TARIM BİLİMLERİ DERGİSİ 2001, 8 (2) 167-170

Antagonistic Effect of *Aspergillus melleus* Yukawa on Soilborne Pathogens of Chickpea

F.Sara DOLAR[1]

Geliş Tarihi : 25.10.2001

Abstract: Effect of *Aspergillus melleus* Yukawa on mycelial growth and infection of chickpea by *Marcophomina phaseolina*, *Fusarium oxysporum* f. sp. *ciceri*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. moniliforme*, *F. sambucinum* and *Rhizoctonia solani* was studied. *A. melleus* inhibited the colony growth of *F. sambucinum*, *F. equiseti*, *R. solani*, *M. phaseolina*, *F. oxysporum* and *F. moniliforme* (52.2, 51.1, 46.6, 46.6, 46.3 and 42.1%, respectively) on PDA. Root rot and wilt diseases caused by *F. sambucinum*, *F. moniliforme*, *M. phaseolina*, *F. equiseti* and *F. oxysporum* f. sp. *ciceri* in *A. melleus*-amended soil were reduced significantly. However, there was no effect of *A. melleus* on infection by *F. acuminatum* and *R. solani* in chickpea. The highest reduction value was obtained with *M. phaseolina* (72.78%).

Key Words: chickpea, *Aspergillus melleus*, antagonist, soilborne pathogens

Toprak Kökenli Nohut Patojenlerine *Aspergillus melleus* Yukawa'nın Antagonistik Etkisi

Özet: Nohut kök patojenlerinden *Marcophomina phaseolina*, *Fusarium oxysporum* f. sp. ciceri, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. moniliforme*, *F. sambucinum* ve *Rhizoctonia solani* 'nin miseliyal gelişimi ve enfeksiyonu üzerine *Aspergillus melleus* Yukawa'nın etkisi çalışılmıştır. *F. sambucinum*, *F. equiseti*, *R. solani*, *M. phaseolina*, *F. oxysporum* f. sp. *ciceri* ve *F. moniliforme*'nin PDA üzerindeki koloni gelişmesi *A. melleus* tarafından önemli derecede (sırasıyla %52.2, 51.1, 46.6, 46.6, 46.3 ve 42.1) engellenmiştir. *F. sambucinum*, *F. moniliforme*, *M. phaseolina*, *F. equiseti* ve *F. oxysporum* f. sp. *ciceri* 'nin neden olduğu kök çürüklüğü ve solgunluk hastalığı *A. melleus* içeren topraklarda önemli ölçüde azalmıştır. Fakat *A. melleus*'un nohutlarda *F. acuminatum* and *R. solani*'nin enfeksiyonu üzerine hiç bir etkisi olmamıştır. *A. melleus* en fazla etkiyi *M. phaseolina* üzerinde göstererek bu etmenin neden olduğu enfeksiyonu % 72.78 oranında engellemiştir.

Anahtar Kelimeler: nohut, *Aspergillus melleus*, antagonist, toprak patojenleri

Introduction

Chickpea (*Cicer arietinum* L.) is the most produced legume crop of Turkey (Anonymous 1998). Diseases are the most important factor limiting its production. Of the many diseases that have been reported, those of economic importance are *Ascochyta rabiei*, *Fusarium oxysporum* f. sp. *ciceri*, *F. solani*, *F. acuminatum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* (Haware et al. 1986, Nene and Reddy 1987, Dolar 1996). *Fusarium oxysporum* f. sp. *ciceri*, *F. solani*, *F. acuminatum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Pythium ultimum* are important root diseases of chickpea in Turkey (Soran 1977, Maden 1987, Dolar 1996). It is difficult to control soilborne plant pathogens by the use of fungicides. Several attempts have been made in recent years to control soilborne pathogens with the use of antagonists, since application of fungicides in agriculture may create environmental problems. The use of antagonistic micoorganisms such as *Penicillium oxalicum*, *Bacillus subtilis*, *Trichoderma harzianum* to control the chickpea diseases has been reported (Kaiser and Hannan 1984, Haral and Konde 1986, Parakhia and Vaishnav 1986). Rhizosphere and rhizoplane mycoflora of chickpea was investigated by some workers (Mathur and Chauhan 1972, Khan and Prakash 1982, Satyaprasad 1982). *Aspergillus melleus* was found in the rhizosphere of *Cicer arietinum* (Domsch et al. 1980). *A. melleus* appears to be widespread in soils of tropical and subtropical regions and it is found on seeds of groundnut and soybean (Joffe and Borut 1966, Joffe 1968, Ellis et al. 1974). *A. melleus* was also isolated from the cotton rhizosphere as an antagonistic fungus (Gazikhodzhaeva and Bekker 1968). But there is no report on antagonistic effect of this fungus to chickpea pathogens.

