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Research Article

Efficacy of granular Pesta mucilage formulation of *Fusarium oxysporum* for biological control of Egyptian broomrape *Phelipanche aegyptiaca* (Pers.) Pomel. in tomato fields

Moslem Taghipour[1], Ghorbanali Asadi[1*], Mehdi Rastgoo[1] and Mahmoud Reza Karimi Shahri[2]

1. Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Plant Protection Research, Khorasan Razavi Province, Agricultural Research and Training Center, Iran Agricultural Research, Education and Extension Organization.

Abstract: One of the employed formulations in the biological control of broomrape is the application of a Pesta granular formulation using semolina, sucrose, and kaolin. In this study, the efficacy of Pseta granular formulation of *Fusarium oxysporum* by using 3, 5, and 10% (w/w) Psyllium seed mucilage (PSM) on Egyptian broomrape *Phelipanche aegyptiaca* (Pers.) Pomel. control was investigated. Natural materials were used to stimulate the germination and growth of fungal spores by providing more available foodstuffs and moisture (during the dew period) for the spores. For this purpose, an experiment was conducted in the research greenhouse during the spring and summer of 2020. The results of this study showed that the application of PSM as mucilage Pesta granules (MPGS) resulted in more effective control of Egyptian broomrape, thereby increasing the fresh and dry weight of the tomato. The lowest dry weight of Egyptian broomrape (0.22 g pot^{-1}) and the highest dry weight of tomato (27.20 g pot^{-1}) were observed under MPG3 treatment (10% w/w). The highest dry weight of Egyptian broomrape (1.40 g pot^{-1}) and the lowest dry weight of tomato (21.12 g pot-1) were obtained under the MPG2 treatment (5% w/w), which was not significantly different from the control treatment (1.34 g pot^{-1}). It was concluded that the application of PSM at 10% w/w may increase the efficacy of the Pesta granule formulation in controlling Egyptian broomrape in tomatoes.

Keywords: Fungal suspension, Greenhouse, Mycoherbicide, Semolina, Spore

Introduction

Parasitic weeds, particularly root parasites such as broomrape (Phelipanche sp.), pose a significant threat to a wide range of economically important crops. Among the various species of broomrape, Egyptian broomrape *P. aegyptiaca* (Pers.) Pomel. It often attacks the potato and tomato, which are the most vulnerable crops to this parasitic plant, and causes significant economic losses every year (Joel DM, 2000; Rubiales, D., Alcantara, and Sillerog, 2004). Various methods have been proposed to control broomrape, but none of them have shown adequate or reliable results. One of the control strategies for broomrape is the application of biocontrol agents, especially fungal biocontrol

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*Corresponding authors: asadi@um.ac.ir
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agents. Fungal biocontrol agents can be used where chemical pesticide application is not possible (due to organochlorines) or obsolete (such as methyl bromide), or the weed is highly resistant. One of the fungal species used as a mycoherbicide in the biological control of weeds is *Fusarium* spp. Among the various species of this genus, *Fusarium oxysporum* has many special forms that induce disease in their hosts (Michielse and Rep, 2009). The advantages of *Fusarium* species for biological control include their easy culture in artificial media and the production of abundant propagules in the fermentation fluid. For the control of Egyptian broomrape, various isolates of Fusarium fungi have been identified and utilized as potential biocontrol agents. Still, none of them has been commercialized as a commercial mycoherbicide (Muller *et al*., 2008).

It is believed that some environmental conditions, especially long dew periods or leaf wetness requirements, can be met synthetically by appropriate formulation techniques (Boyetchlco *et al*., 1999; Greaves *et al*., 2000; Green *et al*., 1998). For microbial agents, the formulation may increase pathogen survival, contamination, propagation stability, and shelf life, and the components of the formulation may vary based on the type of organism present and the available spray equipment (Rhodes, 1993). In the formulations, many hydrophilic polymers have been reported that have facilitated the application of mycoherbicides on foliage, in particular, improving the creation of a suitable dew period and preventing the drying of biocontrol agents during the infecting process (Auld, 1993b; Connick *et al*., 1990; Lawrie *et al*., 2000; Shabana, 1997). The mycoherbicide formulation comes in various forms, including soil application. For the application of mycoherbicides in the soil, internal encapsulation of fungi in the solid matrix is more suitable than liquid formulations. Calcium alginate, mixed with fungal spores and a group of carriers such as kaolin clay, oat flour, soy flour, and corn flour, can be used in this formulation (Boyette and Walker, 1986). Conidia production and field conditions can be modified or improved by using food (additives) (Daigle and Cotty, 1992; Weidemann, 1988). Pesta, a type of granular formulation, contains semolina flour, kaolin, and a fungal suspension (Connick *et al*., 1991a). The most important issue in applying fungal herbicides in the soil is creating a dew period with high moisture content, which allows the spores to germinate and form spongy or fungal propagules. Most pathogens that can be used as mycoherbicides require a high moisture supply period (more than 80% humidity) for germination, infiltration, contamination, and removal of the target weed. This period varies from 16 to 24 h, depending on the pathogenicity and type of target weed (Minbashi *et al*., 2011). Among the polymers that can be used in mycoherbicide formulations, plant mucilage exhibits properties such as absorption and retention of moisture for an extended period, provides the necessary nutrients for the initial growth and development of fungal propagules, causes jelly formation and suspension, acts as an emulsifier, bulking agent, and enables the creation of a complete coating. These biopolymers offer numerous advantages, including being inexpensive, biocompatible, easily degradable, non-toxic, and providing good food sources. Mucilages have a high capacity for water bonding and water retention due to their abundant hydroxyl groups (Rishabha *et al*., 2011). This study aims to enhance the efficacy of F. oxysporum post-granule formulation for stimulating germination and growth of microconidia, develop a biocontrol agent, and utilize seed mucilage of Psyllium to influence certain morphological characteristics of Egyptian broomrape and tomato yield.

