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# Biological control of *Helminthosporium sativum* the causal agent of root rot in wheat by some antagonistic fungi

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## ABSTRACT

The present study has been undertaken to determine the efficacy of some antagonistic fungi isolated from the rhizosphere of wheat plants grown in Summer region, Diwaniya Governorate, Mid Iraq for the biological control of *Helminthosporium sativum*, the causal organism of root rot disease of wheat *in vitro*. Three different species from the genus *Trichoderma*: *T. harzianum, T. pseudokoningii and T. lignorum*, in addition to *Stachybotrys atra* and *Penicillium* sp. were isolated from the rhizosphere of wheat plants. Laboratory experiments indicated that *T. harzianum* and *S. atra* were highly antagonistic to the pathogen when grown together on potato dextrose agar in Petri plates. Microscopic examination of the mycelia showed that hyphae of *T. harzianum* were parasitized *H. sativum*, coiled around its hyphae and caused its lysis, but did not penetrate inside the hyphae. However, *S. atra* was invaded colonies of *H. sativum* and caused severe hyphal damage. In the experiments of culture filtrates of the antagonistic fungi, *T. harzianum and S. atra,* were able to suppress growth of *H. sativum*, if incorporated in the medium and proved to be effective in controlling the pathogen. Results of effect of filtrate on spore germination showed that about 80 and 95% of them are unable to germinate with high concentrations (15 or 20 %) of culture filtrates of *T. harzianum* and *S. atra* respectively. However, the other antagonists: *T. pseudokoningii, T. lignorum* and *Penicillium* sp. were less effective in inhibiting spore germination of the pathogen. Results of antagonistic effect of the culture filtrates on wheat seed infection, except *T. lignorum*, showed that seed colonization by the root rot fungus was decreased significantly at the concentrations of 15 and 20 % compared with control. Seed coating with antagonistic fungi was the best biological seed treatment for reducing seed rot and diseased seedling caused by *H. sativum*. Antagonistic fungi have no side effects on seed germination except *Penicillium* sp. which reduced the seed activity and germination as compared with the other antagonists and with the control. It can be concluded that *T. harzianum* is a strong mycoparasite and *S. atra* is a good antagonistic agent to control *H. sativum*, but in fields may be their activity are conditioned by soil environment specially the microflora.

**Keywords:** Biological control - *Helminthosporium sativum* -Fungi

## INTRODUCTION

Seed borne diseases of cereals may cause heavy loses to the crops at all stages of growth, seed germination, seedling and maturing plants (Sarhan *et al*., 2001). Root rot disease of wheat (*Triticum aestivum* L.) caused mainly by the fungus (*Helminthosporium sativum* Pam. King and Bakke) is a typical simple interest disease, meaning that throughout the season plants are infected from the same sources of inoculum i.e. seeds, soils and debris (Harding, 1972). An increase in the percentage of diseased plants with time also suggests the occurrence of new infections in all ages of plants (Verma and Spurr, 1987). There are no effective chemical control measures have yet been devised that will completely eradicate the pathogen. It was reported that the pathogenicity of some seed or soil fungi has been influenced greatly by associated microflora (Tveit and moore, 1954). A living, multiplying, biological agents potentially may provide continuous control of a pathogen, whereas chemical applications usually are effective for a limited time period. Several workers have examined the possibilities of using antagonistic fungi for the suppression of *H. sativum* (Campbell, 1956; Biles and Hill, 1988). In recent years there has been much success in obtaining biological control of plant pathogens by mycoparasites and antagonistic fungi (Abdelmonem and Rasmy, 2000; Sarhan and Shibly, 2003; Sarhan, 2006; Al-Chaabi *et al.*, 2007). In a previous investigation we characterized a some native species of *Trichoderma* which have been useful for biological control of barley and rice pathogens (Sarhan and Abood, 1996; Sarhan, 2000; Sarhan and Shibly, 2003). In this study, which is an extension of such work, we have investigated the possibilities of suppressing the activity of *H. sativum*, the causal agent of wheat root rot disease, by five antagonistic fungi i.e. *Trichoderma harzianum, T. pseudokoningii, T. lignorum, Stachybotrys atra* and *Penicillium* sp. isolated from the rhizosphere of wheat plants in Iraq.

