Granular formulation of *Fusarium oxysporum* for biological control of faba bean and tomato *Orobanche*

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Abstract

BACKGROUND: *Orobanche* spp. represent a serious threat to a wide range of crops. They are difficult targets for herbicides, and biological control could provide a possible solution. This work therefore aimed to formulate mycoherbicides of *Fusarium* with adequate shelf life and virulence against *Orobanche* but safe to faba bean and tomato.

RESULTS: Only two isolates of *Fusarium oxysporum* Schlecht. (Foxy I and Foxy II) obtained from diseased *Orobanche* shoots were found to be pathogenic to *Orobanche crenata* Forsk. and *Orobanche ramosa* L. Conidial suspension of both isolates significantly decreased germination, attachments and tubercles of *Orobanche*. Microconidia and chlamydospores of both isolates were formulated as mycoherbicides encapsulated in a wheat flour–kaolin matrix (four different formulations). All formulations greatly diminished *Orobanche* emerged shoots, total shoot number, shoot height, attachment of emerged shoots, the germinated seeds that succeeded in emerging above the soil surface and dry weight. Meanwhile, disease incidence and disease severity of emerged shoots were enhanced. The shelf life was adequate, particularly for coarse, freshly prepared, low-temperature-stored, microconidia-rich formulations. The induced growth reduction of *Orobanche*-infected host plants seemed to be nullified by formulations, particularly at the highest dose.

CONCLUSION: These formulations seemed to destroy *Orobanche* but appeared harmless to host plants. Hence, they could be efficiently used as mycoherbicides for biological control of *Orobanche* in faba bean and tomato.

Keywords: biocontrol; faba bean; *Fusarium oxysporum*; mycoherbicides; *Orobanche*; pesta formulations; tomato

1 INTRODUCTION

Root-parasitic weeds such as *Orobanche* represent a serious threat to a wide range of economically important crops. *Orobanche crenata* Forsk. parasitizes major legume crops, while *Orobanche ramosa* L., which is closely related to *O. aegyptiaca* Pers., attacks mainly Solanaceae.[1,2] Besides causing yield loss and reduction in the cultivated area of crops, *Orobanche* also reduces crop quality. The presence of *Orobanche* material in harvested crop products may reduce the value of crop or make it completely unmarketable.[3] Germination of *Orobanche* requires a stimulant excreted by the host roots. On germination, a germ tube moves towards the host roots, forming an appressorium. Subsequently, a haustorium is formed which penetrates the root and connects with the vascular system of the host.[4] After the haustorium has developed into a nodule (tubercle) with crown roots, a bud develops which then forms an emerging reproductive shoot.[3] Because of the intimate host–parasite relationship and the anatomical–physiological connections, *Orobanche* is a particularly difficult target for selective chemical control.[5] Biological control using fungal pathogens could provide a possible solution because of the high specificity of the fungal pathogens used as biocontrol agents.[6–11] *Orobanche* biocontrol takes place using biological agents such as *Ulocladium* spp.[12,13] and *Fusarium* spp.[10,14] *Fusarium oxysporum* Schlecht. f. sp. *orthoceras* (FOO) gave 90% control of *Orobanche* in tomato[15] and 90–97% control in watermelon.[16] Other microorganisms such as *Rhizoctonia*, *Alternaria* and *Sclerotinia* have also been used in the biological control of *Orobanche*.[17] The development of an appropriate formulation that allows storage, handling and successful application of the fungal propagules will determine further success in agricultural applications. As *Fusarium* is able to attack the underground developmental stages of *Orobanche*, a preplanting soil application is most suitable for delivering the fungal antagonist to its target.[18] A granular spore

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(*Received 8 November 2006; revised version received 1 October 2007; accepted 18 March 2008*)
Published online 17 July 2008; DOI: 10.1002/ps.1625

 Pest Manag Sci 1526–498X/2008/$30.00

product can serve as a site for continuous spore production. Two types of formulations, alginate and wheat flour–kaolin granules, have been widely used to make granular products containing living biocontrol agents. The present work is an attempt to formulate mycoherbicides of *F. oxysporum* to biocontrol *Orobanche* in faba bean and tomato instead of using unsafe and/or non-selective chemical substances. The formulations are expected to have adequate shelf life and virulence against *Orobanche* but to be safe to host plants.

2 METHODS AND MATERIALS

2.1 Plant materials and growth conditions

Surface-disinfected seeds of faba bean (Giza 3) and tomato (Supper strain B) were used. Root exudates were produced by growing plants for 14 days, after which ten plants were grouped together, and the root system was washed and completely immersed in water in glass flasks wrapped in black polyethylene. The plants were incubated for 3 days and then removed, and the liquid content of the flasks was stored at −20 °C until use.

Seeds of *O. crenata* (faba bean parasite) and *O. aegyptiaca*/*ramosa* (tomato parasite) were germinated *in vitro* in order to determine the amounts of seed needed to achieve a concentration of approximately 10 000 germinating seeds per pot (2 kg of soil). For the *in vitro* germination, 3 mL of water was added to surface-sterilized *Orobanche* seeds placed on small glass fiber filter paper discs in petri dishes containing one layer of round filter paper. The plates were sealed with parafilm and incubated for 7 days at 20 °C in the dark. The small discs were then transferred to freshly prepared petri dishes with filter paper, and 3 mL of the root exudate was applied. After 10 days, the percentage of germinated seeds was determined using a binocular microscope.

Root chamber and pot experiments were conducted at 15 °C in the night, and at 20–30 °C during the daytime. Surface-sterilized *Orobanche* seeds were evenly sprinkled onto a moistened filter paper strip. The Perspex cover was pressed onto the paper strip, and both were transferred to the back part of the root chamber and fixed with aluminium clips.[19] The chambers were filled with autoclaved sand, irrigated until water saturation, wrapped in black plastic and left for 7 days for preconditioning of the seeds. Two pregerminated host plant seeds were sown in each chamber between the filter paper and the lid. The chambers were placed at an angle of 30° and irrigated as required. From 14 days after sowing, the system was fertilized with 10 mL of 0.2% Wuxal™ (Bayer Crop Science, Langenfeld, Germany) fertilizer solution weekly. For pot experiments, *Orobanche* seeds were sprinkled onto the soil surface of plastic pots filled to two-thirds of their height with sand + clay (1 + 1 by volume) soil, and inoculum was added and mixed into the soil together with the seeds. The pots were then entirely filled with soil, and host plant seeds were sown. Fourteen days after sowing, plants were thinned out to three per pot. The pots were irrigated as required.

2.2 Pathogenicity bioassay

About 300 infected *Orobanche* plants were collected from faba bean and tomato fields. Shoots were carefully collected and stored in a refrigerator. Small pieces of infected shoots were surface disinfected, rinsed with sterile water, blotted dry and placed onto potato dextrose agar (PDA) plates supplemented with 200 mg L^{-1} of chloramphenicol and 100 mg L^{-1} of streptomycin sulfate. The plates were incubated at room temperature until fungal mycelium grew out of the plant pieces. The mycelium was transferred to fresh PDA plates to obtain pure cultures.