Materials and Methods

Fungal isolate

Collection, separation, and identification

Infected and diseased stems of Egyptian broomrape *Phelipanche aegyptiaca* in the late summer of 2019 from plants with disease symptoms such as plant hanging, vascular wilting, chlorosis or necrosis of flowering stems from tomato cultivation fields around the city of Mashhad of Razavi Khorasan province (50 º 58′

N, 36° 18′ E, altitude 966 m; North Eastern Iran) were collected and placed in paper bags and transported to the laboratory. Infected stems of Egyptian broomrape were washed entirely with tap water for 20 minutes. Then, they placed the Whatman filter paper (Whatman GF/A, 30 × 20 cm) on Whatman filter paper (Whatman GF/A, 30 × 20 cm) under a laminar hood (Laminar Air Flow, Class II, Pooya Electronic, Iran) to dry. Then, by using a sterilized scalpel, incisions were made 1 to 1.5 cm from the nearest infection site or the edge of the infection on the Egyptian broomrape stems. The samples were first immersed in 76% alcohol for 90 seconds and then disinfected with a 15% sodium hypochlorite solution for 12 minutes. They were then washed three times with distilled water and placed on Whatman filter paper under the hood to dry. Four pieces of plant samples were placed in Petri dishes with a diameter of 9 cm, containing WA medium on the four sides of the Petri dish, and were sealed with parafilm. They were incubated at 25 ± 2 °C and exposed to 12 hours of alternating light and dark. After 7 days, the subculture was prepared in PDA medium. For this purpose, inoculation needles from seven-day colonies formed around the cultured specimens and small pieces of culture medium with grown mycelium were removed and placed in the middle of the petri dish containing PDA medium. Approximately 36 Petri dishes containing PDA medium with cultured fungi were sealed with parafilm and placed in a laboratory environment for 4 days to promote growth. Eight fungal colonies with a morphology similar to *Fusarium oxysporum* were selected from 36 fungal samples grown in PDA medium, and their hyphae tip was cultured in a water agar medium. For identification, the samples were sent to the Phytosanitary Department of the Khorasan Razavi Agricultural Research and Training Center and the Plant Protection Group, Faculty of Agriculture, University of Tabriz, and then to the Karaj Topaz Institute for more accurate identification using sequencing. To re-identify fungal isolates based on Koch principles and methods, isolation of *Fusarium oxysporum* from infected stems of Egyptian broomrape was performed in pathogenicity test using morphological and microscopic characteristics of fungal colonies and organ grown in natural PDA, CLA and WA medium including color and size of colony, shape and micro and macroconidia, presence or absence of chlamydospores, shape and finally size of phialides according to Snyder and Hansen, (1954); Booth, (1971) and Das *et al*., (2019).

Preparation of spore suspension of *F. oxysporum* for pesta production

Four sterile Petri dishes (6 cm) and small pieces (1-2 mm) of Sucrose Nutrient Agar (SNA) containing *F. oxysporum* under a sterile hood were sterilized and then inoculated on Potato Dextrose Agar (PDA) medium. Petri dishes were then sealed with parafilm and placed in the laboratory environment (at a temperature of about 25 °C) for further growth. To prepare *F. oxysporum* suspension, a Potato Dextrose Broth (PDB) medium was prepared. Then, under the hood, five pieces of 5 mm of 4-day PDA medium containing mycelium and spores were taken and put in 250 ml Erlenmeyer flaks (three pieces of PDA medium for each Erlenmeyer flask), then the lid of Erlenmeyer flask was sealed with aluminum foil and was placed on the flask shaker (Flask shaker, FL 83, IRAN) with a rotation of 130 rpm at 25 °C for 6 days to produce conidia. To filter and separate the mycelium pieces from the spores (conidia), an 8-layer cheesecloth was used. The acquired suspension was then centrifuged (EBA 21, Germany) and subsequently centrifuged twice with distilled water. Finally, the concentration was adjusted using a hemocytometer to 1.2×10^8 spores ml^{-1}, and the suspension was used.

Plant Surveys

Phelipanche aegyptiaca seeds were collected from the field near Mashhad in Razavi Khorasan province, Iran (50 º 58′ N, 36° 18′ E, altitude 966 m; North Eastern) in 2019. This location had a history of growing tomato and being infected with the Egyptian broomrape (EBR). EBR plants with capsules containing seeds at the ripening

stage were identified and collected. After transferring them through paper bags to the laboratory, the capsules were separated, and the seeds were removed by hand. After that, the seeds and above-ground parts of the plant were passed through a sieve to separate the plant wastes from the seeds. Finally, the seeds were placed in an opaque plastic container and stored in a dry and dark place at 4 °C until application.

Extraction of Psyllium seeds-mucilage

For the extraction of mucilage from the seeds of Psyllium, the shell is separated from the seeds. 100 grams of Psyllium seeds (prepared from the Research and Training Center for Agriculture and Natural Resources of the Ministry of Jihad Agriculture, Medicinal Plants Department) cleaned with a laboratory mill model Krups type 210, Germany, for 30 s, and then passed through a 30 mesh sieve (the ratio of husk to seed is 1 to 4). Mucilage extraction was performed using alcohol and cold methods. The obtained bran was first mixed with distilled water (30 times the volume of the bran) using a laboratory electric stirrer (IKA RH basic 2 model) at 600 rpm and 25 °C. The resulting mixture was left to stand for 24 hours to achieve a homogeneous mixture. After 24 h, 30 mL of the resulting mixture was poured into 50 mL Falcon tubes. The tubes were then placed in a centrifuge (Farzaneh Arman Co., Farthest model, Iran) at 11000 g and 25 °C for 15 min to remove impurities. The sediment inside the falcons was mixed with 96% ethanol at three times its volume. They were centrifuged again at 6000 rpm for 10 min using 50 mL Falcon tubes. Afterward, the sediment was rewashed three times with 76% ethanol to remove colored hydrocarbons and residual impurities. The resulting sediment was finally placed in a 40 °C oven for 72 hours to dry, and the resulting powder was stored in a substantial container in the refrigerator for later use.