## MATERIALS AND METHODS

**Isolation of the pathogen:**

The isolate of *Helminthosporium sativum* used in this study was obtained from a naturally infected roots of a winter wheat plants collected from a commercial fields in Summer region, during disease surveys of Diwaniya governorate, Mid Iraq. The pathogen was isolated on potato dextrose agar (PDA) medium, purification and identification of the fungus were done as reported previously (Sarhan and Abood, 1996).

**Isolation of the antagonistic fungi:**

A dilution plate method was used to isolate the antagonistic fungi from soil of different fields in Diwaniya governorate, Mid Iraq. Acidified potato dextrose agar, rose bengal–streptomycin agar and malt agar were used for isolating five antagonistic fungi i.e. *Trichoderma harzianum, T. pseudokoningii, T. lignorum, Stachybotrys atra* and *Penicillium* sp. (Sarhan and Abood, 1996; Sarhan and Jasim, 2000).

***In vitro* tests for antagonism:**

For testing antagonism in cultures, a 8-mm disc cut from a 5-day-old culture of *H. sativum* was put near the periphery of the PDA in a Petri dish. The disc of test antagonist was then placed at the periphery directly opposite the disc of pathogen on agar medium in the same plate. Distances between pathogen and antagonist were made standard. Cultures were incubated at 25°C for 5, 10 and 15 days before the degree of antagonism, if any, was measured. A 1 – 5 scale was used to determine the degree of antagonism on PDA medium, where:
1= antagonist covers the whole plate,
2= antagonist covers 3/4 of the plate,
3= antagonist covers 1/2 of the plate,
4= pathogen covers 3/4 of the plate and
5= pathogen covers the whole plate. (Bell *et al*., 1982). There were four replicates of each treatment, and the test was repeated three times.

**Interactions between paired cultures (antagonist / pathogen):**

Petri plates with PDA were inoculated with an agar disc (8-mm) from an actively growing colony of pathogen and with one of test antagonist placed 40-mm apart. Cultures were incubated at 25°C. Hyphal interactions were examined microscopically from the time of confluence every 24 hr. for 7 – 10 days and the hyphal alterations were recorded. There were four replicates of each treatment, and the test was repeated three times.

**Quantification of the effect of antagonist filtrates on pathogen:**

The possible involvement of metabolite (s) in antagonism produced by fungal antagonists filtrate was tested as follows. Antagonist cultures were grown in 250 ml flasks containing 100 ml potato dextrose broth (PDB) for one week at 25°C, the liquid cultures were filtrated first through cheesecloth then followed by filtering through Millipore filter (0.45 µ pore size) to give sterile and cell free culture filtrate (Abdel-Rahim and Surrieh,1988). Three methods of applying filtrates were tried: (I) Filtrates, at different concentrations (5, 10, 15 and 20 %), were mixed with autoclaved PDA medium before solidify (at around 45°C) then pored in Petri plates, inoculated with the pathogen, incubated at 25°C for one week and examined for inhibition of mycelial growth and sporulation of *H. sativum*. To prepare spore suspension and disperse conidia evenly, 5 ml of autoclaved distilled water was added to each culture plate, conidia were removed by gentle scraping with a glass microscope slide. The spore suspensions were strained through cheesecloth and counted under 20X magnification. Four 0.1-ml counts were made from the suspension in each plate. (II) Filtrates were tested, towards spore germination and length of germ tubes of pathogen, by placing drop of filtrate in concave glass slide then adding a drop of spore suspension, keeping the slides in a moist conditions inside Petri plates for 16 hr. and examined to observe and calculate the spore germination and germ tube lengths of *H. sativum* using the ocular microtome. (III) Wheat seeds, naturally infected with *H. sativum*, were immersed in filtrate for 30 min. and germinated on germination blotters (Sarhan, 2003). After 7-10 days from planting percentage of seed germination and seed colonization, by pathogen, were recorded. There were four replicates of each treatment, and the test was repeated three times.