A total of 169 fungal isolates obtained from infected shoots collected from infested fields of four governerates in Egypt (Damietta, Dakahlia, Sharkia and Ismailia) were tested for their pathogenicity to *Orobanche*. Only two isolates, classified by YM Shabana of the Plant Pathology Department, Mansoura University, according to Bedi and Donchev,[20] as *F. oxysporum* isolate I (Foxy I) and isolate II (Foxy II), caused reduction in *Orobanche* germination *in vitro*, in root chambers and in plastic–bag systems.

For *in vitro* experiments, *Orobanche* seeds were sprinkled onto filter paper in petri dishes, and 3 mL of an aqueous conidial suspension of isolated fungi (10^6 mL^{-1}) was added. All petri dishes were sealed with parafilm and incubated in the dark at room temperature for 7 days. Root exudate was added, and the evaluation was carried out as described in Section 2.1.

Root chamber experiments were conducted according to Abbasher and Sauerborn[21] using about 30 mg of *Orobanche* seeds per chamber by dropping 10 mL of an aqueous conidial suspension (10^6 mL^{-1}) onto the glass filter paper strip. After 7 days, the chambers were transferred to the greenhouse, and two pregerminated host seeds were placed in each, held for 14 days and then fertilized with 10 mL of 0.2% Wuxal fertilizer weekly. The experiment was evaluated 4 times at 9 day intervals using a binocular microscope.

For assessment of the pathogenicity and virulence of selected isolates, transparent plastic envelopes (A4 size) with one side window (13 × 25 cm) were used, with a moistened sheet of filter paper placed inside.[22] *Orobanche* seeds (about 25 mg) were dispersed uniformly on the surface of the filter paper sheet. Three pregerminated host seedlings were transferred to the plastic bag at the second-leaf stage, leaving shoots and leaves out of the bag and placing roots on the glass fiber sheet. The cut was sealed with tape. A plastic pipe was inserted into the bag and sealed on one side, allowing addition of water or nutrient solution with a syringe. Bags were placed vertically to keep roots and seeds in the dark at 25 °C for 35 days.

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DOI: 10.1002/ps

2.3 Production of mycoherbicide formulations

Fusarium oxysporum isolates Foxy I and Foxy II were grown on potato dextrose broth (PDB) for 6 days on a reciprocating shaker at 125 strokes per minute (spm) at room temperature to produce microconidia, or for 20 days at 100 spm to produce chlamydospores. The contents of the culture flasks were blended, and the number of spores was determined using a haemacytometer. The resulting suspensions were centrifuged at $4000 \times g$ for 10 min and adjusted to 3.5×10^8 and 5×10^8 microconidia mL^{-1} and to 2.5×10^7 and 2.7×10^7 chlamydospores mL^{-1} for Foxy I and Foxy II respectively.

The two types of spore were used as active ingredients for pesta formulations, along with three adjuvants (sucrose, yeast extract and glycerol). Dough was prepared according to Shabana *et al*.[9] by blending 38 g of semolina, 4 g of kaolin, 6 g of yeast extract, 2 g of sucrose, 20 mL of spore suspension and 2 mL of glycerol. The dough was then rolled through a pasta machine, folded and extruded several times. A sheet 1 mm thick was produced, air dried under room conditions, ground and sieved to specific sizes (151–500 μm and 501–2000 μm). Microconidia and chlamydospores of both isolates (Foxy I and Foxy II) gave rise to four formulations; PM I and PM II containing microconidia of Foxy I and Foxy II respectively, and PC I and PC II containing chlamydospores.

2.4 Evaluation of mycoherbicide formulations

Colony-forming units were determined by weighing 0.1 g of granules into 10 mL of distilled water with three glass beads (5 mm diameter), soaking for 15–20 min and vortexing until dispersed. Three serial dilutions were made. A quantity of 100 μL of the last two dilutions were plated onto half-strength PDA supplemented with 30 mg of streptomycin sulfate and 10 mg of chloramphenicol. Fungal colonies were counted after incubation at 23 °C for 3 days.

Plastic pots were filled with sand + clay (1 + 1 by volume) soil and divided into groups. A quantity of 60 mg of *O. crenata* or *O. aegyptiaca/ramosa* seeds together with pesta formulation of Foxy I or Foxy II at 0.5, 0.75, 1.0 and 1.25 g kg^{-1} soil were well mixed with the subsoil layer. A set of pots containing *Orobanche* seeds but inoculated with fungus-free pesta was used as a control. Five faba bean seeds were placed in *O. crenata*-infested groups and covered with an additional 3 cm thick layer of soil. Faba bean seedlings were thinned to three per pot 14 days after sowing. The *O. aegyptiaca/ramosa*-infested pots were planted with three tomato seedlings. All pots were kept moistened for the first 14 days. Faba bean pots were fertilized with 0.55 g of NH_4NO_3, 2.2 g of P_2O_5 and 1.1 g of K_2O, while tomato pots were fertilized with 4.5 g of NH_4NO_3, 3.3 g of P_2O_5 and 2.2 g of K_2O. Disease incidence (DI), disease severity (DS) and the numbers of emerged *Orobanche* shoots were determined 2 and 3 months after planting. DI was assessed as the percentage of emerged shoots showing signs of disease, and DS on a numerical scale 0–100: 0, no disease; 1–50%, discolouration of *Orabanche* shoots; 51–80%, wilting but not dieing; 81–99%, partially dead; 100%, completely dead shoots. At the end of the experiment, when host plants had stopped development owing to severe infestation, the *Orobanche* shoot height, number of attachments and aboveground and underground dry weights were determined. In addition, growth parameters of host plants (shoot height, root length and shoot and root dry weight) were determined.

All values are means of at least six replicates from two independent experiments (± SD). The full data were firstly subjected to an analysis of variance (ANOVA), followed by a least significant difference (LSD) test at the 5% level.[23]

3 RESULTS

Only two fungi isolated from diseased *Orobanche* shoots, classified as *Fusarium oxysporum* isolate I (Foxy I) and isolate II (Foxy II), inhibited *Orobanche* germination *in vitro*, in root chambers and in plastic–bag systems (Fig. 1). Germination of *Orobanche* seeds was significantly decreased after inoculation with conidial suspensions of *Fusarium oxysporum* isolates, *in vitro* germination of *O. crenata* being reduced by 80 and 76% following application of Foxy I and Foxy II respectively, at the beginning of the conditioning phase, while *O. ramosa* germination was decreased by 77 and 76% respectively. In root chambers, inoculation with Foxy I and Foxy II reduced the germination of *O. crenata* seeds by 49 and 47% respectively, and the germination of *O. ramosa* by 50 and 46% respectively. Foxy I and Foxy II inhibited *O. crenata* germination in plastic bags by 55 and 53% respectively, and *O. ramosa* germination by 50 and 40%.