Preparation of *F. oxysporum* pesta granules

Seven mucilage Pesta granule formulations were produced to determine their efficacy against EBR under greenhouse conditions (Table 1). To prepare standard Pesta granules according to Connick *et al*. (1991) and Shabana *et al*. (2003), 32 g of semolina (durum wheat flour; Zarmacaron co., Iran), 6 g of kaolin (Merck, Germany), 2 g of sucrose (Kimia Tehran Acid, Iran) and 20 ml of microconidia suspension (1.2×10^8 spores ml^{-1}) were mixed and blended. The resulting dough was kneaded several times to obtain a uniform dough. The dough obtained was passed through thin rollers several times using a Pesta machine (Pesta Machine, MERCETO®, Allpie 150, Italy) until it was entirely uniform and shaped into sheets 1 mm thick. These sheets were then passed through Pesta maker rollers into thin strands with a diameter of 1 mm and were placed at room temperature (22 ± 3 °C) to dry. After drying, the strands were ground in a laboratory mill (Krups, Typ 210, Germany) for 30 seconds, and then the ground material was passed through a 30-mesh sieve. The aggregates between 1 and 2 mm were collected as standard Pesta granules and refrigerated. They were stored until the application (one week after preparation). To prepare the Pesta granule mucilage formulations according to the standard preparation method for Pesta granules, 32 g of semolina, 6 g of kaolin, and 2 g of sucrose, along with the determined percentages of Psyllium seed mucilage (3, 5, and 10% w/w) for each formulation, were mixed. Then, 20 mL of *F. oxysporum* suspension and deionized water, as needed, were added to form a dough, and the mixture was stirred until a uniform consistency was obtained. Finally, for the complete preparation of mucilage granules, 32 g of Psyllium seed mucilage powder was blended with 20 ml of Fusarium oxysporum suspension and 20 ml of deionized water. The obtained dough was thoroughly kneaded, and a uniform dough was prepared. The prepared dough was poured into the manual juicer (Jahao Lime Squeezer Manual Juicer Hand Press, Malltina, Turkey) with small 2 mm holes. Using hand pressure, the dough was pushed out of the holes in the form of strings and placed on aluminum foil at room temperature (25 ± 2 °C). It was then dried and ground as explained above.

Table 1 Ingredients of mucilage Pesta granule formulations encapsulated *Fusarium oxysporum*.

Code No.	Ingredients
1	40 g standard Pesta granule* + 1.2 g Psyllium seed mucilage powder (PSMP)+ 20 ml microconidia suspension (1.2×10^8 spores/ml) + 6 ml DI water
2	40 g standard Pesta granule + 2 g PSMP + 20 ml microconidia suspension (1.2×10^8 spores/ml) + 8 ml DI water
3	40 g standard Pesta granule + 4 g PSMP + 20 ml microconidia suspension (1.2×10^8 spores/ml) + 10 ml DI water
4	32 g semolina + 1 g PSMP + 20 ml microconidia suspension (1.2×10^8 spores/ml) + 6 ml DI water
5	32 g semolina + 1.6 g PSMP + 20 ml microconidia suspension (1.2×10^8 spores/ml) + 8 ml DI water
6	32 g semolina + 3.2 g PSMP + 20 ml microconidia suspension (1.2×10^8 spores/ml) + 10 ml DI water
7	32 g PSMP + 20 ml microconidia suspension (1.2×10^8 spores/ml) + 20 ml DI water

* Standard Pesta Granules: 32 g semolina (Zarmacaron) + 6 g kaolin (Merck) + 2 g sucrose (Kimia Acid).

For the survival test of *F. oxysporum* isolate spores after preparation of Pesta granules, 0.1 g of fresh Pesta granules containing spores of *F. oxysporum* isolate from each treatment were placed in a microtube containing 10 ml of sterile distilled water for 15 to 20 min. The sample was then stirred until it was completely dispersed and dissolved. One milliliter of the resulting mixture was removed and transferred into another microtube containing 9 milliliters of sterile distilled water. The mixture was then stirred again. This was repeated two more times to dilute it by a factor of three. Hundred µl of the last dilution obtained from each sample was placed into 6 cm disposable petri dishes (8 samples and 24 petri dishes) and then placed in a PDA semi-food medium with streptomycin sulfate (0.3 g L^{-1}) at room temperature (23 ± 2 °C) for 4 days to grow. After that, the number of colonies grown on the PDA medium was counted as 1.2×10^7 cfu g^{-1} of Pesta granules.

