

Effect of Some Broomrape Control Methods on Growth and Seed Yield Attributes of Faba Bean (*Vicia Faba* L.) Cultivars

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Abstract: Broomrape (*Orobanche crenata* Forsk.) is a major root-parasite faba bean (*Vicia faba* L.), that seriously limits crop cultivation in Egypt. This parasitic weed is difficult to control and difficult to evaluate and the resistance identified so far is of polygenic nature. The present investigation was carried out at the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt during 2011/2012 and 2012/2013 seasons. Four faba bean cultivars i.e. Giza 3, Misr 1 and Giza 843 compared with Triple white cultivar (susceptible) were evaluated on an *Orobanche* naturally infested soil and an *Orobanche* free soil. Three controlling methods (Coverage with polyethylene, foliar application of glyphosate herbicide and biological control with *Trichoderma* fungi) were applied for resistance to broomrape in addition to control (without any treatment). At harvest date, all the weed control methods recorded significantly lower broomrape population than weedy control. Application of glyphosate recorded the lowest broomrape population. Our results showed host plant specific qualitative differences in the composition of carbohydrates, proteins and phenols. Detection of carbohydrates, proteins and phenolic acids in leaves of faba bean, indicated the increasing carbohydrates, proteins and phenols in tolerant host plants than susceptible. Results showed the superiority of Giza 843 in *Orobanche* and therefore these cultivars is recommended for breeding to tolerant *Orobanche*. There was a negative and high correlation between total dry weights of *Orobanche*, infection severity and both of total carbohydrates and phenols.

Keywords: Faba bean, *Orobanche*, resistance, infection severity and correlation.

INTRODUCTION

Faba bean (*Vicia faba* L.) is the most important food legume in Egypt. It is very important as source of plant protein and plays a good role in farming systems as a break crop in intensive cereals systems. There is a need to increase productivity and total production to meet the increasing demand for faba bean in Egypt. This could be achieved through enhancing crop breeding and agronomy research. Legume production in the Mediterranean countries suffers considerable damage from infestation with the root-parasitic weed (*Orobanche crenata*) which can inflict devastating yield losses on faba bean. In Egypt, broomrape can cause total crop failure whereas the percentage of infection by *Orobanche* Spp. could reach up to 90-100% (Anonymous, 1994). On the other hand, most of the life cycle of broomrape occurs below the ground, where it connects to the root of the host plant such fabe bean and is, therefore very difficult to be controlled either by agronomic practices or herbicides (Goldwasser *et al.*, 2003). Due to this, the available methods of control against root parasitic plants have not proven as effective, economical and applicable as predicted (Joel, 2000b; Goldwasser and Kleifeld, 2004). Although several potential control measures were developed over the past few decades for some crops, 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 weedy root parasites to date is through an integrated approach, combining a variety of measures in a concerted manner. Many attempts have been made to devise control methods against *Orobanche* spp.

Hence, various methods techniques were suggested to control broomrapes and /or alleviate it effects. Unfortunately, the majority of these methods do not offer satisfactory results. Therefore, the search for control methods has become increasingly important (Pavan *et al.*, 2009). The methods for controlling the parasite (*Orobanche*), which used including: mechanical control, chemical control, physical control, biological control and integrated control. These different controlling methods (agricultural, chemical, biological) have generally yielded unsatisfactory results. The only exception seems to be the application of glyphosate to control *O. crenata* on *V. faba* (Kharrat, 2004).

Biological control using *Trichoderma harzianum* could a play major role in the management of broomrapes in crop production depending on the potential of natural enemies as bioagents and can be appropriate means of control due to its high degree of potentiality and soil environment safety (Boari and Vurro, 2004).

Soil solarization is achieved by mulching moistened soil with plastic sheets during summer months, thereby raising soil temperature and controlling broomrapes and other weeds. However it is well documented that long periods of solarization (6-7 weeks) are required for controlling *O. crenata* (Abu-Irmaileh, 1991)

Glyphosate as chemical method is the most common herbicide used to control broomrape. Hence, broomrape was found to be efficiently controlled in broad by glyphosate at 0.08 kg/ha applied 2 or 3 times at 15 days intervals (Kukula and Masri, 1984) or at 60 g/ha at the stages of visible flowering buds (Garcia and Vazquez, 1985). Application of glyphosate was

effective against broomrape, where it reduced number and dry weight of broomrape spike.

