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Research Article
Arbuscular Mycorrhizal Fungi Improved Host-plant Resistance Against Crenate Broomrape in Faba Bean

[1]D.A. Komeil and [2]H.H. Badry

[1]Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby 21545, Alexandria, Egypt
[2]Department of Soil and Water Science, Faculty of Agriculture, Alexandria University, El-Shatby 21545, Alexandria, Egypt

Abstract

Background and Objective: *Orobanche crenata* parasitism on faba bean (*Vicia faba* L.) is the most destructive factor for this crop in Mediterranean regions. Research aimed to study the alleviation of broomrape stress on faba bean utilizing arbuscular mycorrhizal fungi (AMF) in controlled experimental conditions. **Materials and Methods:** Two cultivars of faba bean (Giza 843-tolerant and Nubaria1 susceptible) were infected with AMF in the presence and absence of *O. crenata* in pot experiment. The measured growth parameters were shoot and root dry weight, nutrients' (nitrogen, phosphorous and potassium) concentration in the shoot, chlorophyll content, number of *O. crenata* attachments. Response of plants was evaluated in terms of induction of defence-related marker enzyme activities, namely, peroxidase (POX), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and total phenolics. **Results:** The obtained results showed that soil treatment with AMF gave effective control of *O. crenata* infection. Plants infected with AMF reduced the number of orobanche attachments and retarded the development of established tubercles. Significant increases were recorded in nutrients' content, chlorophyll content and phenolics in treated plants relative to untreated control plants. POX, PPO, PAL activities were keyed up in the host plant roots especially in the susceptible cultivar Nubaria1. The significance of induction was more obvious in infected than the non-infected plants. **Conclusion:** Current findings demonstrated the contribution of AMF to improve the faba bean growth and reduce the number of attached *O. crenata* specially in susceptible cultivar. The improvement of the resistance of faba bean to the aggressive broomrape could be related to specific defence responses in the host plant.

Key words: *Orobanche crenata*, mycorrhiza, broomrape, faba bean, defence response

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Corresponding Author: Doaa A. Komeil, Department of Plant Pathology, Faculty of Agriculture (El-Shatby), Alexandria University, P.O. Box 21545, Alexandria, Egypt, Tel: +201006653518 Fax: +20-3592-2780

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Faba bean (*Vicia faba* L.) belongs to the family Fabaceae and is one of the utmost globally important pulse crops, especially in Egypt. The winter-sown legume crop is the sixth most important grain legume with 4.3 M ton annual production worldwide as it is cultivated in more than 58 countries[1]. Thus, it is a widely consumed legume because of the high nutritional value of its seeds[2] whereas the finely chopped straw is used as livestock feed[3]. The main value of seed legumes, including faba bean, in cropping systems, is their ability to fix atmospheric N in association with soil rhizobacteria[4]. This unique ability reduces the dependence of farmers on extensive use of chemical fertilizers and hence, protecting soil and water quality. Besides, legumes play a critical role in crop rotation, improving soil physical conditions and decreasing the number of diseases and weed populations which in turn leads to lower consumption of herbicides and fungicides[5].

Faba bean area decreased worldwide from 1962-1991 since then has more or less stabilized at 2.1-2.7 M ha, mostly across Asia and the Mediterranean region[1]. One of the reasons for the decline is the prevalence of *Orobanche crenata* F., a major threat to faba bean growth and productivity[6]. Crop yield losses can reach up to 100% depending on infection severity and the broomrape-crop association. Crenate broomrape afflicts also many other dicotyledonous crops like peas, lentil, chickpeas and lupines, along the Mediterranean region, central and Eastern Europe and Asia[7]. Broomrapes belong to angiosperms that are directly linked to other plants by haustoria, a specialized organ. After seed germination, *O. crenata* is dependent on a host plant for all water, carbon and mineral nutrients[8]. Later, a stem arises from the tubercle and emerges above the ground producing an inflorescence, resulting in thousands of new seeds able to survive in the soil for many years. As infection and pathogenesis take place underground, damage to the crop occurs before the emergence of the parasite and diagnosis of infection. The farmer's awareness of the problem arises only when the parasite is to be seen above the ground; at that time considerable damage to the crop plants has already occurred[7]. In Egypt, infested fields are being abundant and for the farmers, as it is difficult to grow faba bean in these fields[9]. The particular characteristics, like underground development and attachment to the host roots of this weed, would make its management extremely challenging[10]. Generally, the use of resistant/tolerant cultivars is the most desirable control strategy for tackling the broomrape problem[11]. This attractive control method reduces losses without having to increase the use of chemical pesticides but unfortunately, complete alleviation of losses is not achieved through only this approach. Resistance ought to be integrated with other control methods, such as physical, chemical, cultural and biological methods, to ensure adequate protection and durability[12]. No single method can give satisfactory control for broomrapes; they only allow the reduction of infestations. Control measures used in Egypt include hand-pulling, directed spraying with glyphosate and the use of resistant and tolerant cultivars[13].