Figure 2 shows that the relative proportions of germ tubes of *O. crenata* and *O. ramosa* that attached to the host roots were significantly reduced by both Foxy isolates, although there was no significant difference in the decrease in attachment of the two *Orobanche* species. *Orobanche* attachment to the host was reduced more by Foxy I (up to 13%), either in root chambers or in polyethylene bags, than by Foxy II (up to 9%). In addition, both isolates led to decreases in the number of *Orobanche* tubercles. The pattern of sequence was similar to that of attachment. The numbers of tubercles of *O. crenata* and *O. ramosa* were decreased by Foxy I (up to 39%) and Foxy II (up to 32%). The magnitude of decrease was more pronounced in root chambers than in polyethylene bags.

Figure 3 shows the shelf life of the formulated Foxy pesta. There were considerable effects of the granule size of the formulations, the spore type and the storage temperature on the viability of fungal propagules. The figure clearly indicates that, in general, the viable fungal propagules of pesta formulations decreased

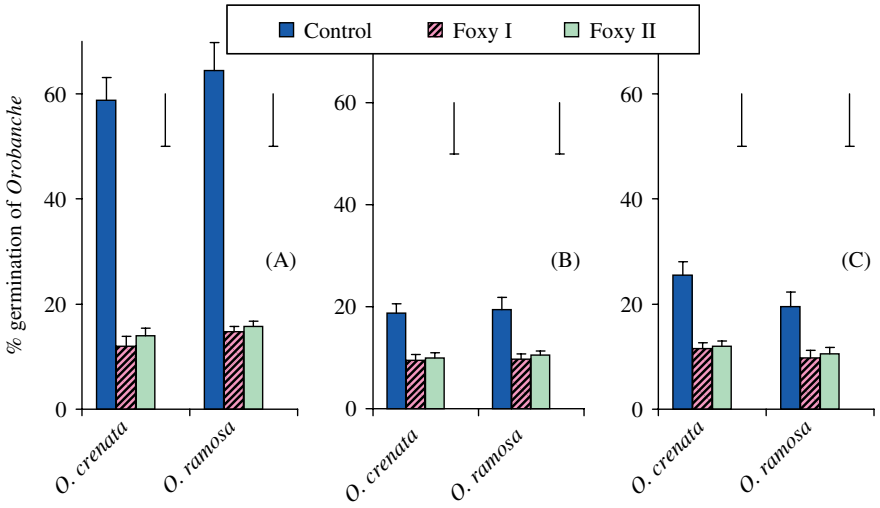

Figure 1. Effect of conidial suspension (10^6 mL^{-1}) of two *Fusarium oxysporum* isolates (Foxy I and Foxy II) on *Orobanche* germination *in vitro* (A), in root chambers (B) and in polyethylene bags (C). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

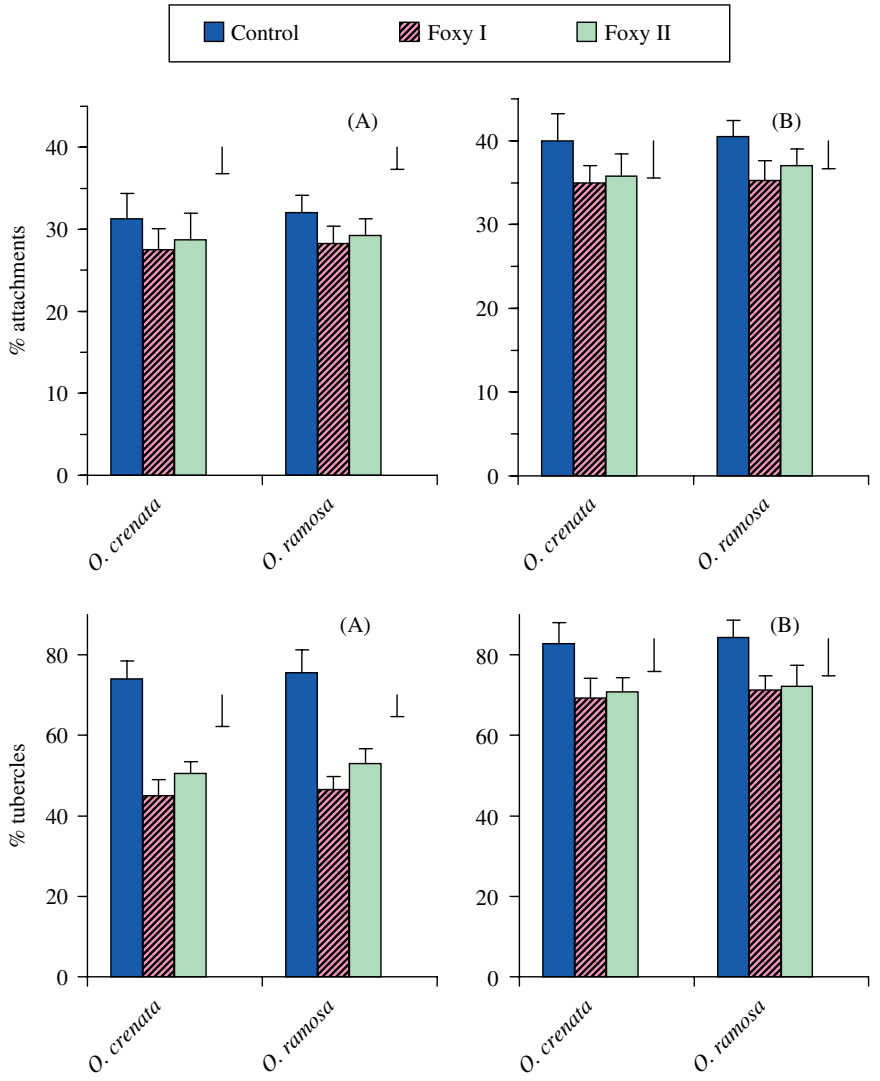

Figure 2. Effect of conidial suspension (10^6 mL^{-1}) of two *Fusarium oxysporum* isolates (Foxy I and Foxy II) on attachment and tubercles of *Orobanche* in root chambers (A) and in polyethylene bags (B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