The development of effective broomrape control systems requires a better understanding of the growth of the crop, growth of the parasite and their interaction under different environmental conditions in a quantitative way. A comment on how to measure resistance seems necessary. Several indices have been used by different authors: total weight of broomrapes per host plant, height of the tallest (or the average) parasitic shoot, dry mater of parasitic plants per host plant, number of broomrapes per unit sown surface, etc. Rubiales *et al.*, (2004) have used the broomrape rate of reproduction as a measure of effectiveness of host resistance. Even though such an index may be perfect for a plant pathologist, its applicability by breeders is not so clear as even with a reduced rate of reproduction, a broomrape plant can yield an incredible amount of seeds. This index could be useful in combination with other better ones than as a single measure of resistance. Orobanche-induced yield reductions are not primarily due to competition for water, but rather due to carbohydrate loss to the parasite. As a consequence, the capacity for host-root water uptake is reduced. Competition for water can be regarded as a secondary cause of yield reduction (Ashrie *et al*, 2010).

Therefore, the purpose of the present investigation was to study the effect of infection with Orobanche on four faba bean cultivars, to identify the best methods for control Orobanche, to measure the relationship between infection severity and other plants traits

MATERIALS AND METHODS

Experimental site and faba bean genotypes:

This investigation was carried out at the experimental Farm Fac., of Agric., Suez Canal Univ., and Ismailia, Egypt during two successive seasons starting in 2011/12 and 2012/13.

Four faba bean cultivars (*Vicia faba*) i.e. Giza 3, Misr 1 and Giza 843 compared with Triple white cultivar (susceptible) were choosing from Food Legumes Research Department, Agriculture Research Center, and Giza, Egypt which represent different levels of resistance to broomrape.

Strategy application methods:

Three strategy application methods for controlling *Orobanche crenata* – parasitic weed were used in this study. These methods were applied for resistance to broomrape in addition to control (without any treatment) as follow.

a- Control of Orobanche by Coverage with polyethylene film

During late spring, the soil was ploughed several times to provide a uniform surface and then leveled. One day before mulching, the soil was irrigated to field capacity in order to increase thermal sensitivity of resting structures and improved heat conduction (Katan, 1981). Solarized plots were covered with 30-mm-thick Black polyethylene film from June, to October 15.

b- Control of Orobanche by Foliar application of glyphosate herbicide

75 cm³ as recommended dose (54 g /fed.) was sprayed twice times. The first spray was applied at 25% of onset flowering date and the second was conducted after 3 weeks from the first one.

c- Control of Orobanche by fungi

One candidate isolate of fungi *Trichoderma harzianum* given a code of T9 was selected and used as a biological agent to evaluate their potential for controlling broomrape on faba bean parents. This isolate (T9) was prepared laboratory at the Botany Department, Faculty of Sciences, Suez Canal University. The *T. harzianum* treatment application was applied to the experimental soil by soil drenching plus foliar spray of biological agent (T9). This method was followed as described earlier by Abdel-Kader and El-Mougy (2002).

Experimental design:

The experimental design of this study was randomize complete design with three replication. The experimental plot consisted of three ridges 3 m long, 60 cm apart, with single seeded hills, 20 cm apart on both sides of ridges. Cultural practices were applied at the proper time as recommended. At harvest ten guarded plants were taken at random from each experimental plot to record the following traits.

Measuring of Orobanche resistance:

Several indices have been used in this study to measure the effectiveness of host resistance such as number of broomrape per plot, number of broomrape tubercles per faba bean plant, total dry weight of broomrapes per host plant. Host (faba bean) root length (cm), where, three months after sowing the plants were extracted from the experimental pots after irrigation, the roots gently washed in water and attachments recorded, and classified according to their infection for determining infection severity.

The infection severity was calculated as a difference between infected treatments and control divided on control and multiplies 100.

Agronomic characters:

The agronomic characters, which studied were included days of 50% flowering, days to harvest date, plant height (cm), number of pods/plant, numbers of branches/plant, 100-seed weight (g), seed yield (g/plant).

Physiochemical characters:

a-SPAD Readings

The chlorophyll meter or SPAD meter simple ,portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves [Minolta Camera Co. Osaka, Japan] according to Castelli et al., 1996. Meter readings are given in Minolta Company defined SPAD (soil plant Analysis Development) values that indicate relative chlorophyll content. SPAD reading was determined for faba bean parents and their crosses at flowering stage. The youngest fully expanded leaf of 5 plants in each plot was used for SPAD measurement. Triplicate reading were taken on side of the midrib of

each single leaf blade, midway between the leaf base and tip and then averaged

b- Protein accumulation in faba bean seeds.