Mycorrhizal symbiosis has become a focal point of research as an alternative to chemical fertilizers and pesticides in sustainable agriculture[14]. The arbuscular mycorrhizal fungi (AMF) has demonstrated a considerable potential to reduce crop damages from broomrapes, as a biocontrol approach[15]. AMF has been shown to reduce the incidence of root parasitic plants of the Orobanchaceae, including the genera *Striga*, *Orobanche* and *Phelipanche* by reducing the production of host stimulant[15] and lytic enzymes such as chitinases, proteases and (add beta sign)-1,3 glucanases[16,17]. Altered root exudation gives rise to the effect of AMF on plant interactions with parasitic plants. Although colonization of host plant by AMF can protect against parasitic weeds, the application of AMF in agriculture is not applicable in controlling parasitic flowering plants to a large extent. AMF belonging to the phylum Glomeromycota develops mutually beneficial relationships with over 90% of terrestrial plants including agricultural and horticultural crops[18]. Concerning its biofertilizer properties, it plays a very important role in promoting plant growth[19] by providing nutritional and structural benefits, AMF also imparts other benefits to plants including improved nitrogen fixation, enhanced photosynthesis rate, production/accumulation of secondary metabolites such as phytohormones, vitamins, amino acids and/or mineralization and solubilization processes, the osmotic adjustment under stress and increased resistance against abiotic and biotic stresses[20].

The objective of this investigation was therefore to evaluate whether the AMF colonization, associated with the roots of faba bean plant susceptible cultivar, can ameliorate the performance of the host crop toward *O. crenata*. The practice of combining two environmentally-friendly control methods, namely, AMF and resistant/tolerant cultivars in combating *O. crenata* may offer a key to successful control management.

MATERIALS AND METHODS

Study area: The experiment was carried out in the greenhouse of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt to assess the effect of arbuscular mycorrhiza fungi (AMF) against *Orobanche crenata* in two winter growing seasons (2018/19 and 2019/20) and data were combined.

Biological materials: Two faba bean cultivars, Giza843 and Nubaria1 were chosen for this study because of their respective tolerance and susceptibility to *O. crenata*. The certified seeds were obtained from the Field Crop Research Institute, Agricultural Research Center, Giza, Egypt.

The *O. crenata* seeds, used in this study were collected from parasitic plants growing under faba bean cultivation at Experimental Station of the Faculty of Agriculture, Alexandria University, Alexandria, Egypt, during the winter seasons of (2017/18 and 2018/19). Seed viability test was performed using 2,3,5-Triphenyl Tetrazolium Chloride as described by Thorogood *et al.*[21]. Broomrape seeds were treated with a 1% solution of TTC in water and the Petri dishes were sealed and incubated in darkness at 30°C for 24 hrs. Seeds were considered viable if they displayed a pink/red colour.

A mixture of AMF strains, *Glomus intraradices*, *G. clarodium*, *Paraglomus* spp., *Acaulospora* sp., *Scutellospora* sp., *Entrophospora* sp. and *Glomous* sp. were used in this study. The local strains were isolated from maize (*Zea mays* L.) rhizosphere of agricultural soils in Borg El-Arab, Alexandria, Egypt. The AMF consortium was confirmed by a molecular technique using VAACAU primer (5'-TGATTCACCAATGGGAAACCC-3') and VAGLO primer (5'-CAAGGGAATCGGTTGCCCGAT-3') which as specific to the AMF[22]. To produce AMF inoculum, fungal spores were surface-sterilized as described in Bécard and Fortin[23] and cultured onto onion using sterile sand as a substrate. About 30 days after sowing, substrate, mycelia, spores and colonized onion roots were harvested and used as inoculum. Root colonization by AMF was determined using the gridline intersect method[24].

Pot experiment: Plastic pots (25 cm diameter), containing 3 kg of soil collected from Agriculture Research Station, Abees, belong to the Faculty of Agriculture, Alexandria University. The analysis of the used soil is presented in Table 1. All the fertilizers were broadcasted on all pots and incorporated below the soil surface before the beginning of the experiment at a rate of 0.3 g pot^{-1} ammonium sulfate, 0.15 g pot^{-1} potassium sulfate and 0.15 g pot^{-1} calcium superphosphate (15% P_2O_5). These rates were applied according to the recommended program by the Ministry of Agriculture and Land Reclamation. As for the superphosphate, only half of the recommended dose was added to allow the AMF to play its role in facilitating the phosphorus element for absorption by the plant roots. The pots were arranged according to a completely randomized block design (RCBD) in an environmentally controlled greenhouse. Six milligrams of *O. crenata* seeds were thoroughly mixed with the top 6 cm soil in each pot of the treatments receiving *O. crenata*. Five grams containing approximately 5000 spores of AMF inoculum were mixed with the top 6 cm of pot soil in the relevant treatments. Four faba bean seeds from both cultivars (Nubaria 1 and Giza 843) were sown at 3 cm depth from the soil surface of each pot. Faba bean was thinned to two plants per pot 15 days after sowing (DAS). *O. crenata* infested and uninfested controls were included in each experiment for comparison. Four pots were used as replicates for each treatment as well as the controls and subsequent irrigations were made every 2 days. At harvest, effects of the tested bio-control agent in reducing numbers of the attached *O. crenata* were recorded after carefully washing off the roots. The number of *O. crenata* attachments per pot and their dry weight (g per plant) were recorded 12 weeks after plant emergence. The growth parameters of faba bean plants as plant height (cm) and plant dry weight (g) were recorded at 90 DAS.