Pot experiment

To investigate the effect of Psyllium seed mucilage (PSM) application on the soil formulation of Pesta granules for biological control of EBR by Fusarium oxysporum, an experiment was conducted in 2020 at the research greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad. The experiment was designed as a randomized complete block design (RCBD) with nine treatments, including seven main treatments, and two positive and negative controls (Table 2), with 12 replications. About 108 plastic pots with dimensions of 17 × 36 cm with a capacity of 4 kg of soil in a ratio of 1: 1: 0.5 (farm soil, sand, and animal manure) at pH = 8.33 and EC = 1.13 ds m^{-1} were used to perform pot experiments. The soil was sterilized in an oven at 80°C for 4 h before the experiment. After that, each pot was filled with 4 kg of sterile soil and 200 mg of EBR seeds from the previous year (seeds with 86.75% survival based on tetrazolium test and 26.8% germination using tomato root extract) and was mixed with the top 5 cm of each pot and spread well, then 5 disinfected tomato seeds were planted at top 2 cm of the soil of pots and irrigated. Irrigation was performed with a low flow of water to prevent the washing away of EBR seeds and going to lower soil levels. Before sowing the seeds, a 20-20-20 N-P-K base fertilizer is applied according to the fertilizer recommendations at a rate of 100 ml per pot. After two weeks of sowing, two plants were kept in each pot. Mucilage pesta granules as 0.6 g kg^{-1} of soil (2.4 g of each formulation pot^{-1}) at a depth of 2 cm were applied 21 days after sowing and then gently irrigated. Up to 72 hours after inoculation, the moisture in the pots was maintained at saturation capacity, allowing the biocontrol fungus to germinate and function effectively. The ambient temperature of the greenhouse was set at 25 °C during the day and 18 °C at night; however, 45 days after sowing, the temperature increased to 27 °C during the day and 20 °C at night, remaining constant until the end of the experiment. Sixty days after sowing, two 400 W Sodium lamps (HPS LAMP 400W, OSRAN

VIA LOX, GREAT BRITAIN 0518) were used for 3 h every day from 5 pm to 8 pm as additional light until the end of the experiment. Greenhouse pests, such as Trialeurodes vaporarium and *Liriomyza huidobrensis*, were treated with various pesticides, including Acetamiprid at 500 ppm, Dichlorvos at 1500 ppm, and Thiacloprid at 500 ppm. The greenhouse experiment was repeated, and the variances among experiments were homogeneous.

Statistical analysis

Data were collected 100 days after sowing and analyzed. Statistical analysis of the data (ANOVA) was performed using the SAS software package (SAS, version 9.1.3), Minitab 17, and Excel 2013. Mean comparisons were performed at the 5% significance level ($P \leq 0.05$) using the Least Significant Difference (LSD) test. After harvest, a sample of soil around the roots was taken from each treatment to assess the impact of mucilage application on soil salinity and acidity.

Results

Pot experiments

The application of PSM, significantly reduced the total number EBR shoots compared to the negative control. Total number of EBR shoots in MPG3 (lowest total number of EBR shoots pot^{-1}) was 4.9 times lower with respect to the positive control. EBR shoots started to emerge 46 days after sowing and continued to emerge until the 78th day. Results showed that the fewest EBR shoots emerged in MPG3 (0.41 shoots per pot), which was 4.6 times lower compared to the positive control (1.91 shoots per pot) (Fig. 1).

EBR shoot height treatments were significantly different, so that EBR shoot height from MPG1 to MPG3 was decreased and, after that, started to increase. The highest length of the EBR shoots was observed in the positive control (4.46 cm), which was about 5 times higher than the lowest EBR (0.91 cm) (Fig. 2).

The experimental results showed that the EBR number of shoots per plant significantly decreased from 2.12 to 0.45 shoots per plant. The number of decreased EBR shoots per plant in MPG3 was 5 times that of the positive control (Fig. 3). The number of healthy EBR shoots reduced in different treatments from 4.5 to 0.2 shoots per pot. The total shoots that emerged and non-emerged in the positive control were healthy (100%), whereas the percentage of healthy EBR shoots decreased in MPG3 and MPG6 (Table 3). The percentage of diseased and lost EBR shoots increased rapidly during the experiment. The first infected EBR shoot emerged in MPG6, and died after two weeks, in other treatments, some EBR shoots that were infected by *F. oxysporum*, died after three weeks. The highest percentage of dead or infected EBR shoots was observed in MPG3 (78.03%), while the lowest percentage was found in the positive control (Table 3).

Table 2 Specifications of treatments used in the greenhouse trial.

No.	Treatment code	Description
1	MPG [a] 1	Soil was inoculated with mucilage standard Pesta granules (3% w/w)
2	MPG 2	Soil was inoculated with mucilage standard Pesta granules (5% w/w)
3	MPG 3	Soil was inoculated with mucilage standard Pesta granules (10% w/w)
4	MPG 4	Soil was inoculated with mucilage semolina Pesta granules (3% w/w)
5	MPG 5	Soil was inoculated with mucilage semolina Pesta granules (5% w/w)
6	MPG 6	Soil was inoculated with mucilage semolina Pesta granules (10% w/w)
7	MPG 7	Soil was inoculated with mucilage granules (100% w/w)
8	C^+	Positive control: Standard Pesta granules without PSM[b] and conidial suspension free
9	C^-	Negative control: Standard Pesta granules without PSM

[a] MPG: Mucilage Pesta Granule; [b] PSM: Psyllium Seed Mucilage.