The "Lowry Assay: Protein by Folin Reaction" (Lowry *et al.*, 1951) has been the most widely used method to estimate the amount of proteins (already in solution or easily-soluble in dilute alkali) in biological samples. First the proteins are pre-treated with copper ion in alkali solution, and then the aromatic amino acids in the treated sample reduce the phosphomolybdatephosphotungstic acid present in the Folin Reagent. The end product of this reaction has a blue color. The amount of proteins in the sample can be estimated via reading the absorbance (at 750 nm) of the end product of the Folin reaction against a standard curve of a selected standard protein solution (in our case; Bovine Serum Albumin-BSA- solution).

c- Carbohydrates accumulation in faba bean seeds.

The total sugar was determined according method described in A.O.A.C. (1995).

d- Total phenolic content (TPC) accumulation in faba bean seeds.

TPC was calculated in the methanolic extracts, according to the Folin-Ciocalteu method with slight modifications (Jaramillo-Flores *et al.*, 2003). The absorbance was measured at 725 nm using a spectrophotometer (Shimadzu, UV-2450, Tokyo, Japan).

Statistical analysis:

The regular analysis of variance of RCBD with three replications was performed for each character measured in each field experiment for controlling methods. After that, data over seasons were subjected to combine according to Steel *et al.* (1997). Moreover, the data were further subjected to analysis to calculate correlation among all the character.

RESULTS AND DISCUSSION

Agronomic characters mean:

Broomrape infection caused reduction in the mean values of plant height, root length, number of branches per plant, number of pods/plant, number of orobanche per plot, dry weight of Orobanche spikes, 100-seed weight (g) and seed yield /plant in the four studied cultivars Table (1). Although none of the tested cultivars was absolutely free from this parasite, differences in responses of faba bean cultivars to Orobanche infestation were observed for all measured parameters. Expressing resistance by dry weight of Orobanche shoots showed considerable variation among cultivars and ranged from 2.89 to 3.75 g per faba bean plant. The level of Orobanche infection was high and uniform in the field, with an average of 37.33 emerged broomrapes per plot of the susceptible check cv. Triple white. Similar findings were detected by Abdalla and Darwish (2002).

Giza 843 surpassed the other cultivars and gave the highest mean values in all the studied traits under nature broomrape infection. While the susceptible cultivar (Triple white) gave the lowest values for all studied traits (Table 1). As number of attachments might be

influenced by root length. Giza 843 had much bigger root system than Triple white. The effects of plant vigor and root length were determined, where, the height of faba bean plants ranged from 82.52 to 99.88 cm and root length varied from 11.81 to 16.2 cm. Both number and weight of Orobanche spikes indicating that the cultivar Giza-843 effectively shared in transmitting its properties of high yield and its immense ability of resisting broomrape. This result was in harmony with the result reported before by Hassanein *et al.*, (2000).

Significant differences among the cultivars for days to 50 % flowering and days from sowing to maturity. The averages of number of days from sowing to maturity ranged from 149.08 days for Giza 843 to 156.58 days for Misr 1. Host plants might escape broomrape infection by reduced plant maturity. The occurrence of phenolics, carbohydrates, proteins and chlorophyll content were also recorded in extracts of the host plants. Giza 843 accumulated more phenolics, carbohydrates and proteins as a defence mechanism against broomrape. These finding were more or less agreement with the results reported by each of Bora *et al.* (1998).

It might be concluded that such substances metabolites can be assumed as existing normally at genetically determined levels either in the host root or the parasite as a means of routine mechanical support and natural defense. But, in the presence of broomrape, it is tentatively suggested that a signal is received and transduced to the host genome, resulting in a general up-regulation of genes encoding the phenylpropanoid pathway, thus providing the host cells with extra precursors of defensive compounds.

Seed yield in faba bean is challenged by many pathogens and Broomrape is weedy root parasite that represents a major constraint for faba bean production. Significant differences among cultivars for seed yield and its components were observed. The average seed yield per plant for Giza-843 was 48.36 g that out yielded all other parents. The present results were in the same line, which reported by Morsy and Attia (2002) They found that the susceptible cultivars gave the highest seed yield in the Orobanche free field while Giza 843 significantly exceeded all genotypes for both seed in infested conditions.