Total soluble phenolic content measurements: Total phenol estimation was carried out using Folin Ciocalteu reagent according to the method described by Jiang and Zhang[25].

Defence enzymes assays: Procedures were carried out as described by Mabrouk *et al.*[26] for the phenylalanine ammonia-lyase (PAL), peroxidase (POX) and polyphenol oxidase (PPO) assays. Fresh root tissues from healthy and treated faba bean plants. The extraction buffer (ratio 1:3, wt/v) containing 100 mM KH_2PO_4/K_2HPO_4 (pH7), 1% (v/v) Triton

Table 1: Characteristics of the experimental soil

		Available nutrient (ppm)					Mechanical analysis (%)			
pH	EC				OM	$CaCO_3$				
Soil extract 1:1	(dS m^{-1})	NO_3	P	K	(%)	(%)	Clay	Silt	Sand	Soil texture
7.7	0.31	22	18	560	0.4	5	41	23	36	Clay-loam

X-100 and 2% (wt/v) insoluble polyvinyl pyrrolidone was added. The mixture was homogenized and centrifuged for 20 min at 10,000 g (4°C). The supernatant was used immediately for total protein quantification and enzyme assays[27].

Phenylalanine ammonia-lyase (PAL) activity was determined by measuring the absorbance at 290 nm of the amount of cinnamic acid formed after incubation of the crude enzyme with L-phenylalanine for a fixed time. The reaction mixture containing 1.4 mL of borate buffer (100 mM, pH 8.8), 0.6 mL of L-phenylalanine (100 mM) and 0.3 mL of the crude enzyme. Following 2 hrs of incubation at 40°C, the reaction was stopped by the addition of 0.05 mL of 5 M HCl. The activity was expressed as EU mg^{-1} root fresh weight per hour.

Soluble peroxidase (POX) activity was determined by measuring the absorbance at 470 nm in a reaction medium containing 9 mM guaiacol, 1 mM hydrogen peroxide and crude enzyme extract and expressed as EU mg^{-1} root fresh weight min^{-1}.

Polyphenol oxidase (PPO) activity was determined by measuring the absorbance at 410 nm using catechol as substrate. Initially, 200 µL of crude extract was mixed with 700 µL of phosphate buffer 0.1 M pH 7 and reaction was started by adding 100 µL 0.2 M catechol and expressed as EU mg^{-1} root fresh weight min^{-1}.

Plant and soil analysis

Determination of nutrients' contents in faba bean: Total nitrogen in faba bean shoots was determined by the Kjeldahl distillation method according to Bremner and Mulvaney[28]. Total phosphorus was colourimetrically determined at 410 nm using a spectrophotometer according to Murphy and Riley[29]. Potassium concentration was determined by flame emission spectrophotometer at 766.5 nm using flame photometer according to Chaves *et al.*[30].

Determination of total chlorophyll contents: Chlorophyll a and b in fresh faba bean leaf extracts were determined according to Albanese *et al.*[31]. Using 200 mg FW, leaf material ground in a prechilled mortar in the presence of 3 mL acetone 100% (v/v). After complete extraction, the mixture was filtered and the volume adjusted to 10 mL with cold acetone. The absorbance of the extract was read at 664, 645 nm and pigment concentrations were calculated. Total chlorophyll contents (a+b) were calculated using the equation represented by Ritchie[32].

Statistical analysis: Analysis of variance was carried out using Proc Mixed in the SAS software package (SAS 9.4). A factorial experimental design was used to investigate the variations in the studied parameters as affected by the soil treatments, faba bean cultivars and their interactions. Means were compared using the Least Significant Difference (LSD) procedure at 0.05 level of probability.

RESULTS

Data presented in Table 2 revealed that arbuscular mycorrhizal fungi (AMF) promoted almost all growth criteria of the two faba bean cultivars (Nubaria 1 and Giza 843) compared to the corresponding untreated control plants at 90 days after sowing (DAS). The increase in growth parameters was often significant in comparison with the untreated control plants, either alone or in presence of *O. crenata*. Furthermore, the differences between cultivars were significant concerning the above-mentioned parameters. Results cleared that the cv. Giza843 was superior in most growth parameters compared with the cv. Nubaria1.

