*	ns	ns	ns	ns	*	*	*	*	*	*
Micronutrients	X	*	**	ns	ns	ns	*	*	**	**	**	**
cultivars												
Roundup	X	*	*	ns	ns	ns	**	**	*	*	*	*
Micronutrients	X	*	*	ns	ns	ns	**	**	*	*	*	*
cultivars												

\*\*\*, significant at 0.05 level of probability.

ns: not significant at 0.05 level of probability.



Table 4. Means of number of broomrape/m<sup>2</sup>, weight of Broomrape (g)/m<sup>2</sup>, seed Fe, Mn, and Zn content (mg/g) of three faba bean cultivars as influenced by roundup, foliar spray times of micronutrients and their interactions during the two seasons 2012/2013 and 2013/2014.

Treatments	Number of Broomrape/m <sup>2</sup>		Weight of Broomrape (g)/m <sup>2</sup>		Season						
	2012/2013	2013/2014	2012/2013	2013/2014	Fe (mg/g)		Mn (mg/g)		Zn (mg/g)		
<b>Roundup</b>											
No spray	3.28	7.33	0.959	1.23	0.810	0.963	0.121	0.117	0.274	0.269	
Once	1.17	2.06	0.681	0.681	1.26	1.21	0.126	0.114	0.352	0.283	
Twice	0.61	0.83	0.256	0.256	1.44	1.43	0.149	0.142	0.379	0.291	
L.S.D.at 0.05	0.934	1.02	0.335	0.382	0.1109	0.131	0.0096	0.025	0.0151	0.0272	
<b>Micronutrients</b>											
Once	2.78	5.89	0.933	1.09	1.23	1.16	0.120	0.104	0.331	0.268	
Twice	0.59	0.93	0.330	0.356	1.11	1.24	0.143	0.144	0.339	0.295	
L.S.D.at 0.05	0.836	0.897	0.299	0.209	0.104	0.137	0.0193	0.0185	0.0219	0.0443	
<b>Bean Cultivars</b>											
Nubaria 1	2.61	5.06	0.997	1.05	0.942	0.988	0.119	0.100	0.304	0.259	
Nubaria 2	1.56	1.94	0.350	0.389	1.04	1.52	0.144	0.152	0.391	0.299	
Nubaria 3	0.89	3.22	0.548	1.05	1.52	1.10	0.133	0.119	0.310	0.286	
L.S.D.at 0.05	0.669	1.16	0.341	0.359	0.161	0.198	0.0158	0.024	0.0297	0.0248	
<b>Interaction</b>											
Roundup X Micronutrients	**	**	*	*	*	*	*	*	**	*	
Roundup X cultivars	**	**	*	*	*	*	*	*	*	*	
Micronutrients X cultivars	*	*	*	*	*	*	*	*	*	*	
Roundup X Micronutrients X cultivars	*	*	*	*	*	*	*	*	*	*	

\*, \*\*, \*: significant at 0.05 level of probability.

The highest mean values of chemical composition (Fe, Mn, and Zn) were recorded with faba bean cultivar "Nubaria 2". While the lowest ones were achieved by "Nubaria 1" cv. The first and second order interactions between "Roundup x Micronutrients", "Roundup x cultivars", "Micronutrients x cultivars" and among "Roundup x Micronutrients x cultivars" were significant, while they were insignificant during both season or only one season, on number of Broomrape/m<sup>2</sup>, Weight of Broomrape (g)/m<sup>2</sup>, seed Fe, Mn and Zn (mg/g) during the two seasons, presented in Table (4).

Iron is a constituent of many enzymes involved in the nutritional metabolism of plant. Zinc plays an important role as a metal component of enzymes (superoxide dismutase, carbonic anhydrase an polymerase) or as a functional, structural, or regulate RNA cofactor of a large number of enzymes (Kabata-Pendias and Pendias 1999).

Resistance to broomrape appears to have multiple components and to be based on a chain of escape and resistance mechanisms that either act alone or in combination and at different stages of the infection process. The increased efforts in delivering the control by resistant cultivars can be more effectively made, and the tools of modern plant breeding and of heterologous gene transfer (Dita *et al.* 2006; Rispail *et al.* 2007; Yoder *et al.* 2009) will be valuable.

Strategies of Broomrape's control have been developed but only marginal successes have been achieved. Most control methods are unfeasible, uneconomical, and hard to achieve or result in incomplete protection. Breeding for resistance is possible, but it is hampered by the lack of sufficient levels of resistance, the complexity of its inheritance and the unreliability of available screening methods. Recent achievements in the identification of resistance levels and their deployment in breeding programmes will be presented and critically discussed (Rubiales, 2014)

Table (4) showed that there were significant interactions "Roundup x Micronutrients", "Roundup x cultivars", "Micronutrients x cultivars" and "Roundup x Micronutrients x cultivars" on number of broomrape/m<sup>2</sup>, weight of Broomrape (g)/m<sup>2</sup>, seed Fe, Mn, and Zn content (mg/g) during the two seasons.

The results of this study will could be used to help the farmers to choose the appropriate cultivar for areas that are infested by *O. crenata* and will form cost effective integrated control package against this ever increasing dreadful weed. Moreover, using these Orobanche resistant genotypes, the dietary requirement

of the household could be fulfilled and the nutrient depleted area could be recovered as they are frequently using mono-cropping of cereals due to the invasiveness of *O. Crenata*.

## CONCLUSION

The present study could lead to conclude that for better faba bean yield and its components by planting cultivar "Nubaria 2" cv., with two sprays from Roundup® (Glyphosate) at (4 and 8 weeks from sowing) and two foliar sprays of micronutrients and reduce number and weight Broomrape/m<sup>2</sup> under study conditions at Abou El-Matamir, El-Behira governorate, Egypt.

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