There are morphological, biological and productive modifications which are generally more accentuated the more precocious and virulent the attacks. The abscission of flowers and young pods is the single most important factor contributing to low yields. However, pests' diseases, weeds and weather conditions also play a major part in determining faba bean yield. When total crop failure due to weed infestation occurs it is due to a parasitic weed broomrape. This was also observed in faba bean and explained by an earlier pod-setting and maturity, which would restrict the dry-matter partitioning into parasites (Manschadi *et al.* 1997). The sink strength of *O. crenata* in the bud stage is comparable with that of seeds in the rapid seed-filling phase as soon as *O. crenata* starts growing an underground shoot, all assimilate are directed towards the parasite, unless the host is in the stage of rapid seed filling of a pod. Beside this possible escape factors, we

Means of Control:

Different methods have been used to control *O. crenata* i.e. covering by polyethylene, Trichoderma fungi and glyphosate herbicide. At harvest, all the weed control treatments recorded significantly lower broomrape population than weedy control. Giza 843 was markedly more resistant than others in the all controlling methods.

Solarization by covering of moist soil with a layer of polyethylene under high-temperature conditions can control broomrape, but the efficiency of this method in faba bean seems to be the less reliable. The biggest limitation to this method however, is the high cost of the polyethylene and need to combine with other treatments such as farm manure or ammonium nitrates fertilizers. Availability of appropriate machinery and cloud-free sunny days may further restrict use of this method. In this connection Abdul-Wahid and El-Bramawy (2010) mentioned that the soil temperature attained by mulching inhibited *O. crenata* germination, killing most of the buried seed and inducing secondary dormancy in the remaining seeds.

Like all other plants, parasitic weeds have natural enemies which can affect their growth and can potentially be used as agents for their control. Biological control of weeds is defined as the use of natural antagonists to exert pressure on the population of their host to reduce it to levels below economic importance.

The fungus Trichoderma was used experimentally to control *Orobancha crenata* in faba beans, destroying emerged shoots and underground tubercles. In the same line, *T. harzianum*, as a potential for controlling of faba bean broomrape and a soil application reduced seed germination of *O. crenata* and increased seed yield of faba bean Abdul-Wahid and El-Bramawy (2010). This fungus penetrates seeds and destroyed seed contents of broomrape, thereby reduced seed bank of this parasitic weed in soil

In general, biological control is an effective method for long period since it is relatively cheap, specific to the target organism and not harmful to the environment but should combine with other treatments.

Glyphosate was a Systemic herbicide which was used in controlling Orobancha after emergence of the shoots for two times.

As shown in figures (1, 2, 3, and 4) spraying glyphosate was very effective in controlling broomrape. A decrease in number and dry weight of broomrape spikes was recorded as compared with control plots. The reduction in the number and dry weight of broomrape spikes were highly significant. The reduction percentage of broomrape dries weight by glyphosate application more than three times (Fig.2). Moreover, application of glyphosate recorded lower broomrape population than other weedy control methods. The competition between crop and broomrape was minimized and this made the crop plants to utilize available resources and synthesized photosynthates more effectively throughout crop growth period which in turn positively influenced the fruit yield by improving yield components. Glyphosate was sprayed on the host crop and was translocated to the parasite to kill it. Its phytotoxicity is the most limiting factor. Necrosis of installed broomrape tubercles was noticed in faba bean plants after application glyphosate. In addition to this, browning and death of attached Orobancha seedlings without the presence of a hypersensitive reaction in the host root has been observed in faba bean field Nassar and Mekky (2002).

Better broomrape control efficiency could be attributed to better infection severity as shown in Fig. (4). where, it was 8.17 % for coverage method, 26.92 % for Trichoderma fungi and 58.85 % for glyphosate herbicide based no. of Orobancha / plant. Higher seed yield can be attributed to higher broomrape control efficiency which illustrated by infection severity. It was 8.28% for coverage method, 17.38 % for Trichoderma fungi and 67.42 % for glyphosate herbicide based on seed yield / plant. The competition between crop and broomrape for glyphosate method was minimized than other methods and this made the crop plants able to utilize available resources and synthesized photosynthates more effectively throughout crop growth period which in turn positively influenced the seed yield by improving yield components.