While the lowest significant biomass resulted from soil treatment with *O. crenata*, the presence of AMF, either alone or in presence of *O. crenata* resulted in a significant increase in the faba bean biomass. Faba bean

Table 2: Effect of AMF inoculation on growth parameters of two *V. faba* L. cultivars infected with *O. crenata* under greenhouse conditions at 90 DAS

	Growth parameters			Total phenol (mg mL^{-1})
Treatments	Shoot dry weight/plant (g)	Root dry weight/plant (g)	Total dry weight/plant (g)	dry weight
Faba bean cultivar				
Nubaria 1	6.06[b]*	4.67[b]	10.37[b]	6.00[b]
Giza 843	6.81[a]	5.14[a]	12.40[a]	8.43[a]
$LSD_{0.05}$	0.57	0.33	0.57	0.37
Soil amendment				
without any treatment (control)	6.97[ab]	5.12[b]	12.09[b]	4.47[d]
+ AMF	7.52[a]	5.65[a]	13.53[a]	7.33[b]
+ *O. crenata*	4.84[c]	3.84[c]	8.68[c]	6.55[c]
+ AMF + *O. crenata*	6.4[b]	5.02[b]	11.96[b]	10.50[a]
$LSD_{0.05}$	0.81	0.46	0.81	0.52

*Means in the same column followed by the same letters are not significantly different at 0.05 level of probability, $LSD_{0.05}$: Least significant difference at 0.05 level of probability. AMF : Arbuscular mycorrhizal fungi, DAS: Days after sowing.

Table 3: Effect of AMF on *O. crenata* growth parameters in two *V. faba* L. cultivars under greenhouse conditions at 90 DAS

Cultivars	Treatments	Total number of *O. crenata* attachments	Total *O. crenata* fresh weight (g)	Total *O. crenata* dry weight (g)
Nubaria1	+ *O. crenata*	11.00^{Aa*}	16.70^{Aa}	8.41^{Aa}
	+AMF + *O. crenata*	4.67^{Ab}	8.44^{Ab}	3.72^{Ab}
Giza843	+ *O. crenata*	5.33^{Ba}	8.71^{Ba}	3.57^{Ba}
	+ AMF + *O. crenata*	2.67^{Bb}	2.89^{Bb}	2.37^{Aa}
$LSD_{0.05}$		1.6	1.82	1.7

*Means marked with the same capital letter within the same parameter indicate no significant difference between cultivar under the same soil treatment. Means marked with the same small letter within the same parameter indicate no significant difference among soil treatments for the same faba bean cultivar. $LSD_{0.05}$: Least significant difference at 0.05 level of probability. AMF: Arbuscular mycorrhizal fungi, DAS: Days after sowing.

dry weight amounted to 13.53 and 11.96 g for treatments with AMF alone and AMF + *O. crenata*, respectively, against only 8.68 g in presence of *O. crenata* alone. The positive effect of AMF on the increase of faba bean dry weight was more significant despite the presence of parasitic weeds and it was even more significant in weed-free treatment.

Number of broomrape attachments: The number of *O. crenata* attachments per faba bean plant was significantly variable among the two tested faba bean cultivars as shown in Table 3. Less number of broomrape tubercles was attached to the faba bean resistant cv. Giza843 (5.33), compared to the susceptible cv. Nubaria1 (11.00). Moreover, inoculation with AMF did not affect only the number of total *O. crenata* attachments but also the fresh and dry weights of *O. crenata* attachments. AMF inoculation led to less fresh and dry weights of broomrapes associated with cv. Giza843, amounting to 2.89 and 2.87g, respectively, while fresh and dry weights of broomrapes associated with cv. Nubaria1 reached 8.44 and 3.72 g, respectively, in presence of AMF.

Analysis of some biochemical parameters

Phenolic compounds accumulation in response to mycorrhiza treatment: Analysis of root phenolic contents in control plants of both tested cultivars showed a significant increase of these compounds' concentration in response to *O. crenata* infection compared to non-infected plants (Table 2). A higher value for total phenols was observed for cv. Giza843 (8.43 mg mL^{-1} DW), than for cv. Nubaria1 (6.00 mg mL^{-1} DW). Application of AMF significantly increased the phenolic compounds' production and accumulation in non-infected (7.33 mg mL^{-1} DW) and infected (6.55 mg mL^{-1} DW) plants as compared to the control (4.47 mg mL^{-1} DW).

PAL, POX and PPO activities: Figure 1a represents the means of PAL activity of both faba bean cultivars as affected by the different soil treatments. It was clear that the highest significant PAL activity was reported for the AMF colonized plants, in presence of broomrape infection, amounting to 4.5 and 7.1 UE mg^{-1} root FW h^{-1} for cv. Nubaria1 and cv. Giza843, respectively. The intermediate PAL activity was detected in roots colonized only by AMF and roots infected by *O. crenata* infestation alone, while the lowest significant PAL activity for both cultivars was associated with no soil treatments and reached 1.3 and 1.5 UE mg^{-1} root FW h^{-1}, for cv. Nubaria1 and cv. Giza843, respectively.