**Coating seeds with antagonistic fungi:**

Treatments with antagonists were done by dipping 10 g. wheat seeds, naturally infected with *H. sativum*, in a flask with appropriate volume of spore suspension of test antagonist amended with autoclaved 0.5 % methyl cellulose (as adhesive material) for 30 minutes. The seeds of control treatments were immersed in autoclaved 0.5 % methyl cellulose only. Spore suspensions of antagonists were prepared from 1-week-old cultures as previously described and calibrated to 2 x $10^5$ conidia /ml of autoclaved distilled water with the aid of a hemacytometer (Sarhan, 2006). Treated seeds were dried in an room temperature then planted on germination blotters. After 7-10 days from planting the percentage of seed germination, seed rot and infected radicles were recorded. There were five replicates of each treatment, and the test was repeated four times.

## RESULTS AND DISCUSSION

**Antagonism:**

In *in vitro* tests, all 5 fungal antagonists restricted the growth of the pathogen of wheat on PDA medium. The degree of antagonism ranged from 1.0 to 2.9. *Trichoderma harzianum* and *Stachybotrys atra* were highly antagonistic to *H. sativum* when grown together in culture plates. Maximum antagonism, of 1.0 degree, was achieved by these two antagonists after10 days of incubation (Table 1). Typical inhibition of *H. sativum* caused by *T. harzianum* and *S. atra* proved to be strongly effective in inhibiting the pathogen. However, *T. pseudokoningii, T. lignorum* and *Penicillium* sp. were less effective in controlling the pathogen. Circumstantial evidence indicated that *S. atra* was antagonistic to *H. sativum* by the production of an toxic substances (Domch *et al.*,1980). On PDA, *T.*

*harzianum* inhibited *H. sativum* and then grew over it, which indicates that *T. harzianum* is the more aggressive saprophyte in culture, and also appeared to be antagonistic by its occupation of the substrate, production of an antibiotic substances and perhaps by physically excluding the pathogen (Dossantos and Dhingra,1982). The antagonistic effect of antagonists interferes with successive phases of the development of pathogen.

Table 1: Class of antagonism ( *in vitro* ) of five antagonistic fungi against *H. sativum* on PDA medium.

| The antagonists | *Class of antagonism ( 1 – 5 degrees ) at different incubation periods (days) | | |
|---|---|---|---|
| | 5 days | 10 days | 15 days |
| Control | 5.0 a | 5.0 a | 5.0 a |
| *Trichoderma harzianum* | 2.2 b | 1.0 c | 1.0 c |
| *T. pseudokoningii* | 2.5 b | 1.6 c | 1.7 c |
| *T. lignorum* | 2.7 b | 2.6 b | 2.9 b |
| *Stachybotrys atra* | 1.8 c | 1.0 c | 1.0 c |
| *Penicillium* sp. | 2.4 b | 1.9 c | 1.8 c |

* A 1-5 scale was used to determine the degree of antagonism on PDA.
Numbers in the same column with the same letters are not significantly different at p= 0.05.

**Mode of action of antagonists against pathogen (pairing cultures):**

In paired colonies of antagonist and pathogen on PDA, hyphae of *T. harzianum* coiled around hyphae of *H. sativum*, parasitized its hyphae and caused their lysis, whereas *T. pseudokoningii, T. lignorum* and *Penicillium* sp. appeared to be antagonistic by its production of an antibiotic, as suggested also by Ikuotum and Agboola (1992). Coiling, however, does not necessarily imply hyperparasitism, competition for the available substrate or antibiosis could be other possible biological control mechanisms (Sarhan, 2000). No hyphal interactions were observed between other antagonists and *H. sativum*, this means that if antagonist does not hyperparasitized pathogen, it may compete for nutrients with the pathogen in the medium. Two days after contact between colonies of *S. atra* and *H. sativum*, antagonist invaded the colonies of pathogen and caused severe mycelial damage. Hyphae of pathogen exhibited high vacuolation of the cytoplasm followed by disappearance of hyphal contents, this probably due to enzymatic substances produced by the antagonist. This is in accordance with earlier investigations on growth of *S. atra* (Jermyn, 1953 ; Youatt, 1958) and on species of *Trichoderma* ( Elad *et al.*, 1982; Ahmad and Baker, 1987) which showed that these fungi able to produce cell wall-degrading enzymes, such as cellulase, chitinase, in addition to B-glucosidase and protease.