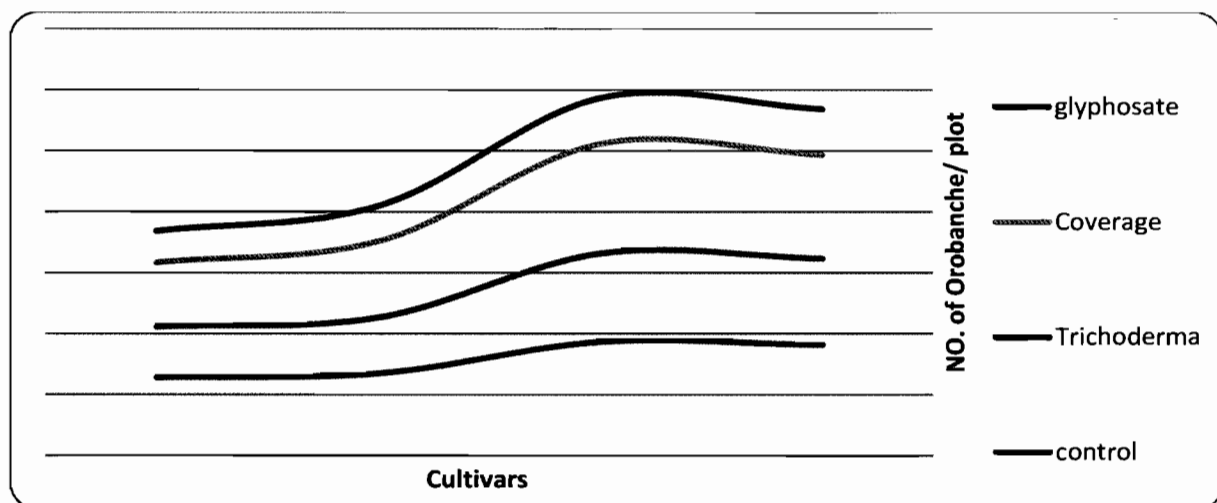


Fig. (1): Effect of some Methods of resistance on NO. of Orobancha/ plot in some faba bean cultivars.

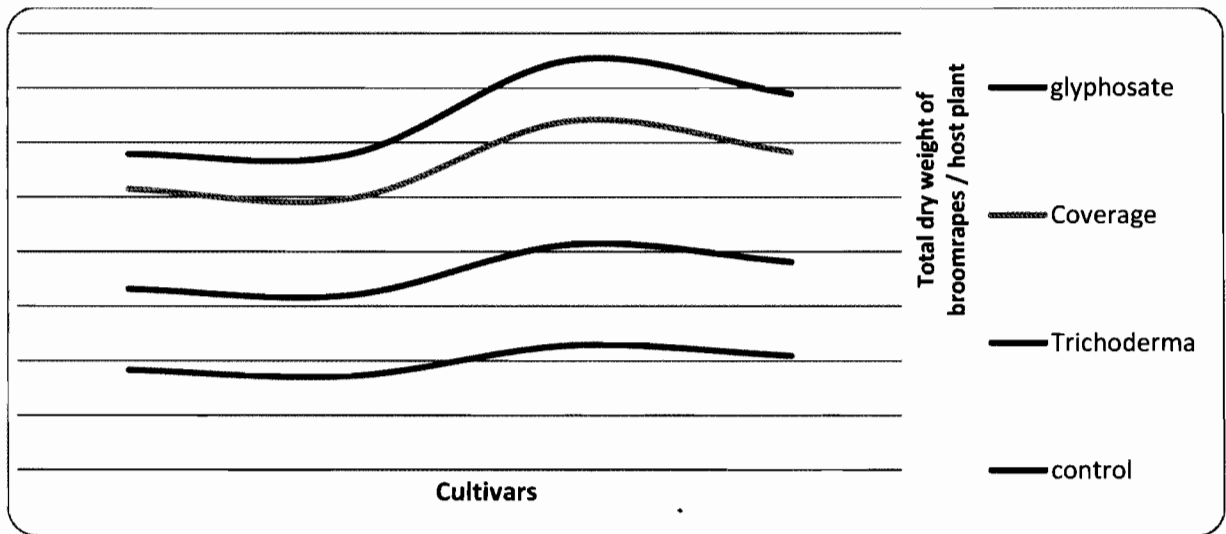


Fig. (2): Effect of some Methods of resistance on dry weight. of Orobanche/ plot in some faba bean cultivars.