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DOI: 10.1002/ps

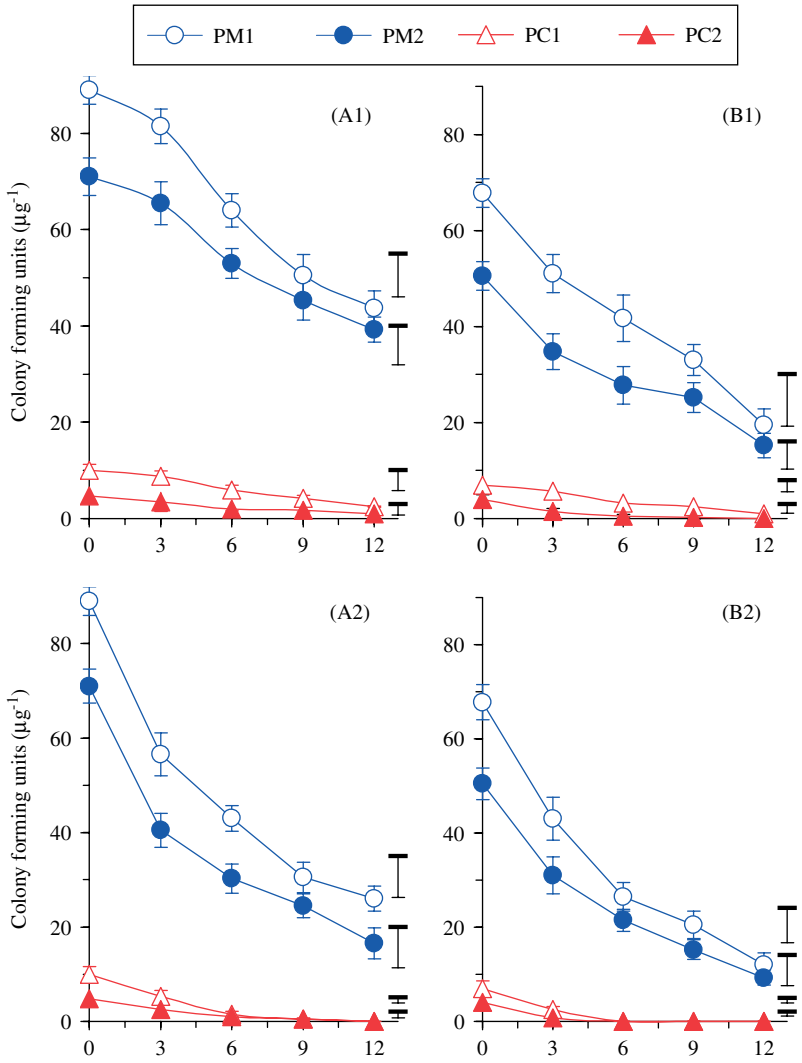

Figure 3. Effect of granule size (>500 µm, A; <500 µm, B) and storage temperature (3 °C, 1; 25 °C, 2) on propagule viability of microconidia and chlamydospore pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates at 3 month intervals. Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

with the elapse of storage time. However, coarse granules (>500 µm) had a larger number of viable fungal propagules than smaller granules (<500 µm). More propagules remained viable on storage at 3 °C than at room temperature (25 °C). However, there were great differences among the formulations, and the sequence PM I > PM II > PC I > PC II was displayed with respect to the number of viable fungal propagules.

Shoots of *O. crenata* and *O. ramosa* started to emerge respectively 65 and 52 days after sowing, and continued to emerge until day 95 and day 86. The number of emerged and the total number of *Orobanche* shoots decreased after the application of pesta formulations (Fig. 4). However, low doses had, in general, no significant effect on the number of emerged shoots and the total shoot number of either *Orobanche* species. Increase in pesta dose retarded *Orobanche* emergence and the total number of *Orobanche* shoots. Furthermore, high doses resulted in marked diminutions in emerged shoots, up to 86 and 80% in *O. crenata* and *O. ramosa* respectively, and in total shoot number, up to 80 and 68%. In general, all formulation types seemed to have very similar effects on emerged shoots and total shoot number.

The emerged shoots of the pesta-treated *Orobanche* exhibited disease symptoms at emergence, which continued to develop up to the flowering stage. Figure 5 shows that treatment with any of the different formulations greatly enhanced disease incidence (DI) of the emerged *Orobanche* shoots either with faba bean or with tomato. The DI appeared to be mostly related to the formulation dose, reaching 97% in *O. crenata* and 95% in *O. ramosa* (18-fold and 15-fold, respectively, relative to the fungus-free pesta) at the highest dose. In the same pattern, all the formulations of Foxy highly augmented disease severity (DS) of *Orobanche* shoots emerged in faba bean or tomato, this index increased with increasing in the mycoherbicide dose. The highest dose resulted in 96 and 92% (15-fold and 31-fold relative to the fungus-free pesta) DS for *O. crenata* and *O. ramosa* respectively. However, there were no differences among the effects of the different formulation types.

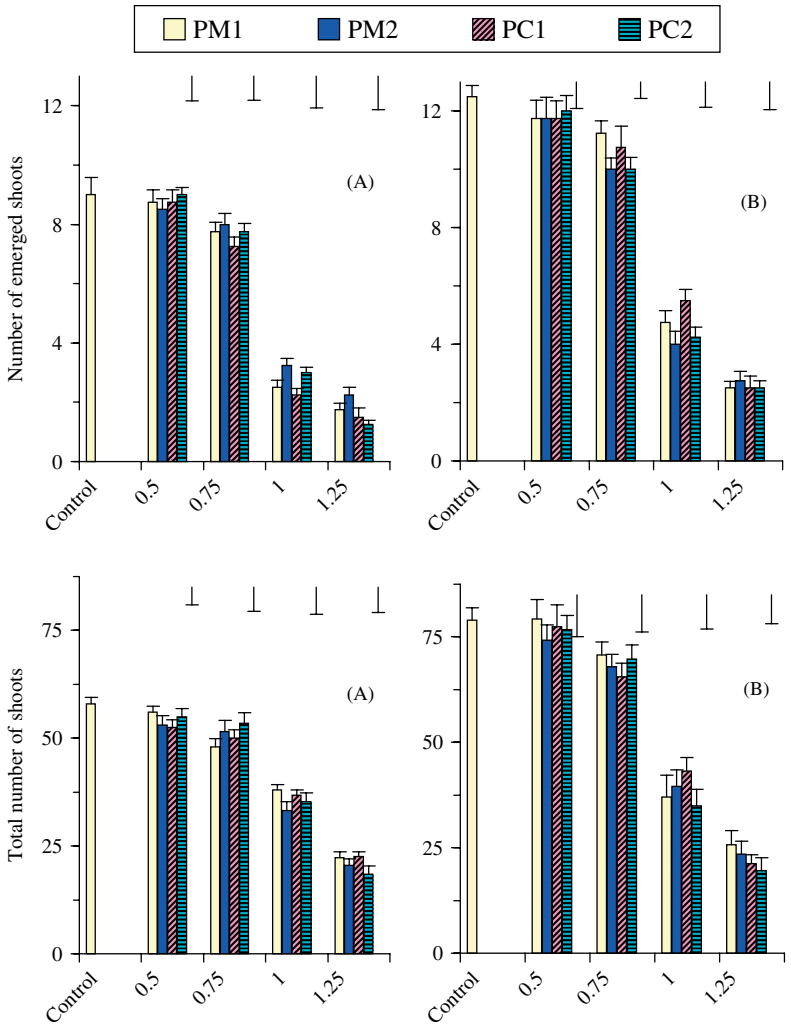

Figure 4. Effect of microconidia and chlamydospore granular pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates on emerged and total shoot number of *O. crenata* (A) and *O. ramosa* (B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

In confirmation, treatments with pesta formulations significantly reduced shoot height and the number of attachments of both *Orobanche* species compared with their respective control (Fig. 6). The magnitude of reduction increased with increasing pesta doses. The lowest dose resulted in a slight reduction in shoot height, not exceeding 5% in *O. crenata* and *O. ramosa*, whereas the highest dose induced up to 79% reduction in both *Orobanche* species. In the same manner, the number of attachments of *O. crenata* or *O. ramosa* was decreased up to 5 or 14% by 0.5 g pesta kg^{-1} soil, as against 79 or 70% by the highest dose (1.25 g pesta kg^{-1} soil). The effects of the different types of formulation seemed mostly variable.