**Effect of culture filtrates:**

In this test, culture filtrate of the antagonists was able to suppress growth and sporulation of *H. sativum*, if incorporated in the medium (Table 2). The higher concentrations, of all antagonistic fungi, were able to suppress significantly growth and sporulation of pathogen on PDA medium as compared with the control, whereas no significant effects were observed at the low concentration (5%) of culture filtrate. Mycelial growth of the pathogen decreased sharply with increasing the concentrations of culture filtrate in PDA medium, radial growth reduced from 8.8 to 1.9 and 1.5 cm in treatment with 15 % filtrate of *T. harzianum* and *S. atra* respectively. The average number of conidia of *H. sativum* was significantly reduced from 38.550 in control to 6.500 and 2.590 conidia/ml after treatment with 15% filtrate of *T. harzianum* and *S. atra* respectively, this means that sporulation capacity decreased by 74 and 91 % respectively. Similar results were obtained from treatments of 20 % filtrate. *Penicillium* sp. was the least effective one.

Table 2: Effect of culture filtrate of antagonists on mycelial growth and on sporulation of pathogen on PDA medium.

| The antagonists | Radial growth and average number of conidia of *H. sativum* at different concentrations of antagonist filtrates (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5% | | 10% | | 15% | | 20% | |
| | Radial growth (cm) | Average* no. of conidia | Radial growth (cm) | Average no. of conidia | Radial growth (cm) | Average no. of conidia | Radial growth (cm) | Average no. of conidia |
| Control | 8.5 a | 39.255 a | 9.0 a | 34.605a | 8.8 a | 38.550a | 9.0 a | 40.250a |
| *Trichoderma harzianum* | 7.4 ab | 33.100 a | 3.2 c | 11.250cd | 1.9 d | 6.500d | 1.8 d | 5.650d |
| *T. pseudokoningii* | 7.2 ab | 35.350 a | 5.1 b | 14.333cd | 4.4 c | 9.722d | 4.0 c | 9.178d |
| *T. lignorum* | 7.7 ab | 36.266 a | 6.0 b | 19.621bc | 5.0 b | 16.250cd | 5.2 b | 15.410c |
| *Stachybotrys atra* | 5.9 ab | 20.532 b | 2.2 cd | 7.319d | 1.5 d | 2.590d | 1.2 d | 2.330d |
| *Penicillium* sp. | 6.8 ab | 34.111 a | 4.8 b | 26.750a | 3.9 c | 25.404ab | 3.6 c | 19.210b |

* Calibrated to 2 x $10^5$ conidia /ml of autoclaved distilled water.
Numbers in the same column with the same letters are not significantly different at p= 0.05.

In the spore germination experiments, filtrates of *T. harzianum* and *S. atra* proved to be effective in inhibiting spore germination of the pathogen. However, other antagonists were less effective in reducing the spore germination of *H. sativum*. All treatments with *T. harzianum* and *S. atra* resulted in significant reduction of germ tube length compared with control; maximum effect occurred at 20% filtrate treatment of *S. atra* which causing 95 % germ tube length reduction (Table 3). Metabolic by-products of antagonists and metabolites from cells within the culture filtrates have also been shown to affect plant pathogenic fungi (Dossantos and Dhingra,1982). Bell *et al.* (1982), also demonstrated that species of *Trichoderma*, or their antibiotics suppressed growth of six fungal plant pathogens. In seed treatment trial, results of antagonistic effect of culture filtrate on wheat seed germination and infection (Table 4) showed that all treatments with filtrates of *Trichoderma* spp. increased significantly seed germination compared with the non-infected /non-treated control.