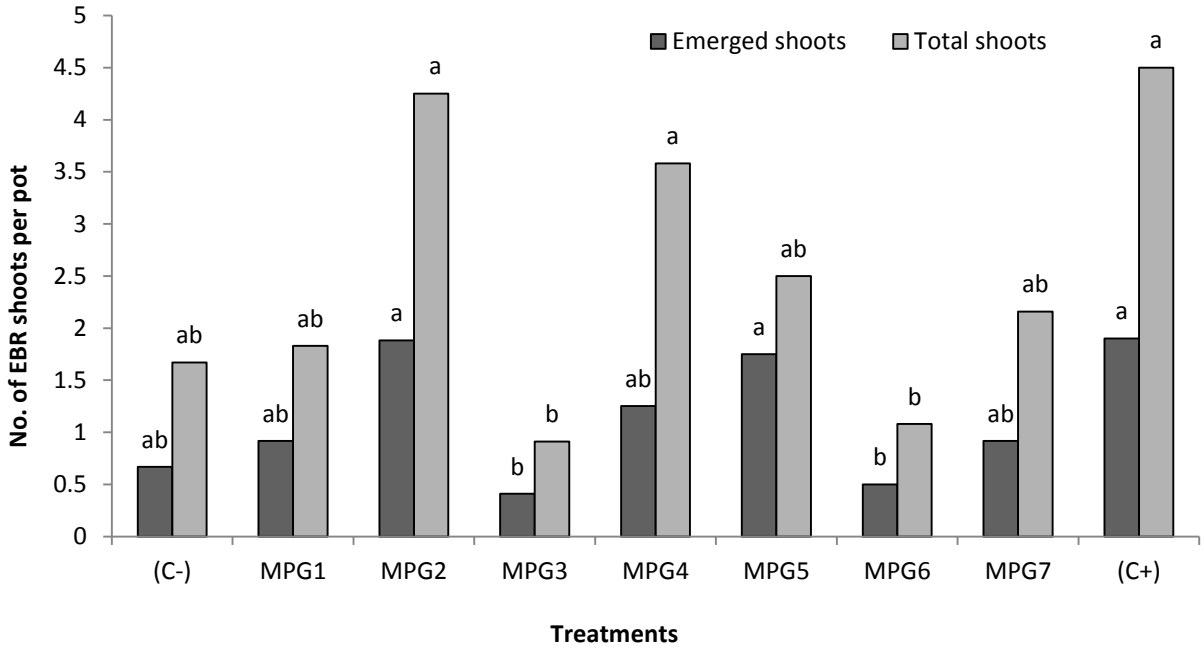

Figure 1 Egyptian broomrape (EBR) total number and emerged shoots in different treatments**.** Those values by same letter indicate do not differ significantly (LSD test, α = 0.05); MPG1-MPG3: Mucilage standard Pesta granules (3, 5 and 10% w/w); MPG4-MPG6: Mucilage semolina Pesta granules (3, 5 and 10% w/w); MPG7: Mucilage granules (100% w/w); C^+: Positive control and C^-: Negative control.

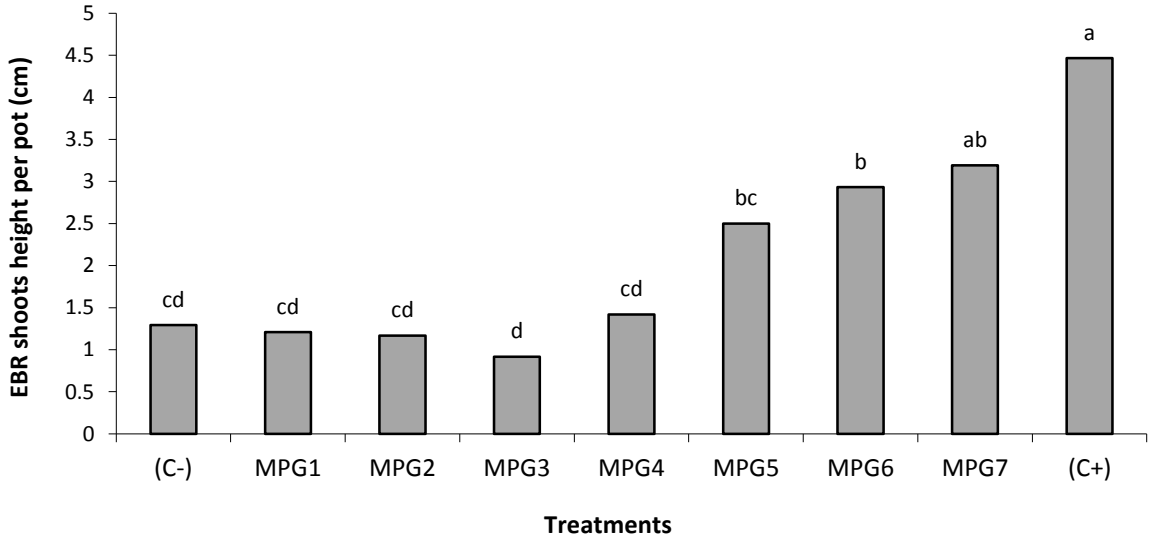

Figure 2 Egyptian broomrape (EBR) shoots height in different treatments. Those values by same letter indicate do not differ significantly (LSD test, α = 0.05); MPG1-MPG3: Mucilage standard Pesta granules (3, 5 and 10% w/w); MPG4-MPG6: Mucilage semolina Pesta granules (3, 5 and 10% w/w); MPG7: Mucilage granules (100% w/w); C^+: Positive control and C^-: Negative control.