The dry weight of *Orobanche* shoots was significantly decreased by all doses of the applied pesta formulations (Fig. 7). The decrease in *O. crenata* or *O. ramosa* dry weight was greater with the highest dose (91 and 90% respectively) than with the lowest dose (not exceeding 16 and 17% respectively). Total dry weight was similarly decreased by 90 and 85% in both species by the highest dose, as against 17 and 16% by the lowest dose. The trend of response to the formulations was generally similar in the two *Orobanche* species, but PC II seemed to be the most effective formulation type, particularly at the highest dose.

The responses of growth parameters of *Orobanche*-infected host plants (shoot height, root length and dry matter content) to Foxy pesta are presented in Figs 8 and 9. *Orobanche* infestation resulted in a reduction in shoot height of faba bean or tomato as compared with normally grown control host plants (Fig. 8). However, treatment with either Foxy pesta formulation seemed to overcome the *Orobanche*-induced reduction in host growth. Such effects tended to increase at high pesta doses. The effects of the PC II formulation on shoot height enhancement were greater than those of the other formulations. However, the effect of pesta formulations was more pronounced in tomato plants than in faba bean. Infestation resulted in 93 and 81% reduction in shoot height of faba bean and tomato respectively, and Foxy pesta increased shoot heights to 107 and 127% of those of the infected plants. Similarly, root lengths of *Orobanche*-infected faba bean and tomato were markedly lower than those of the controls, reaching 68 and 74% respectively. The

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DOI: 10.1002/ps

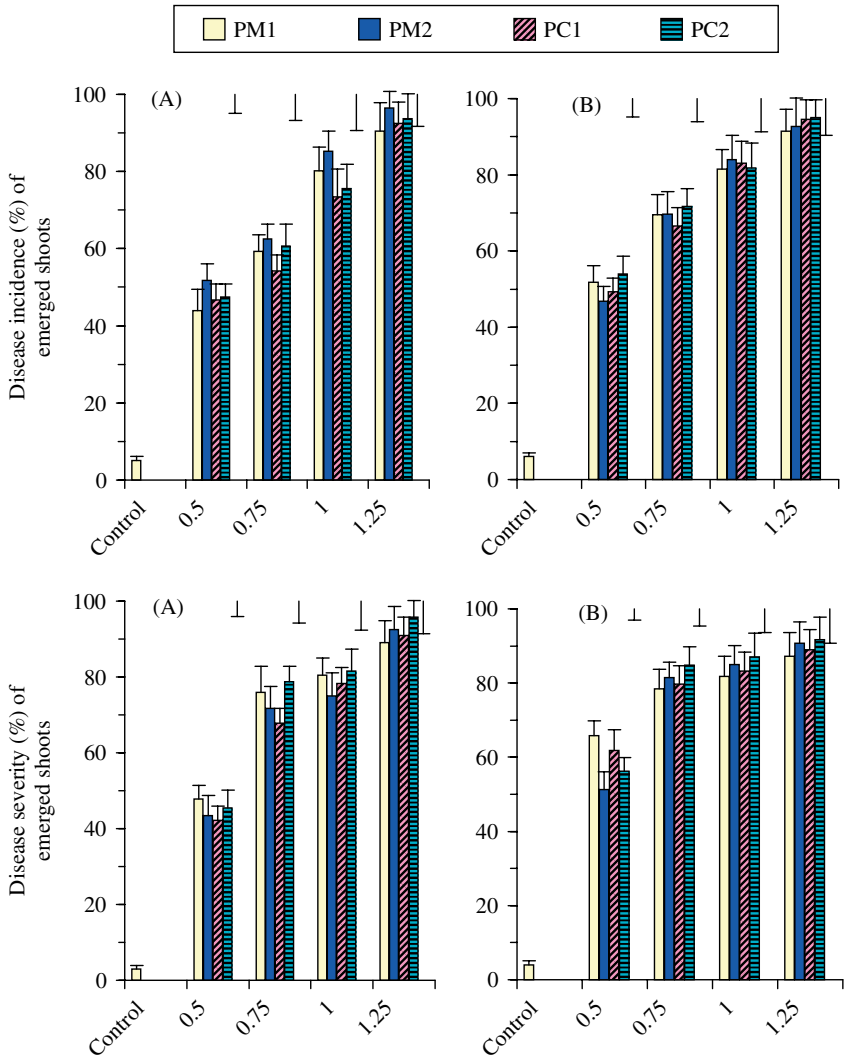

Figure 5. Effect of microconidia and chlamydospore granular pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates on disease incidence and disease severity on emerged *O. crenata* (A) and *O. ramosa* (B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

application of pesta formulations of all types induced elongation in plant root length of 154 and 143% in the infected faba bean and tomato plants. The increase was more pronounced with increasing pesta dose. The pattern of response was generally similar in faba bean and tomato.

In the same manner, *Orobanche* caused a reduction in shoot and root dry weight of host plants (Fig. 9). *Orobanche* decreased shoot and root dry weight of faba bean by 81% and of tomato by 74%. Nevertheless, the drop in dry matter content appeared to be counterbalanced following the application of all doses of Foxy pesta formulations; the magnitude of increase was related to increase in dose. Shoot dry weight of pesta-treated faba bean and tomato reached 129 and 143% respectively in the infected plants, and root dry weight reached 129 and 141%.