It was found that these antagonists produced a growth-regulating factor that increases the rate of seed germination (Windham *et al.,* 1986; Sarhan and Shibly, 2000).

Table 3: Effect of culture filtrate of antagonists on percentage of spore germination and length of germ tubes of the pathogen

| The antagonists | Percentage of spore germination and length of germ tubes of *H. sativum* at different concentrations of antagonist filtrates (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5% | | 10% | | 15% | | 20% | |
| | Spore g. (%) | Leng. of* germ. tube (µm ) | Spore g. (%) | Leng. of germ. tube (µm ) | Spore g. (%) | Leng. of germ. tube (µm ) | Spore g. (%) | Leng. of germ. tube (µm) |
| Control | 90.2 a | 39.1 a | 85.5 a | 32.5 a | 88.1 a | 35.7 a | 86.3 a | 38.4 a |
| *Trichoderma harzianum* | 73.1 b | 20.3 b | 50.0 c | 19.0 bc | 21.4 d | 8.7 c | 19.5 d | 7.9 c |
| *T. pseudokoningii* | 70.6 b | 25.0 ab | 62.7 bc | 23.6 ab | 55.8 c | 18.8 b | 50.1 c | 18.0 bc |
| *T. lignorum* | 76.5 b | 29.5 ab | 76.2 b | 24.1 ab | 68.4 bc | 20.6 b | 66.7 bc | 20.5 b |
| *Stachybotrys atra* | 51.1 c | 20.5 b | 32.8 cd | 18.7 bc | 5.3 e | 2.8 c | 4.9 e | 2.5 c |
| *Penicillium* sp. | 66,2 bc | 30.9 a | 59.1 c | 28.9 a | 40.0 c | 27.2 a | 38.5 cd | 27.5 a |

* Length of germ tubes were measured with ocular micrometer scale ( 50 divisions ).
Numbers in the same column with the same letters are not significantly different at p= 0.05.

Table 4: The antagonistic effect of culture filtrates, at different concentrations, on percentages of seed germination and seed colonization by *H. sativum*.

| The antagonists | Percentages of seed germination and seed colonization by pathogen as affected by different concentrations of antagonist filtrates (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5% | | 10% | | 15% | | 20% | |
| | Seed germ. (%) | Seed coloniz. (%) | Seed germ. (%) | Seed coloniz. (%) | Seed germ. (%) | Seed coloniz. (%) | Seed germ. (%) | Seed coloniz. (%) |
| Control(infected /non-treated) | 24.7 d | 36.8 a | 26.1 d | 37.2 a | 24.4 d | 37.0 a | 25.6 d | 36.8 a |
| Control(non-infec.*/non-trea.) | 81.0 b | 0.0 e | 84.5 b | 0.0 e | 81.6 b | 0.0 e | 85.5 b | 0.0 e |
| *Trichoderma harzianum* | 92.4 a | 30.5 a | 94.1 a | 22.1 ab | 95.0 a | 11.0 b | 96.4 a | 10.6 b |
| *T. pseudokoningii* | 93.8 a | 33.7 a | 94.0 a | 25.0 ab | 95.6 a | 15.3 b | 96.8 a | 14.8 b |
| *T. lignorum* | 91.6 a | 35.0 a | 92.3 a | 27.1 ab | 95.3 a | 28.2 ab | 95.6 a | 27.5 ab |
| *Stachybotrys atra* | 80.0 b | 26.4 ab | 82.1 b | 20.2 b | 84.0 b | 0.0 c | 85.0 b | 0.0 c |
| *Penicillium* sp. | 75.1 c | 32.0 a | 71.0 c | 28.7 ab | 70.5 c | 21.1 b | 70.1 c | 20.6 b |

* The naturally infected wheat seeds were surface-sterilized with 2 % sodium hypochlorite.
Numbers in the same column with the same letters are not significantly different at p= 0.05.