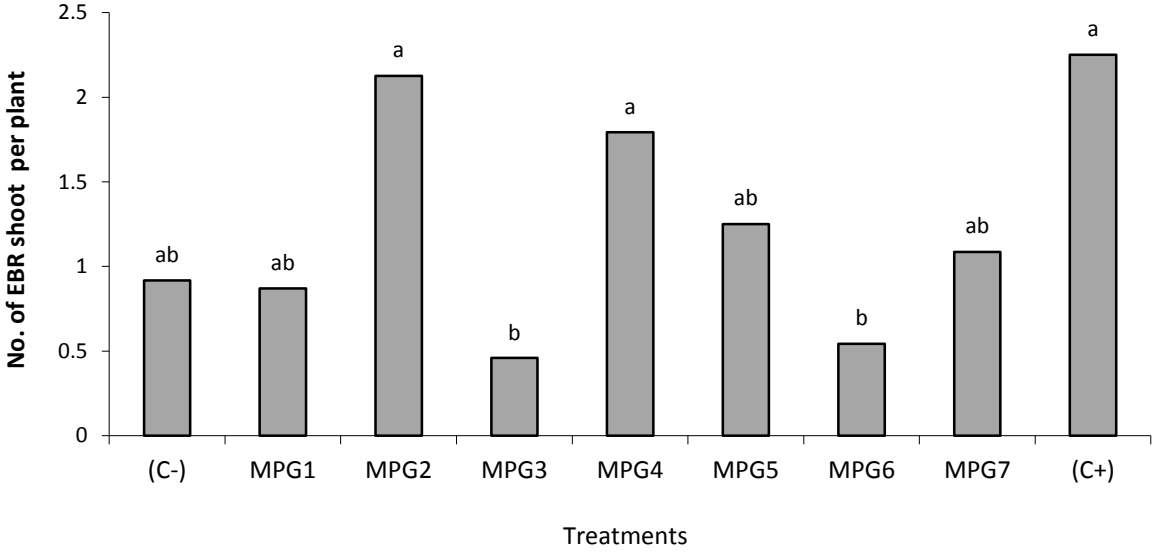

Figure 3 Egyptian broomrape (EBR) number shoots per plant in different treatments. Those values with similar letters indicate not significantly difference (LSD test, $\alpha = 0.05$); MPG1-MPG3: Mucilage standard Pesta granules (3, 5 and 10% w/w); MPG4-MPG6: Mucilage semolina Pesta granules (3, 5 and 10% w/w); MPG7: Mucilage granules (100% w/w); C^+: Positive control and C–: Negative control.

Table 3 Dead and healthy Egyptian broomrape shoots in different treatments.

Treatments	Dead or infected EBR shoots pot^{-1} (No.)	Dead or infected EBR shoots (%)	Healthy EBR shoots pot^{-1} (No.)	Healthy EBR shoots (%)	Total EBR shoots pot^{-1} (No.)
MPG 1	0.83^{b}	45.35	1.00^{b}	54.65	1.83^{ab}
MPG 2	1.92^{a}	45.18	2.33^{ab}	54.82	4.25^{a}
MPG 3	0.71^{b}	78.03	0.20^{c}	21.97	0.91^{b}
MPG 4	1.50^{ab}	41.90	2.08^{ab}	58.10	3.58^{ab}
MPG 5	1.67^{a}	66.80	0.83^{b}	33.20	2.50^{ab}
MPG 6	0.83^{b}	76.86	0.25^{c}	23.14	1.08^{b}
MPG 7	0.50^{bc}	23.15	1.66^{ab}	76.85	2.16^{ab}
C^+	0.00^{c}	00.00	4.50^{a}	100.00	4.50^{a}
C–	0.84^{b}	50.29	0.83^{b}	49.71	1.67^{ab}

Means in the same column followed by the same letters were not significantly different according to LSD test at level $\alpha = 0.05$; MPG1- MPG3: Mucilage standard Pesta granules (3, 5 and 10% w/w); MPG4-MPG6: Mucilage semolina Pesta granules (3, 5 and 10% w/w); MPG7: Mucilage granules (100% w/w); C^+: Positive control and C–: Negative control.

The reduction in the overall EBR shoots due to the application of mucilage Pesta granules by *F. oxysporum* was significantly different, from 5.61 to 0.61 g pot^{-1} and 1.40 to 0.22 g pot^{-1}, fresh and dry weight, respectively. The most effective treatments in this regard were MPG3, MPG6, and the negative control. These treatments also resulted in the fewest number of EBR attachments to the tomato roots, and subsequently, the least number of emerged and EBR biomass in MPG3, MPG6, and the negative control. The maximum fresh and dry weights of EBR shoots were in MPG2 and were not statistically significant from the positive control treatment. The minimum fresh and dry weight of EBR shoots observed in MPG3 was times lower compared to the positive control, and did not show a significant difference with MPG6 and negative control (Table 4).

Table 4 Fresh and dry weight of Egyptian broomrape and tomato in different treatments.

Treatments	EBR fresh weight (g pot^{-1})	Tomato fresh weight (g pot^{-1})	EBR dry weight (g pot^{-1})	Tomato dry weight (g pot^{-1})
MPG 1	3.64^{a}	187.92^{ab}	1.23^{ab}	24.95^{ab}
MPG 2	5.61^{a}	157.88^{c}	1.40^{a}	21.12^{c}
MPG 3	0.61^{b}	198.92^{a}	0.22^{c}	27.20^{a}
MPG 4	5.22^{a}	181.38^{b}	1.26^{ab}	24.45^{ab}
MPG 5	4.86^{a}	186.92^{ab}	1.28^{ab}	24.80^{ab}
MPG 6	1.55^{ab}	191.54^{a}	0.63^{b}	26.58^{a}
MPG 7	5.26^{a}	169.25^{bc}	1.21^{ab}	23.08^{bc}
C $^{+}$	5.40^{a}	161.92^{c}	1.34^{a}	22.20^{c}
C $^{-}$	1.40^{ab}	195.21^{a}	0.45^{b}	26.79^{a}

Means in the same column followed by the same letters were not significantly different according to LSD test at level $\alpha = 0.05$; MPG1- MPG3: Mucilage standard Pesta granules (3, 5 and 10% w/w); MPG4-MPG6: Mucilage semolina Pesta granules (3, 5 and 10% w/w); MPG7: Mucilage granules (100% w/w); C^{+}: Positive control and C^{-}: Negative control.

EBR infestation, especially in MPG2 and the positive control, negatively affected the fresh and dry weights of the tomato. It reduced the fresh and dry weight of tomatoes in pots that were untreated with Pesta granules (positive control) or treated with standard Pesta granules plus PSM at 5% w/w (MPG2). In the positive control treatment, despite the high number of EBR shoots that emerged from the soil compared to other treatments, the high growth rate of EBR shoots resulted in both EBR fresh and dry weights being high enough to reduce the fresh and dry weights of the tomato. However, the highest fresh and dry weights of tomato were observed in MPG3, which was not significantly different from the negative control and MPG6. Compared to the positive control, the dry and fresh weights increased by 18.38% and 18.6%, respectively (Table 4).

As shown in Table 5, increasing the application of the percentage PSM (3 to 10% w/w) in treatments caused a reduction of soil pH and an increment of soil salinity. In comparison, the application of PSM alone with full coverage (100%), increased the pH and soil salinity compared to the positive control. In the negative control, in which only standard Pesta granules were used, acidity and salinity compared to the positive control were increased.