The objective of this study was to investigate the possibility of using *A. melleus* for the control of wilt and root rot diseases of chickpea.

Materials and Method

Fungus isolates : *Aspergillus melleus* was isolated from the chickpea rhizosphere. Identification of the fungus was accomplished using keys provided by Domsch et al. (1980).

Isolates of *Fusarium oxysporum* f. sp. *ciceri*, *F. acuminatum*, *F. equiseti*, *F. moniliforme*, *F. sambucinum*, *Rhizoctonia solani* and *Macrophomina phaseolina* were obtained from wilted and root rotted plants of chickpea.

[1] Ankara Univ. Agric. Fac., Dept. of Plant Protection-Ankara

All of the isolates were maintained on Potato Dextrose Agar (PDA) and petri plates were incubated at 22 ±1°C with a 12 h photoperiod of near UV light. The pathogenicity of all isolates were tested using susceptible chickpea cultivar AUG-424 obtained from NIAB (Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan).

Screening of *Aspergillus melleus* for antagonism towards some pathogens of chickpea: A single mycelial disc (0.7 cm dia) was taken from the edges of 7 day-old pure cultures of the test fungus and the pathogens. Both mycelial discs were inoculated at opposite sides of the same PDA plate, about 3 cm apart. The mycelial discs of slow-growing fungi were placed on the agar plates 5 to 7 days before inoculation with *A. melleus*. Respective controls were also made with the test organism (without pathogens) and pathogens. All plates were incubated at 22±1°C for 7 days. The colony diameters of dual cultures were measured and compared with the control treatment.

Antagonistic effect of *A. melleus* to chickpea pathogens was tested by using pot-culture inoculation methods developed by Nene and Haware (1980). To produce the inoculum of the pathogens *Fusarium oxysporum* f. sp. *ciceri*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. moniliforme*, *F. sambucinum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and the test organism *A. melleus*, 50 g mixture of sand and chickpea meal (45 g sand+5 g chickpea flour+ 10 to 15 ml distilled water) in 150 ml flasks was sterilized and inoculated with a 0.7 cm diameter plug obtained from the margin of 7 day-old cultures of each fungus grown on PDA. Flasks were incubated for 15 days at 25±2°C with an illumination of 12 h per day. The inoculum of the test organism and each of the pathogens in each flasks were thoroughly mixed with 1 kg autoclaved soil in each pot at the same time. The soil in the pots was slightly watered after the inoculum was incorporated and sowing was done 7 days later. Seeds of the susceptible chickpea cultivar (Canitez 87) were surface sterilized with sodium hypochlorite (1%) for 3 min and then five seeds were sown in each pots.

Three set of control pots were prepared. One control set contained only pathogens (without *A. melleus*), second set included only *A. melleus* and third control plants were grown in a mixture of non infested sand+chickpea flour and autoclaved soil. Plants were grown in a growth room at 25±1°C with a 12 h photoperiod of fluorescent light at approximately 11 000 lux.

Percent of inhibition and disease assessment: The inhibition of mycelial growth of pathogens by *A. melleus* was calculated using the following formula (Gokulapalan and Nair 1984).

$$I = \frac{100\,(C-T)}{C}$$

I: percent of inhibition

C: growth in control plates

T: growth in dual cultures

For pot experiments, the observations were made at weekly intervals for five weeks. Disease incidence was recorded by counting the number of infected plants. All experiments were performed twice and data for the two experiments combined.

Results and Discussion

Aspergillus melleus, as most of the rhizosphere fungi isolated from chickpea, is known to survive saprohytically in nature. *A. melleus* for biological control of some of the chickpea diseases was tested in this study.

The antagonistic effect of *A. melleus* on pathogenic fungi was evaluated by comparing the diameter of pathogen colonies in dual cultures with the control treatment. The results are shown in Table 1. The inhibitory effect of *A. melleus* on *F. sambucinum* and *F. equiseti* was the highest. *A. melleus* inhibited the growth of *F. sambucinum* and rapidly grew over the colony. Percentage of inhibition in growth of *M. phaseolina*, *R. solani*, *F. oxysporum* f. sp. *ciceri* and *F. moniliforme* was found to be 46.6, 46.6, 46.3 and 42.1%, respectively.. A light inhibition zone was occurred between *A. melleus* and *M. phaseolina*, *R. solani*. *R. solani* was supressed by the growth of the antagonist whereas growth of *M. phaseolina* was inhibited and was overgrown by *A. melleus*. A narrow inhibition zone, 1-2 mm wide, was also produced at the region of contact between *A. melleus* and *F. solani*. Any antagonistic effect of *A. melleus* on *F. acuminatum* was not observed.