Whereas, seed colonization by the root rot fungus was significantly decreased as the filtrate concentration increased except in treatments of *T. lignorum* which showed no significant differences between treated seeds and control. Filtrate from culture of *T. harzianum* and *S. atra* protected completely the infected wheat seeds from colonization by the pathogen, indicating that an antibiotic or toxic substance may also be produced that effectively hamper development of root rot fungus and protect the seeds from infection.

Similar results were reported by Luz (1994), who suggested that colonization of infected wheat seeds may be controlled by secretion of small quantities of antibiotics from the antagonistic microorganisms associated with seeds. It is possible that not all the antifungal activity on seeds is due to antagonists growth and antibiotic production. Some inhibition could result from toxic compounds accumulated in the culture medium showing biological activity against pathogens. In these tests, *T. harzianum* and *S. atra* have good effect on sporulation capacity of *H. sativum* and significantly reduced infection efficiency. The most effective antagonist for controlling pathogen was *S. atra.*

**Seed coating test:**

The effect of seed coating, with fungal antagonists, on wheat root rot fungus is shown in Table 5. In seeds naturally infected with *H. sativum*, where seed infection was high, biological treatment gave maximal germination and survival.

Table 5: The effect of seed coating with fungal antagonists on *H. sativum* as measured by percentages of seed germination, seed rot and diseased radicles.

| Inoculum: pathogen | Inoculum: antagonist | Seed germination (%) | Seed rot (%) | Diseased Radicles (%) |
|---|---|---|---|---|
| *H. sativum* | None | 21.5 d | 58.8 a | 69.7 a |
| *H. sativum* | *T. harzianum* | 95.2 a | 4.2 c | 7.1 cd |
| *H. sativum* | *T. pseudokoningii* | 91.0 a | 9.4 c | 16.5 c |
| *H. sativum* | *T. lignorum* | 93.4 a | 34.6 b | 40.8 b |
| *H. sativum* | *Stachybotrys atra* | 86.3 b | 0.0 c | 0.0 c |
| *H. sativum* | *Penicillium* sp. | 61.1 c | 39.5 b | 47.6 b |
| None* | None | 83.5 b | 0.0 c | 0.0 c |

* Seeds of wheat, surface-sterilized with 2 % sodium hypochlorite.
Numbers in the same column with the same letters are not significantly different at p= 0.05.

All treatments decreased significantly seed rot and radicle infection as compared with the control, however, seed germination was increased significantly only by

*Trichoderma* spp. compared with control. In this test, *H. sativum* controlled completely on wheat seeds by *S. atra* which inhibited seed rot and radicle infection completely. The most effective treatments for reducing seed infection were *T. harzianum* and *S. atra* causing 93% and 100% seed rot reduction. However, *T. lignorum* and *Penicillium* sp. Treatments were the least effective ones for reducing seed infection. The seed coating with antagonists ensure quicker and more effective utilization of the antagonists by the plants than the addition of antagonists to the soil. The action of biological agents at the seed surface seems to be more effective than soil application of fungal antagonists. Abdelmonem and Rasmy (2000) found that seed coating with *Trichoderma* spp. was the best biological treatment for reducing seed and seedling infections of mangrove caused by fungi and bacteria. The root rot fungus, *H. sativum*, appeared to colonize first the pericarp and endosperm of wheat seed and from there to infect developing roots. By protecting the pericarp and the root system from the pathogen, disease may be controlled. Results in Tables 4 and 5 showed that the cell-free filtrate from *S. atra* culture protected seeds from *H. sativum* to the same extent as seed coating with spore suspension. Since the wheat seed coating harbored the most pathogens, removing it, minimized seed infection and maximized seed germination. This illustrates a well-known principle that is the basis for chemical seed treatment. The advantage of biological seed treatment is that protection can be prolonged, whereas chemical protects the seeds, the antagonists protect the seeds and roots. The rate of seed germination was increased only when seeds were either treated with culture filtrate (Table 4) or coated with spore suspension (Table 5) of *Trichoderma* spp. Compared with controls. In this study, *S. atra* appeared to be a more promising antagonist, as seed protecting bio-agent, than *Trichoderma* spp. because it protected completely wheat seeds and radicles against the infection of *H. sativum*, the causal agent of root rot disease of wheat.