Similarly, POX activity illustrated in Fig. 1b, revealed that faba bean roots produced the lowest significant enzyme values in non-treated soils, amounting to 11.5 and 16.3 UE mg^{-1} root FW h^{-1}, for cv. Nubaria1 and cv. Giza843, respectively. On the other hand, in presence of AMF and *O. crenata* in the soil, the POX activity significantly increased for the two respective cultivars reaching 25.7 and 32.4 UE mg^{-1} root FW h^{-1}.

Moreover, Fig. 1c demonstrated the PPO activity for both faba bean cultivars. In the case of cv. Nubaria1, the highest significant PPO activity accompanied roots colonized by AMF alone (1.5 UE mg^{-1} root FW h^{-1}) or roots infected with *O. crenata* (1.9 UE mg^{-1} root FW h^{-1}), while, cv. Nubaria1 alone, without any soil treatment, produced the lowest significant PPO activity (0.7 UE mg^{-1} root FW h^{-1}). As for cv. Giza843, the highest significant PPO activity was detected in presence of both AMF and *O. crenata* in the soil (3.8 UE mg^{-1} root FW h^{-1}), followed by the *Orobanche crenata* infested condition (3.2 UE mg^{-1} root FW h^{-1}) and then AMF treated soil (2.7 UE mg^{-1} root FW h^{-1}), while the lowest significant PPO was a characteristic of the faba bean cultivar alone (1.5 UE mg^{-1} root FW h^{-1}).

Nutrients' concentration and photosynthetic performances: The results of total chlorophyll content showed highly significant differences among the two tested cultivars in Fig. 2. In general, cv. Giza843 possessed the highest content of total chlorophyll compared to cv. Nubaria1. The presence of

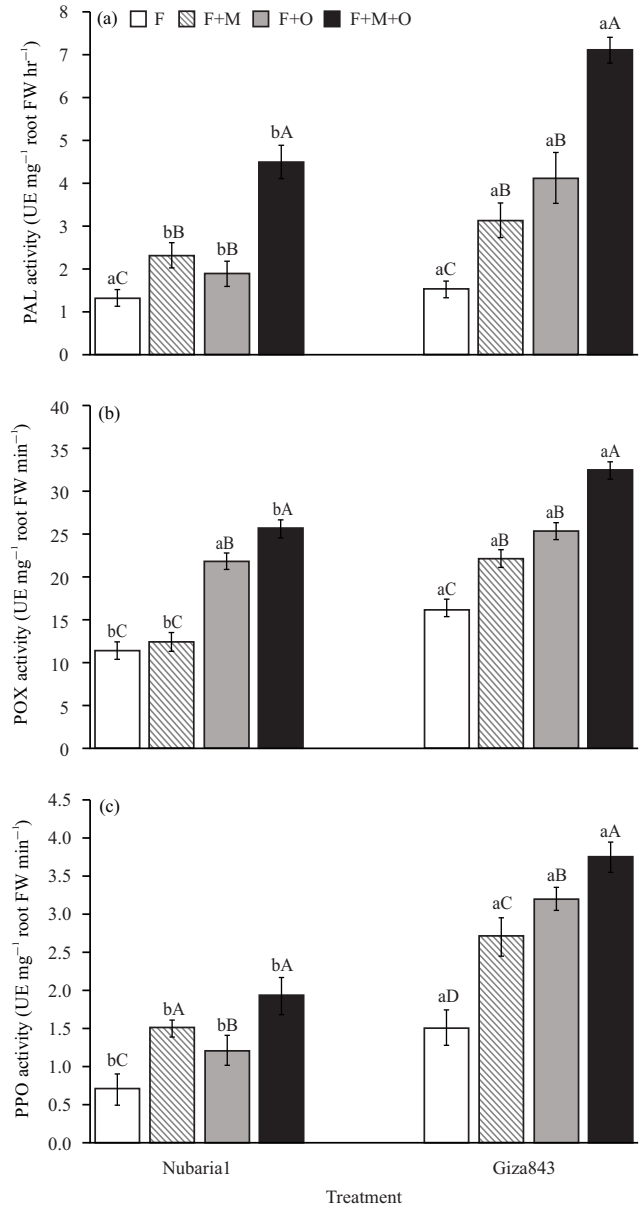

Fig. 1(a-c): (a) Phenylalanine ammonia-lyase (PAL), (b) Polyphenol oxidase (PPO) and (c) Peroxidase (POX) activities in roots of the susceptible (cv. Nubaria1) and resistant (cv. Giza843) faba bean cultivars, infested/non-infested by *O. crenata* in presence/absence of AMF after 90 DAS.