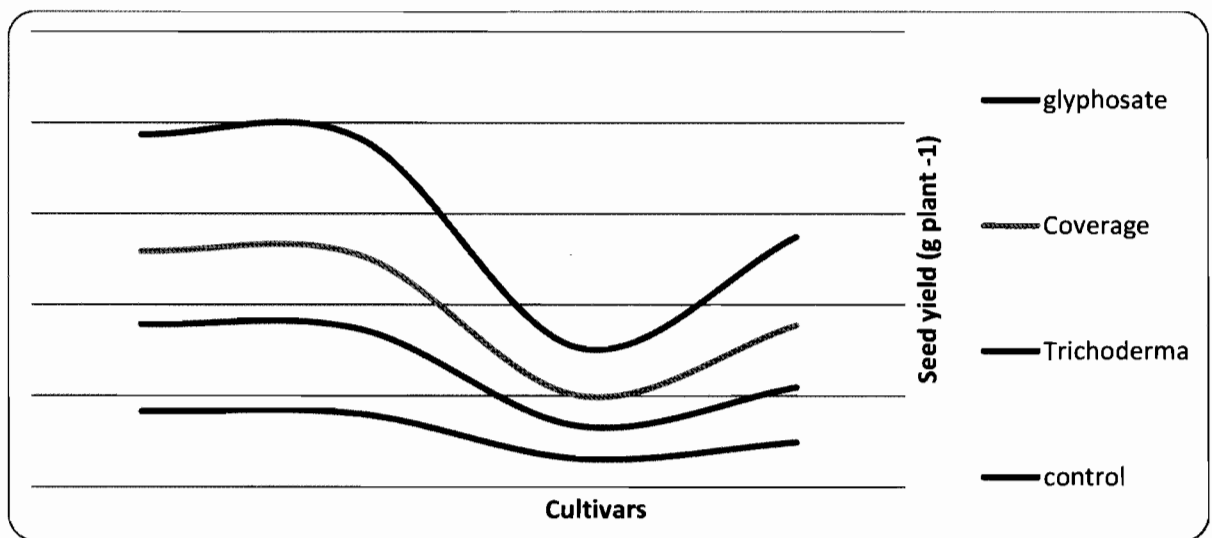


Fig. (3):Effect of some Methods of resistance on seed yield in some faba bean cultivars

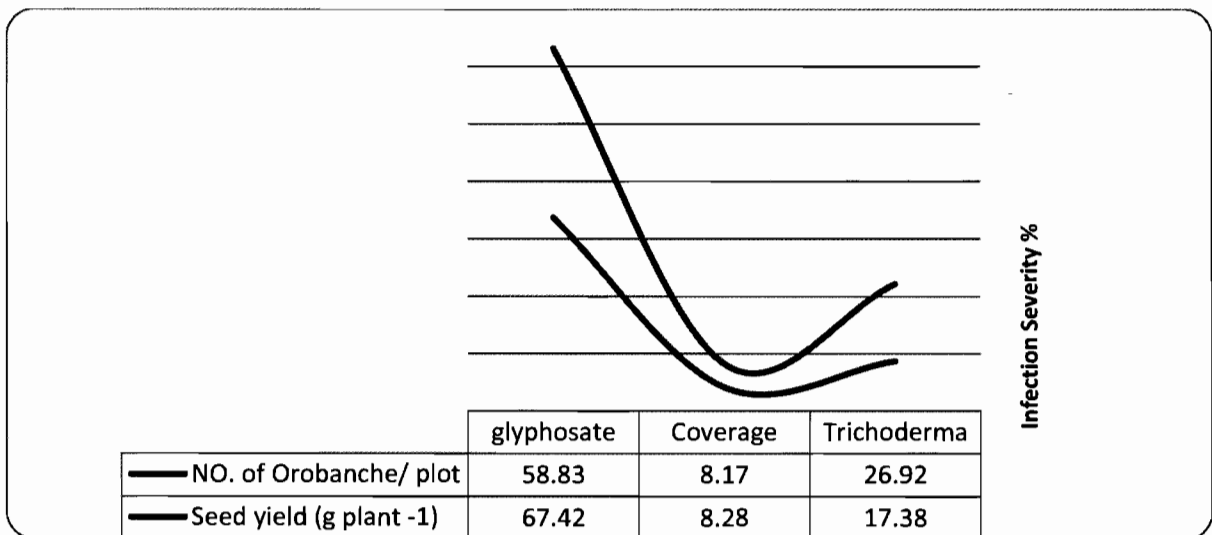


Fig. (4): The relationship between Methods of resistance and Infection Severity % for NO. of Orobanche/ plot and Seed yield (g plant -1).

CONCLUSION

For effective management of broomrape in faba bean application of glyphosate 75 cm fa-1 is proved to be highly effective than solarization or *Trichoderma* fungi. This was evidenced by lower number of spikes per plot, spike height and dry weight of broomrape at harvest. Good broomrape management practices had resulted in improving growth components of faba bean crop which in turn supported to reveal better yield components. Giza 843 was markedly more resistance to *Orobanche* than others and gave the best yield under this condition. 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

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