4 DISCUSSION

Parasitic plants are among the most problematic weeds that are responsible for major losses to many crops. Early growth stages, such as seed germination stimulated by host root exudates and tubercle development, are key phases for the development of these parasites. Inhibition of these early phases by naturally occurring compounds could be a general strategic option for management of parasitic plants.[24] Most of the isolated fungi from *Orobanche* in the area investigated were soil borne. These findings are in accordance with other reports stating that a high proportion of soil-borne fungi were associated with *Orobanche*.[10,25] This might be attributed to the long underground development phase of the parasitic weed, in addition to its reduced leaf area.[26] In the present study, two isolates of *Fusarium oxysporum* showed pathogenicity to *O. crenata* and *O. aegyptiaca/ramosa*. Germination of *Orobanche* seeds was obviously decreased after inoculation with conidial suspension of *Fusarium oxysporum* (Foxy I or Foxy II) *in vitro* (up to 80%), in root chambers (up to 50%) and in plastic bags (up to 55%). Similarly, Müller-Stöver *et al*.[27] had reported that pesta granules of *Fusarium oxysporum* f. sp. *orthoceras* (FOO) reduced the

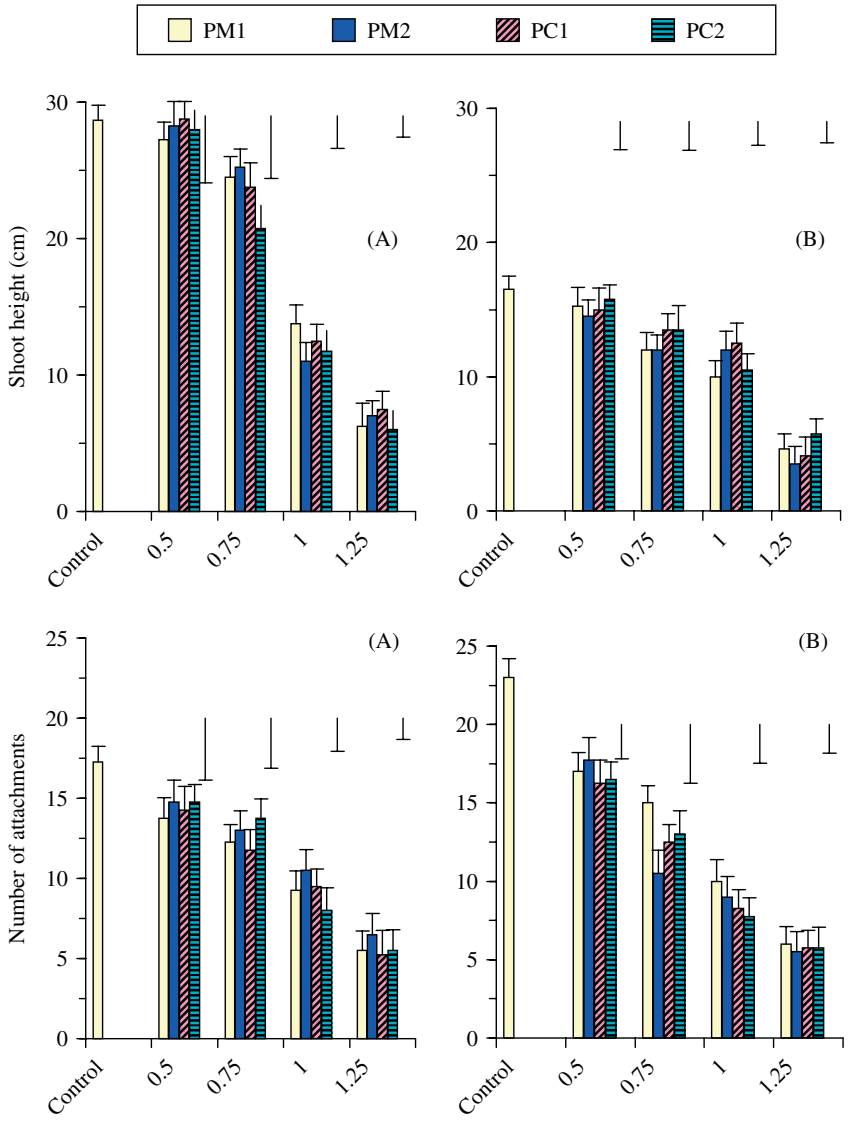

Figure 6. Effect of microconidia and chlamydospore granular pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates on shoot height and attachment number of *O. crenata* (A) and *O. ramosa* (B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

emergence of *O. cumana* shoots by 64%. Moreover, Müller-Stöver and Kroschel[13] found that *in vitro* and in root chambers, germination of *O. crenata* seeds was decreased after inoculation with *Ulocladium botrytis* Preuss. Boari and Vurro[10] observed a 60% reduction in the number and weight of emerging *O. ramosa* shoots, and also a 70% reduction in the number of tubercles attached to tomato roots after application of *F. oxysporum* and *F. solani* isolates in a plastic–bag system. The reduction in *Orobanche* seed germination by Foxy isolates may be due to the production of toxic metabolites.[14,28]

Because of the high specificity of *Fusarium* to *Orobanche* spp., which are major constraints to vegetable crop production,[29] it is preferable as a more specific mycoherbicide formulation agent. Host specificity is of great importance when considering a pathogen for biological weed control. A narrow host range provides higher environmental safety of a bioherbicide, but can also limit effectiveness if more than one weed species has to be controlled.[11,30] Formulation and processing strategies to reduce loss of fungal viability that may be caused by severe grinding, low water content in formulations or drying are desirable. The present results indicated that small Foxy pesta granules lost much more of their viable propagules than the coarse granules. These findings are in accordance with Connick *et al.*[31] who proposed that grinding may damage the spores to a greater extent in fine particles. However, different fungal species and spore types may be affected in different ways by grinding. For instance, FOO microconidia were found to be more susceptible to grinding than chlamydospore-rich biomass in pesta.[12] This could be attributed to the differential thickness of chlamydospore walls, which could retard the germination of chlamydospores, so forming lower colony-forming units than microconidia that seemed to be more susceptible to grinding. Müller-Stöver[12] observed a greater bioherbicidal efficacy of small-sized