F: FB, F+O: FB + *O. crenata*, F+M: FB + AMF, F+M+O: FB + AMF + *O. crenata,* FW: Fresh weight, FB: Faba bean AMF : Arbuscular mycorrhizal fungi. Data are means of four replicates for each treatment with SE indicated by vertical lines. Bars marked with the same lower-case letters within the same parameter indicate no significant difference between cultivars under the same soil treatment. Bars marked with the same upper letters within the same parameter indicate no significant difference among soil treatments for the same faba bean cultivar.

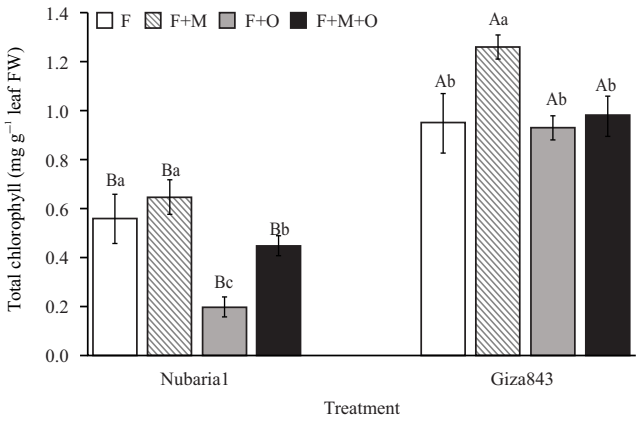

Fig. 2: Total chlorophyll in the leaves of the susceptible (cv. Nubaria1) and resistant (cv. Giza843) faba bean cultivars, infested/non-infested by *O. crenata* in presence/absence of AMF after 90 DAS.

F: FB, F+M: FB + AMF, F+O: FB + *O. crenata*, F+M+O: FB + AMF + *O. crenata,* FW: Fresh weight, FB: Faba bean, AMF: Arbuscular mycorrhizal fungi. Data are means of four replicates for each treatment with SE indicated by vertical lines. Bars marked with the same lower-case letters within the same parameter indicate no significant difference between cultivars under the same soil treatment. Bars marked with the same upper letters within the same parameter indicate no significant difference among soil treatments for the same faba bean cultivar.

AMF with both cultivars increased the values of total chlorophyll reaching 0.65 and 1.26 mg g^{-1} leaf FW for cv. Nubaria1 and cv. Giza843, respectively. Compared to the non-infested conditions, *O. crenata* significantly decreased the chlorophyll contents in the infested plants for the susceptible cultivar only (0.2 mg g^{-1} leaf FW), whereas no significant change was recorded for the tolerant cultivar (0.93 mg g^{-1} leaf FW). However, in *O. crenata* - infested condition, plants infected with AMF exhibited a significant increase in chlorophyll content for both tested cultivars, Nubaria1 (0.45 mg g^{-1} leaf FW) and Giza843 (0.98 mg g^{-1} leaf FW).

It was clear from Table 4 that the soil treatment with AMF had a positive significant effect on faba bean shoot nutrients' concentration, where the highest significant nitrogen, phosphorous and potassium (N, P and K) concentrations in the plant tissues were reported for the AMF infection alone and AMF + *O. crenata*, against the soil treatment with *O. crenata* that negatively affected the contents of the three nutrients. No significant difference was recorded in nutrients' concentration in both tested cultivars except K% which was significantly higher in cv. Giza843. The highest nutrients' concentration (N, P and K) was observed under AMF condition in the

Table 4: Nutrients' concentration (N, P and K) in shoot and AMF infection percent in the root of two *V. faba* L. cultivars infected with *O. crenata* under greenhouse conditions at 90 DAS

Treatment	AMF infection (%)	Nutrient concentration (%)		
		N	P	K
Faba bean cultivar				
Nubaria 1	29.45[a]*	2.17[a]	0.24[a]	1.88[b]
Giza 843	30.28[a]	2.23[a]	0.26[a]	2.03[a]
$LSD_{0.05}$	2.37	0.12	0.03	0.12
Soil amendment				
Without any treatment (control)	15.00[b]	1.99[c]	0.24[bc]	1.84[c]
+ AMF	46.12[a]	2.73[a]	0.30[a]	2.26[a]
+ *O. crenata*	15.00[b]	1.75[d]	0.19[a]	1.67[c]
+ AMF + *O. crenata*	43.35[a]	2.34[b]	0.27[ab]	2.04[b]
$LSD_{0.05}$	3.35	0.17	0.05	0.17

*Means in the same column followed by the same letters are not significantly different at 0.05 level of probability. $LSD_{0.05}$: Least significant difference at 0.05 level of probability, AMF: Arbuscular mycorrhizal fungi. DAS: Days after sowing.

cultivated faba bean which recorded 2.73, 0.30 and 2.26%, respectively. Whereas, the nutrients' concentration of N, P and K by both cultivars under the stress of *O. crenata* alone were significantly lower compared to other treatments. The nutrients concentration (%) under *O. crenata* infestation in presence of AMF was found to be high when compared to its controlled condition free of AMF.