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## ARABIC SUMMARY

### المكافحة الحيوية للفطر *HELMINTHOSPORIUM SATIVUM* المسبب لمرض تعفن جذور الحنطة بوساطة بعض الفطريات المضادة

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هدفت هذه الدراسة إلى اختبار كفاءة بعض أنواع الفطريات المضادة المعزولة من المنطقة المحيطة بجذور الحنطة rhizosphere من حقول منطقة سومر، محافظة الديوانية، وسط العراق في المكافحة الحيوية للفطر *Helminthosporium sativum* ، المسبب لمرض تعفن جذور الحنطة، تحت الظروف المختبرية. حيث تم عزل خمسة فطريات مضادة ، ثلاثة أنواع منها تعود للجنس هي : *T. harzianum* , *T. pseudokoningii* و *T. lignorum* ، بالإضافة إلى كل من الفطر *Stachybotrys atra* والفطر *Penicillium* sp. من المنطقة المحيطة بجذور الحنطة.

أوضحت نتائج التضاد بين الفطريات المضادة والفطر الممرض لنبات الحنطة بعد نموهما سويا" على الوسط ألزرعي بطاطا دكستروز آجار PDA أن عزلات كل من الفطر المضاد *T. harzianum* والفطر المضاد *S. atra* قد أوقفت تماما" نمو الفطر الممرض وقضت عليه. وقد أوضحت الفحوصات المجهرية للغزل الفطري أن خيوط الفطر المضاد *T. harzianum* قد التفت حول خيوط الفطر الممرض *H. sativum* وتطفلت عليها ثم أدت إلى تحللها. وتمكن غزل الفطر المضاد *S. atra* من النمو فوق مستعمرات الفطر الممرض ومنعها من النمو. كما أظهرت نتائج تجارب تأثير رشاحة الفطريات المضادة المختبرة وبتركيزات مختلفة على نمو الفطر الممرض وقدرته على تكوين الابواغ الكونيدية ، أن لرشاحة كل من الفطر المضاد *T. harzianum* والفطر المضاد *S. atra* القدرة على تثبيط نمو الفطر *H. sativum* عند إضافتها إلى الوسط ألزرعي، وفي نفس الوقت قللت معنوياً من معدل إنتاج الأبواغ الكونيدية . ومن دراسة تأثير الرشاحة على نسبة إنبات الابواغ الكونيدية وأطوال الأنابيب الجرثومية للفطر الممرض، وجد أن ما يقارب 95 % منها غير قادرة على الإنبات عند التركيزين 15 و 20 % من رشاحة الفطر المضاد *S. atra* ، أما الفطريات المضادة الأخرى فقد أظهرت كفاءة أقل في خفض نسبة إنبات الأبواغ، في حين انخفضت معنويا" أطوال الأنابيب الجرثومية عند جميع المعاملات ماعدا رشاحة الفطر *Penicillium* sp. . وعند معاملة البذور بالرشاحة، وجد أن أنواع الفطر *Trichoderma* أدت إلى زيادة معنوية في نسبة إنبات البذور كما أن التركيزات العالية من رشاحة الفطر المضاد *S. atra* تمكنت من حماية البذور تماما" من الإصابة بالفطر الممرض. حيث بينت نتائج هذه الدراسة إن أفضل المعاملات لتقليل عفن البذور وإصابة الجذير ، إضافة إلى تحسين وزيادة نسبة إنباتها تتم عن طريق تغليف البذور بالفطريات المضادة قبل زراعتها. نستنتج من هذه الدراسة أن الفطر المضاد *T. harzianum* هو طفيل فطري جيد والفطر المضاد *S. atra* هو فطر تضادي قوي ويمكن استخدامهما كعوامل حيوية لمكافحة الفطر *H. sativum* المسبب لمرض تعفن جذور الحنطة.