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DOI: 10.1002/ps

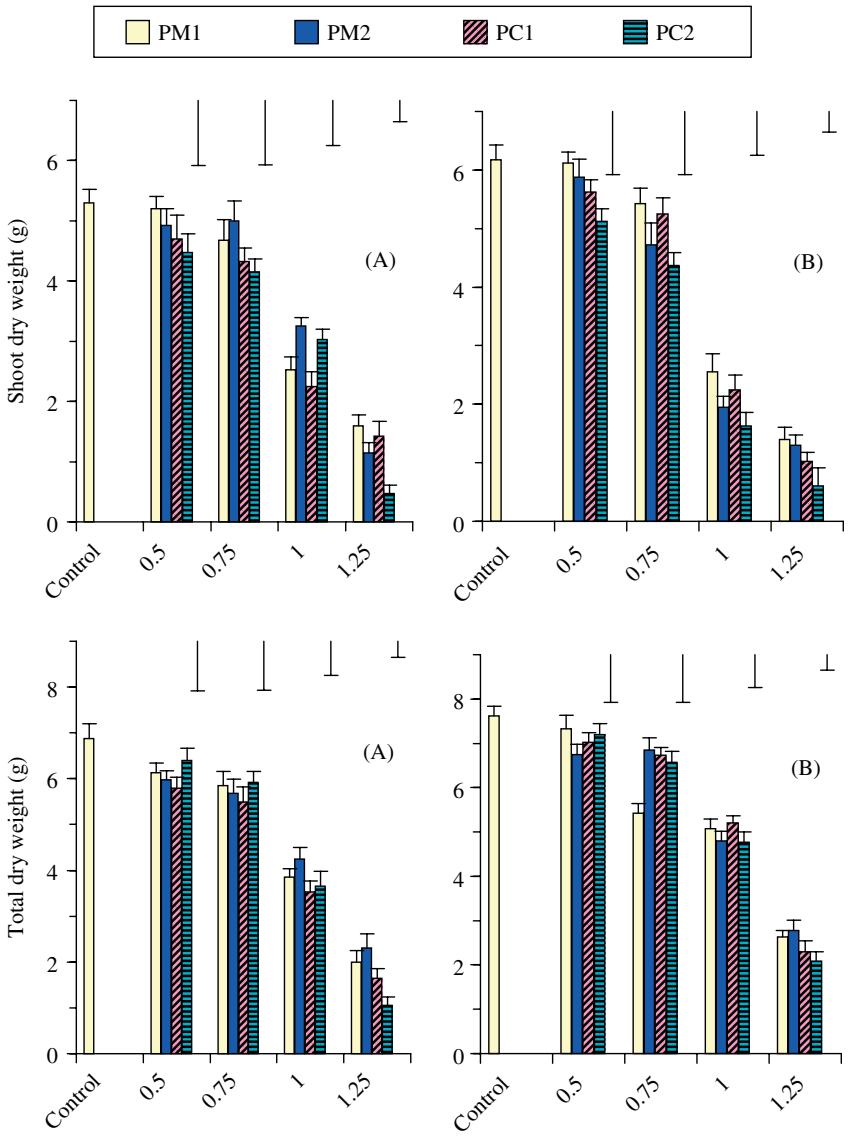

Figure 7. Effect of microconidia and chlamydospore granular pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates on shoot dry weight and total dry weight of *O. crenata* (A) and *O. ramosa* (B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

granules containing air-dried, chlamydospore-rich biomass of FOO than in the case of larger granules, probably owing to a better distribution in the soil.

Shelf life is very important for a commercial bioherbicide. The viability of living organisms in granular formulations during storage may be influenced by the nutritional amendments added to the formulation, which can, for example, prevent desiccation of the fungal propagules.[32] However, a loss of virulence after 6 months of storage was also observed in sodium alginate pellets in a greenhouse experiment. Likewise, storage temperature is another critical factor for the shelf life of a mycoherbicide. The propagule viability of all formulations in the present study decreased minimally by 8 and 18% for coarse and fine formulations stored for 3 months at 3 °C. However, the viability decreased by 37% on storage at 25 °C. Nevertheless, the viability dropped after storage for 1 year to 45 and 70% at 3 °C and to 71 and 82% at 25 °C. These findings indicate that low temperature is essential for longer shelf life of Foxy pesta mycoherbicides; propagules were damaged during storage at high temperature, probably owing to the high fungal metabolic activity. Therefore, the formulation granule size and storage at cool temperatures all contribute significantly to the preservation of the viability of the fungal inoculum.

Disease incidence (DI) and disease severity (DS) of *Orobanche* were highly accelerated. The highest dose (1.25 g kg^{-1} soil) augmented DI in *O. crenata* and *O. ramosa* by 97 and 95% respectively (Table 1). Similarly, DS was enormously enhanced to more than 96 and 92%. In the same pattern, more than 86 and 80% reductions were obtained in shoot emergence of *O. crenata* and *O. ramosa*, respectively, by application of the highest dose of all Foxy pesta formulations. Similar reductions were observed in total number of shoots, number of attachments, shoot height, shoot dry matter and total dry weight (Table 1). In this connection, Shabana *et al.*[9] reported that application of pesta formulations containing microconidia and

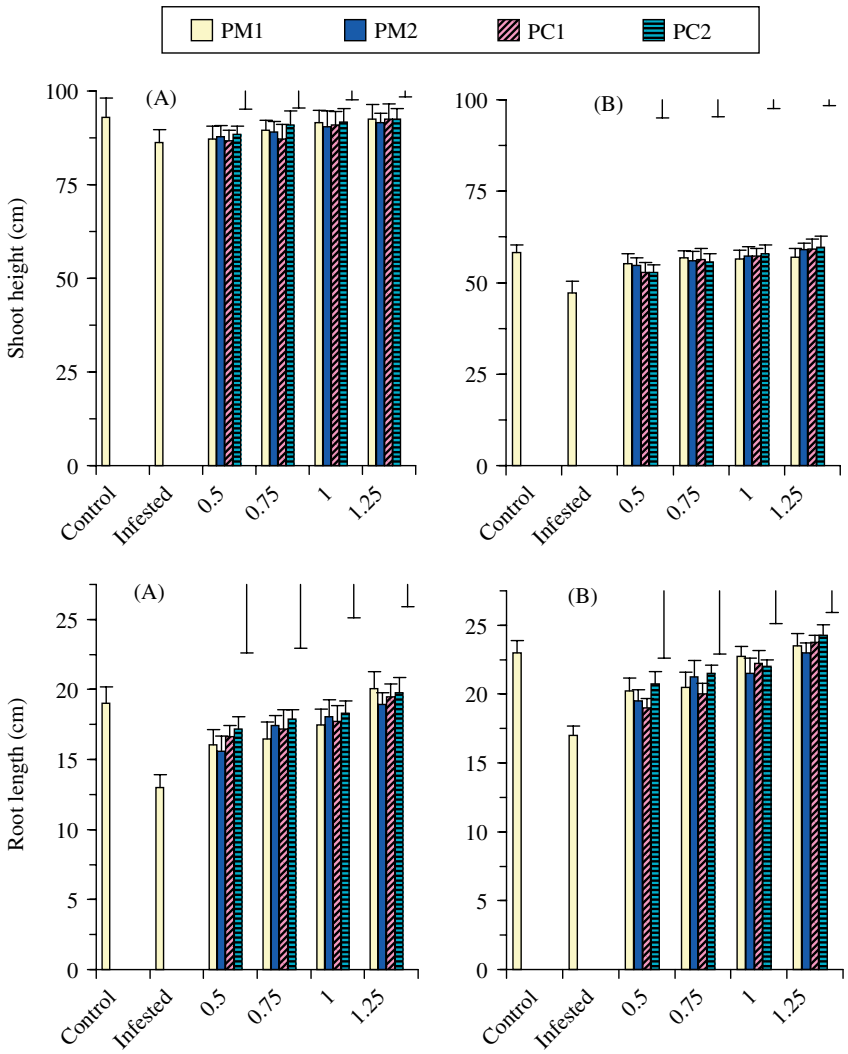

Figure 8. Effect of microconidia and chlamydospore granular pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates for biocontrol of *Orobanche* on host shoot height and root length (faba bean, A; tomato, B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

chlamydospores of FOO as a bioherbicide for *O. cumana* resulted in a reduction in *Orobanche* biomass (67–80%) and an increase in disease severity. Moreover, pesta granules reduced the emergence of *O. cumana* shoots by 64%.[27] Similar results were also observed by Müller-Stöver and Kroschel[13] on *O. crenata* by inoculation with *U. botrytis*.