DISCUSSION

The application of resistant cultivars only to control *Orobanche crenata* will reduce the diversity of faba bean strains and deprive the farmer of the advantages of other cultivars, such as high productivity and quality of grains. Therefore, the integration of arbuscular mycorrhizal fungi (AMF) as a biological agent with tolerant/susceptible cultivars for the management of broomrapes' infection was investigated.

To confirm the behaviour of two faba bean cultivars (Giza843 - tolerant cultivar and Nubaria1 - susceptible cultivar) to *O. crenata*, artificial infestation experiments were carried out in pots due to the difficulty to control the environment, the inoculum density, its origin and its distribution uniformly in the soil. Tolerant and susceptible faba bean cultivars showed different responses to *O. crenata* infestation. The tolerant cv. Giza843 exhibits a better control of *O. crenata* infestation than the susceptible cv. Nubaria1 as the number of *O. crenata* attachments developed on the host roots and *O. crenata* dry weight was lower for the tolerant cultivar. It was noticed that cv. Giza843 had a good resistance level compared to susceptible cultivar but no complete resistance was detected. Various mechanisms of resistance against broomrape have been explained by different studies such as physicals barriers and bio-chemicals related to germination stimulants or inhibitors production[9]. The resistance avoidance mechanism of cv. Giza843 to prevent *O. crenata* parasitism is based on both low infection level and low impact of parasitism on vegetative development and yield components[33]. Reduction of stimulant and/or production of inhibitors of broomrape seeds germination is not recorded[34]. Morphological parameters values seem to be affected by *O. crenata* too. Thus, for both cultivars, the parasitism effect was greater with plant biomass reaching a 28.21% reduction. This significant reduction of dry weight was following the results of Mesa-Garcia and Garcia-Torres[35], where four *O. crenata* spikes per plant were sufficient to reduce susceptible faba bean seed yield to half. Besides, the destructive effects of *O. crenata* on the physiological characters of the faba bean plant were recorded. In general, total chlorophyll content was significantly reduced under broomrape parasitism. The decrease of chlorophyll content became clearer in the susceptible cultivar more than in the tolerant one. These results might be due to the negative effect of *O. crenata* on the photosynthetic process, consequently decreasing chlorophyll concentration. The germinated *O. crenata* seeds compete with the host and depend totally on the host for all nutrition, drawing sugars and nitrogen compounds from the phloem and also drawing most of their water from the host xylem[7]. Similarly, Mauromicale *et al.*[36] indicated that *O. crenata* caused a significant and progressive decrease of tomato leaf chlorophyll content. It was shown that the chlorophyll content depended on the *Orobanche* infection; it was lower in the host plant infected with broomrape than the uninfected one.

Obtained results showed that both cultivars are extensively colonized by AMF. Colonization by AMF improved the growth of faba bean and this growth enhancement was not influenced by the presence of *O. crenata* in both cultivars.

Despite the number of broomrape attachments, an increase in the dry weight of faba bean plants was recorded. The increase in plant dry weight of mycorrhizal faba bean infected with *O. crenata* compared to the control without AMF may be due to its ability to cope with the competition created by the parasite to the host which increases the metabolism activity of the host plant. This research work reported that, despite the high susceptibility of cv. Nubaria1 to *O. crenata* infection, the usage of AMF inoculation reduces the number of *O. crenata* attachments by 57.55%. Besides, the use of AMF with the tolerant cultivar improved the broomrape control by reducing the number of attachments by 49.91%. A previous study by Akiyama *et al*.[37] showed that parasitic weeds and AMF hyphal branching are induced by the same signal, which is plant strigolactones sesquiterpenes. Results showed that the well-established AMF colonization did not prevent *O. crenata* infection, however, application of AMF reduced parasitic weed infection. Both cultivars, cv. Giza 843 and cv. Nubaria 1 produce root exudates able to induce germination of *O. crenata* seeds. The reduction of the number of attachments in both cultivars in the present investigation may be due to the decrease in germination stimulant formation by faba bean roots after AMF colonization. The low number of established infection events with tolerant cultivars allows suggesting that AMF raised up resistance of cv. Giza843 to escape from *O. crenata* injury. The decrease in the germination stimulant produced by other hosts, such as sorghum, pea and tomato, after AMF colonization were reported[15,38-40]. Moreover, the good establishment of AMF in faba bean roots suggests that the plants had become more tolerant as they grew older. Further studies are needed on cv. Nubaria1 with less number of *O. crenata* events, higher dry weight under AMF condition can be considered for cultivation in naturally infested soil. Moreover, inoculated plants with AMF under *O. crenata* stress reached levels of photosynthetic capacity (estimated by the chlorophyll content) near to those of non-stressed plants, showing that in this respect, AMF is capable to counterbalance *O. crenata* infection. Accordingly, the use of AMF for integrated management of parasitic weeds was proposed[15,41].