Table 5 Salinity and acidity of soil around the tomato roots in different treatments.

Treatments	pH	EC ds m^{-1})
MPG 1	8.43	0.45
MPG 2	8.41	0.47
MPG 3	8.36	0.51
MPG 4	8.41	0.44
MPG 5	8.41	0.45
MPG 6	8.32	0.79
MPG 7	8.51	0.64
C $^{+}$	8.10	0.48
C $^{-}$	8.36	0.55

Discussion

Based on this experiment, encouraging and positive results were obtained through the application of PSM, which can increase the efficacy of the Pesta granule formulation of F. oxysporum in controlling EBR in tomato. Among the MPG1, MPG2, and MPG3 treatments, which used 3, 5, and 10% w/w PSM, respectively, the control of EBR and biomass of the tomato in MPG1 was lower compared to the negative control (0% w/w PSM), but higher than the positive control. Control of EBR and biomass of the tomato in MPG2 was lower than that of the negative control and did not show a significant difference compared to the positive control. In contrast, in MPG3 (10% w/w PSM), the control of EBR and biomass of the tomato was higher than that of the positive and negative

controls, MPG1 and MPG2. While comparing MPG4, MPG5, and MPG6 treatments, only semolina flour and PSM were used at 3, 5, and 10% w/w. The control of EBR and biomass of the tomato were higher, respectively, as the application of PSM increased. In MPG6 (10% w/w), control of the EBR and biomass of the tomato was better and higher compared to the positive control, MPG4 and MPG5 (0, 3 and 5% w/w PSM, respectively). For MPG7, the F. oxysporum suspension was completely covered by PSM, and the control of EBR and biomass of the tomato was lower compared to the negative control but higher than that of the positive control. Shabana *et al.* (2003) reported that some adjuvants may facilitate faster regeneration of the fungus, a greater number of conidia on the surface of granules, improved spore viability and germination, and/or enhanced metabolic activity. Natural polymeric materials in agriculture can be used in the formulation of mycoherbicides as adjuvants to retain moisture and enhance the viability, germination, and efficacy of fungal agents in mycoherbicides. Among these products, the application of Xanthan gum and Gellan gum in bioherbicides using *Alternaria cassia* and *A. eichhorniae* pathogens has also been mentioned (Shabana *et al*., 1997). Substances such as Psyllium (e. g. Metamucil®) are known to have the property of retaining moisture and reducing the rate of moisture loss. One of these products uses a combination of polyvinyl alcohol and Metamucil for controlling Lamb's quarters (*Chenepodiom album*) (Greaves *et al*., 2000).

It appears that the application of PSM at 10% w/w as a supplement to standard Pesta granules or with only semolina flour has been able to increase the efficacy of the Pesta granule formulation and improve it. The study results showed that the characteristics of EBR, such as the percentage of total and emerged EBR shoots, the height of EBR shoots, the number of EBR shoots per plant, the fresh and dry weight of EBR shoots, and the fresh and dry weight of tomatoes, had significant differences compared to the positive control. Therefore, it can be reported that the seed mucilage of the Psyllium plant can play a complementary role and modify and enhance the biological effects of the *F. oxysporum* as the biocontrol agent in control of EBR. Probably, the impact of PSM in supplying and retaining moisture for fungal growth, preventing drought, supplying nutrients, and increasing the adhesion of biocontrol agents to soil particles has increased production efficacy. However, using PSM alone in experiments has little effect on the efficacy of biocontrol fungal granule formulations. Shabana *et al*. (2003) stated that mycoherbicides, using special additives such as yeast extract, sucrose, and Sodium Alginate for the preparation of Pesta granules, improved the impact, exhibited higher stability, and had a longer shelf life than standard Pesta granules. Providing a 12-hour dew period is essential to ensure proper moisture for the fungus to germinate and grow on the granules. As stated, many biocontrol fungi need dew periods ranging from 6 to 24 h to achieve satisfactory control (Boyette *et al*., 1990). Application of materials such as water traps (water locks), which are a type of moisture-absorbing polymer that can retain water, resulted in 100% survival of chlamydospores and microconidia. These results were similar to the findings of Quim *et al*. (1999), who used starch as a desiccant in the formulation of *F. oxysporum* granules. All granular formulations of *F. oxysporum* f. sp. *Orthoceras* that were used against broomrape plants in sunflower fields showed a high occurrence rate of disease and its severity (above 80%), while with 2 g of stillage (which seems to be a kind of gum or mucilage) added to standard post-granules EBR biomass, it decreased by 63.7%. Connick *et al*. (1997, 1998) also reported that the relationship between water activity and the half-life and viability of biocontrol agents in post-granular formulations is a crucial and fundamental issue.

Using the amount of mucilage in the Pesta granule formulations showed that a higher amount of PSM increased the control of EBR. Instead, its dry weight decreased. This effect was higher when used in conjunction with standard Pesta granular and PSM at a 10% w/w concentration. As the results showed, in MPG3

and MPG6, where the applied PSM was 10% w/w, the control of EBR was better, and the number of EBR shoots, as well as the fresh and dry weights, were reduced, whereas the dry weight of the tomato increased. However, in MPG3, due to the improved mucilage performance with standard Pesta granules, less growth and expansion were allowed in the early stages of tubercle development, and EBR connections to plant roots were better controlled. Therefore, fewer shoots of parasites were able to emerge from the soil, resulting in less damage to the tomato. While more EBR shoots and tubercles were established in the early stages of MPG6, in later stages and throughout the experiment, the biocontrol agent was able to infect EBR shoots, damage them, and destroy them. By increasing the amount of PSM in Pesta granules by 10% w/w, the efficacy of the increased Pesta granule formulation of *F. oxysporum* in EBR control. It may be assumed that increased control of EBR in the early stages, when PSM was used in conjunction with standard Pesta granules, occurred with a slight delay and resulted in delayed control of florets when semolina flour was used. The order of performance of treatments in EBR control and biomass tomato is as follows:

EBR control MPG3 > MPG6 = C^- > MPG1 > MPG5 > MPG4 > MPG7 > MPG2 = C^+
Biomass tomato MPG3 = MPG6 = C^- > MPG1 > MPG5 > MPG4 > MPG7 = MPG2 = C^+

As other studies have reported, the variability in weed-control efficacy among the different Pesta formulations may be attributed to the different types and amounts of food base in the formulation, resulting from the various additives added to the product (Shabana *et al*., 2003). Regarding the application of Pesta granules in soil, the results of Nemat *et al*. (2008) should be taken into consideration. In their experiment, using standard Pesta granules with 6 g of yeast extract and 2 ml of glycerol using two isolates of *F. oxysporum* in controlling EBR, a reduction of 5 to 14% (in 0.5 g of Pesta granules kg^{-1} of soil) against 70 to 79% of EBR joints (in 1.25 g of Pesta granules per kg of soil) to the host root was achieved. Furthermore, a 5% reduction in the height of EBR was achieved, compared to a 79% reduction using two values of 0.5 and 1.25 g kg-1 of soil. However, in another study, the application of 1 and 2 g of Pesta granules of *F. oxysporum* did not show a significant difference in the number and dry weight of flowers. Still, a notable difference existed between the control and treatment groups. Application of 2 g of Pesta granules significantly reduced the number of *O. cumana* shoot pot^{-1} and significantly reduced the shoot of broomrape compared to the control treatment (Muller-Stover *et al*., 2008).

Conclusion

Based on the results of this study, the application of PSM by 10% w/w along with standard Pesta granules of *F. oxysporum* or in the form of semolina Pesta mucilage granules, which provide suitable growth conditions for mycoherbicide, improves the efficacy of pesta granule formulations and results in better control of EBR and increased fresh and dry weight of tomato. The application of PSM at 10% w/w has been more effective in controlling EBR and increasing the biomass of tomato compared to Pesta granule formulations containing 3% and 5% w/w PSM.

Conflict of interest

The authors have no conflict of interest.

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اثر کاربرد فرمولاسیون موسیلاژی گرانول پستای‌قارچ *Fusarium oxysporum* بر کنترل گل جالیز مصری *Phelipanche aegyptiaca* (Pers.) Pomel. در مزارع گوجه‌فرنگی

مسلم تقی‌پور[1]، قربانعلی اسدی[1*]، مهدی راستگو[1] و محمودرضا کریمی‌شهری[2]

۱- گروه اگروتکنولوژی دانشکده کشاورزی دانشگاه فردوسی مشهد، ایران.
۲- بخش تحقیقات گیاه‌پزشکی مرکز تحقیقات، آموزش و ترویج کشاورزی و منابع طبیعی خراسان رضوی، سازمان تحقیقات، آموزش و ترویج کشاورزی و منابع طبیعی ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: asadi@um.ac.ir
دریافت: ۲۷ مهر ۱۴۰۳؛ پذیرش: ۱ بهمن ۱۴۰۳

چکیده: یکی از فرمولاسیون‌های به‌کار رفته در کنترل بیولوژیک گل جالیز، فرمولاسیون گرانول پستا با استفاده از آرد سمولینا، ساکارز، کائولین و سوسپانسیون قارچی است. براین اساس، در این مطالعه اثر کاربرد فرمولاسیون گرانول پستای قارچ *Fusarium oxysporum* با استفاده از درصدهای مختلف موسیلاژ بذر گیاه اسفرزه (۳، ۵ و ۱۰ درصد وزنی) جهت کنترل گل جالیز مصری *Phelipanche aegyptiaca* (Pers.) Pomel. مورد بررسی قرار گرفت. مواد طبیعی برای تحریک جوانه‌زنی و رشد اسپور قارچ با فراهم کردن رطوبت بیشتر (دوره شبنم) و مواد مغذی جهت رشد، استفاده گردید. به‌همین منظور آزمایشی در گلخانه تحقیقاتی در بهار و تابستان ۱۳۹۹ انجام گردید. نتایج این مطالعه نشان داد که کاربرد موسیلاژ بذر گیاه اسفرزه به‌عنوان ماده افزودنی در گرانول پستا (MPGs)، منجر به کنترل بیشتر گل جالیز مصری و افزایش وزن خشک و تازه بوته‌های گوجه‌فرنگی شد. کم‌ترین وزن خشک گل جالیز مصری (۰/۲۲ گرم در گلدان) و بالاترین وزن خشک گوجه‌فرنگی (۲۷/۲۰ گرم در گلدان) در تیمار گرانول پستای استاندارد با ۱۰ درصد موسیلاژ (MPG3-10% w/w) مشاهده شد. بیشترین وزن خشک گل جالیز مصری (۱/۴۰ گرم در گلدان) و کم‌ترین وزن خشک گوجه‌فرنگی نیز در تیمار گرانول پستای استاندارد با ۵ درصد موسیلاژ (MPG2- 5% w/w) مشاهده شد که با تیمار شاهد مثبت با ۱/۳۴ گرم در گلدان اختلاف‌معنی‌داری نداشتند. بنابراین کاربرد ۱۰ درصد وزنی موسیلاژ به‌همراه گرانول پستای استاندارد تأثیر مثبتی بر کنترل گل جالیز مصری داشته و می‌تواند به‌عنوان ماده مکمل و افزودنی مورد استفاده قرار گیرد.

واژگان کلیدی: آرد سمولینا، اسپور قارچ، سوسپانسیون قارچی، علفکش قارچی و گلخانه