Growth parameters of host species (faba bean and tomato) were greatly reduced by *Orobanche* infestation (Table 2). However, the reduction appeared to be counterbalanced following the application of all Foxy pesta formulations. These amendments could supplement host species under good growth conditions. In conformity with these results, Müller-Stöver and Kroschel[13] found that dry matter accumulation of faba bean was significantly increased by treatment with *U. botrytis* compared with *Orobanche*-infected control (faba bean plus *Orobanche*). Also, Shabana *et al*.[9] observed an increase in sunflower dry weight as a result of treatments with FOO. The *Orobanche*-induced growth inhibition of faba bean and tomato could be the result of parasitism which might deplete host nutrition. However, the application of pesta could overcome any malfunction in host metabolism caused by *Orobanche* infestation and consequently prevent the translocation of minerals to *Orobanche*, and so might support new syntheses and normal growth of host plants.

In conclusion, the mycoherbicide formulations from microconidia and chlamydospores of two isolates of *Fusarium oxysporum* (Foxy I and Foxy II) gave good results in controlling *Orobanche*. Conidial suspensions of both isolates inhibited germination, attachments and tubercles of *O. crenata* and *O. ramosa*. The formulated spores as pesta granules greatly diminished the emerged shoots of *Orobanche*, total shoot number, shoot height and number of attachments of emerged shoots, as well as shoot and total dry weight, although to varying degrees. Meanwhile, disease incidence and disease severity of emerged shoots were enhanced following the application of Foxy pesta. Moreover, Foxy formulations not only reduced *Orobanche* growth

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DOI: 10.1002/ps

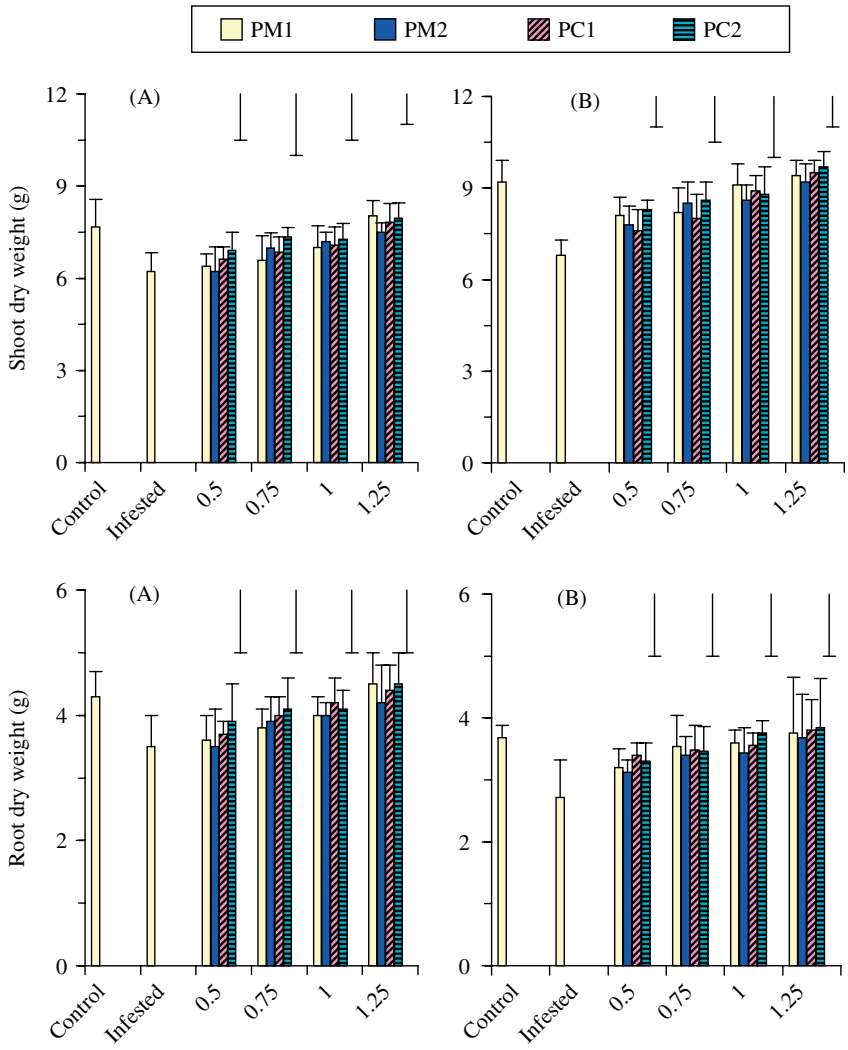

Figure 9. Effect of microconidia and chlamydospore granular pesta formulations (PM and PC) of two *Fusarium oxysporum* isolates for biocontrol of *Orobanche* on host dry weight (faba bean, A; tomato, B). Vertical bars above each data entry represent SD, and those after each group represent LSD at the 5% level.

Table 1. Efficacy of microconidia and chlamydospore pesta formulations (PM and PC respectively) of two *Fusarium oxysporum* isolates at 1.25 g kg^{-1} soil for biocontrol of *Orobanche*

	Percentage of respective control							
	O. crenata				*O. ramosa*			
	PM I	PM II	PC I	PC II	PM I	PM II	PC I	PC II
Disease incidence	1810	1930	1850	1875	1525	1546	1575	1583
Disease severity	1525	1546	1575	1583	2967	3083	3033	3192
Emerged shoots	19	25	17	14	20	22	20	20
Total number of shoots	20	22	20	20	38	35	39	32
Shoot height	22	24	26	21	28	21	25	35
Number of attachments	28	21	25	35	32	38	30	32
Shoot dry weight	30	22	27	9	23	21	17	10
Total dry weight	23	21	17	10	29	33	24	15

but also overcame the reduction in growth of host plant species (faba bean and tomato). These findings therefore suggest that Foxy formulations could be efficiently used as mycoherbicides for the biocontrol of *Orobanche* in faba bean and tomato fields instead of unsafe and/or non-selective chemicals.

Table 2. Efficacy of microconidia and chlamydospores pesta formulations (PM and PC respectively) of two *Fusarium oxysporum* isolates at 1.25 g kg^{-1} soil for biocontrol of *Orobanche* on growth of host plants (faba bean and tomato respectively)

	Faba bean					Tomato				
Parameter	Infested	PM I	PM II	PC I	PC II	Infested	PM I	PM II	PC Iq	PC II
Percentage of infested plants										
Shoot height		107	106	107	107		121	125	126	127
Root length		154	146	150	152		138	135	140	143
Shoot dry weight		129	120	126	128		138	135	140	143
Root dry weight		129	120	126	129		138	135	140	141
Percentage of normally grown plants										
Shoot height	93	99	98	99	99	81	98	101	102	103
Root length	68	106	100	103	104	74	102	100	103	105
Shoot dry weight	81	105	98	102	104	74	102	100	103	105
Root dry weight	81	105	98	102	105	74	102	100	103	104

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