The effective role of AMF to improve crop performance owing to enhanced nutrient content in plants by the successfully colonized roots cannot be overlooked. The reduction in the growth characteristics, such as plant dry weight, of infested faba bean plants is due to the absorption by parasitic broomrape of key nutrients that the host plant requires for growth, especially N, P and K[42]. This investigation reported that AMF can offset this reduction and increase the nutrients' content of faba bean in presence of crenate broomrape by 33.71% N, 42.11% P and 18.14% K which may have a role to avoid damage caused by the parasitic plant, specially in the susceptible cultivar. AMF, after establishing symbiosis, produce extensive underground extra-radical mycelia ranging from the roots up to the surrounding rhizosphere, thereby helping in improving the uptake of nutrients specifically N, P and K the premier's growth-limiting factor in higher plant[43]. The AMF infection enabled the host plant to be more vigorous which resulted in potential resistance to pathogen attacks[44]. Apart from the reduction in parasitic weed infection attributed to AMF, the significant level of faba bean root covered with the fungus may lead to the prohibition of the haustorium penetration of *O. crenata* into the root host.

Current results indicated the significant ability of AMF in lowering oxidative damages in faba bean roots infected by *O. crenata*. AMF can induce resistance by the production of phytoalexin and the antioxidant enzymes in plants as an active defence response[44]. The biosynthesis of phytoalexin, chitinases and glucanases are responsible for pathogen inhibition. Also, certain proteins, peptides and low molecular- weight compounds produced by AMF may act as elicitors of plant resistance and show plant defence responses[17]. Present results showed, that resistant faba bean cv. Giza843 exhibited a higher accumulation of phenolic compounds under *O. crenata* infection, compared to the susceptible cv. Nubaria1. Similar results were reported by Briache *et al*.[45]; Pérez-deLuque *et al*.[46] in response to *O. crenata* infection. These defence responses may include the elaboration of cell wall thickenings usually accompanied by the deposition of lignin, a polymer of aromatic phenolics. This thickening limited the infection process and played an important role as a physical barrier to stop the broomrape invasion. As with all biotic and abiotic stresses, parasitic weeds infection induces oxidative stress in host plant. To endure oxidative damage under unfavourable conditions, plants possess both non-enzymatic and enzymatic antioxidants such as PAL, POX and PPO[47]. The PPO enzyme is involved in the oxidation of polyphenols into quinones (antimicrobial compounds) and lignifications of plant cells during the microbial invasion and also may participate in the responding defence reaction and hypersensitivity by inducing plant resistance against fungi[48]. Also, the PAL enzyme catalyses the first committed step of the core pathway of general phenylpropanoid metabolism. The induction of PAL is correlated with increased resistance to pathogenic infestation[49].

The POX is present in all plants species and has many diverse functions, including H_2O_2 detoxification and the formation of reactive oxygen species. These toxic intermediates cause an oxidative burst in response to pathogens leading to lignin biosynthesis that forms the structural barrier against invading pathogens[50]. In the present results, resistant cv. Giza843 was characterized by high PAL, POX and PPO activities in normal conditions which are in contrast with susceptible cv. Nubaria1. For both cultivars, *O. crenata* infestation further increased the activity of these enzymes. The increase in the activities of the investigated defence enzymes, PAL, POX and PPO, in faba bean plants seems to be a defence mechanism induced by AMF colonization against *O. crenata* haustorium invasion. In the current investigation, plant inoculated with AMF stimulated PAL, POX and PPO activities in roots of both studied cultivars under *O. crenata* infected and non-infected conditions. These stimulatory effects confirm the effectiveness of AMF treatment in improving the resistance of faba bean plants against *O. crenata*, especially for the susceptible cultivar. Other studies reported a significant effect of AMF on increasing antioxidant enzymes activities leading to improved plant resistance against pathotypes with different host plant-pathogen interactions[51,52]. Another mechanism of AMF to protect the plant from biotic disease by increases the accumulation of non-soluble polysaccharides and lignin in the cell walls of plant roots can constitute a physical barrier of fungal disease infections[44].

CONCLUSION

Adoption of an integrated approach encompassing AMF inoculation may provide a sustainable, cheap and easy method to apply for *O. crenata* control under subsistence low-input farming systems. The AMF colonization improves the tolerance of plants to stressful *O. crenata* by bringing about several changes in their morpho-physiological traits. The integration between mycorrhiza and susceptible cultivar increase the protection of faba bean plants against branched broomrape invasion. Because of the high dominance of variable in field conditions such as management, climate and environmental effects can intensify or weaken the effects of treatments.

SIGNIFICANCE STATEMENT

This study discovered that AMF can enhance defence response in the host to reduce *O. crenata* infection in the host. This fact can be beneficial for sustainable agriculture to be integrated into the control program of controlling broomrapes. This study will help the researcher to uncover the critical areas of using AMF as an ecofriendly agent to control broomrapes that many researchers were not able to explore. Thus, a new theory on the relation between AMF and resistance/susceptibility of faba bean to *O. crenata* infection may be arrived at.

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