A. melleus was found to be non-pathogenic to chickpea in pathogenicity tests. It reduced the disease incidence of some of the chickpea pathogens in pot tests (Table 2). Infection by *F. acuminatum* and *R. solani* of chickpea were not affected by *A. melleus*. The effect of *A. melleus* on *F. solani* infection was lower (3.5%). However, root rot diseases caused by *M. phaseolina*, *F. sambucinum* and *F. moniliforme* in *A. melleus*-amended soil were reduced significantly (72.78, 60.02 and 45.5 %, respectively). A reduction in the disease incidence of 39.4 and 33.4 % was observed in the pathogens *F. oxysporum* f. sp. *ciceri* and *F. equiseti* with the presence of the antagonist, compared to the control plants inoculated with the pathogens alone.

Results of petri plate and pot experiments in this study agreed to each other, except *R. solani*. *A. melleus* restricted the growth of *R. solani* on PDA but it was ineffective in pot experiment. *A. melleus* showed the highest antagonistic effect on *M. phaseolina* and *F. sambucinum* in both experiments.

Table 1. Colony gowth of pathogenic fungi with and without addition of *Aspergillus melleus* on PDA and the percentage of inhibition after 7 days

Pathogen name	The colony diameter of the pathogens (mm)*		
	Control (pathogen only)	Dual cultures (Pathogens+*A. melleus*)	Percentage of inhibition
F. sambucinum	90±0.2	43±0.4	52.2
F. equiseti	90±0.3	44±0.3	51.1
F. oxysporum f.sp.*ciceri*	82±0.6	44±0.2	46.3
F. monilliforme	83±0.5	48±0.3	42.1
F. solani	70±0.3	52±1.2	25.7
F. acuminatum	46±0.4	42±0.1	8.7
M. phaseolina	90±0.4	48±0.3	46.6
R. solani	90±0.0	48±0.2	46.6

* Numbers represent the average measurements of 10 petri plates.

Table 2. Effect of *Aspergillus melleus* on the pathogenic fungi of chickpea

Pathogen name	Percentage of infection*		Reduction in the diseases incidence by *A. melleus*
	Pathogen (without *A. melleus*)	Pathogen (with *A. melleus*)	
F. sambucinum	83.3±7.8	33.3±4.8	60.02
F. equiseti	91.0±5.5	60.6±4.5	33.40
F. oxysporum f.sp.*ciceri*	94.3±7.6	57.1±9.3	39.44
F. monilliforme	100.0±0.0	54.5±9.8	45.50
F. solani	100.0±0.0	96.5±2.0	3.50
F. acuminatum	98.0±2.8	98.0±3.1	0.00
M. phaseolina	61.0±9.4	16.6±5.6	72.78
R. solani	100.0±0.0	100.0±0.0	0.00

* Calculation based on 60 plant

Some of the microorganisms such as *Penicillium oxalicum*, *Bacillus subtilis* and *Trichoderma harzianum* has been used to control the chickpea diseases (Kaiser and Hannan 1984, Haral and Konde 1986, Parakhia and Vaishnav 1986). However, there was no record about the antagonistic effect of *A. melleus* on chickpea diseases. Gazikhodzhaeva and Bekker (1968) reported that *A. melleus* was the most specific of the antagonistic fungi isolated from the cotton rhizosphere and it significantly reduced incidence of wilt caused by *Verticillium dahliae*.

Haral and Konde (1986) detemined that culture filtrates of a *Bacillus subtilis* str. reduced the mycelial weight of the *Fusarium oxysporum* f. sp.*ciceri* and *Rhizoctonia solani*. Parakhia and Vaishnav (1986) reported that when chickpea seeds were treated with *Trichoderma harzianum* before sowing in pots inoculated with *Rhizoctonia bataticola* (*Macrophomina phaseolina*), infection was reduced up to 18%. In this study, *A. melleus* was significantly inhibited colony growth and disease incidence of *M. phaseolina* and *F. oxysporum* f. sp. *ciceri*.

Conclusion

A. melleus isolated from the chickpea rhizosphere showed antagonistic effect to some chickpea pathogens and it significantly reduced incidence of root rot and wilt caused by *Macrophomina phaseolina*, *Fusarium sambucinum*, *F. moniliforme* and *F. oxysporum* f. sp. *ciceri*. Results of this study shows that *A. melleus* is an effective biological control agent against some soilborne chickpea pathogens. It may be used to control these diseases in the future.

Acknowledgement

The author is grateful to Dr. Fatma Jamil (Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan) for poviding susceptible chickpea cultivar AUG-424 seeds, to Dr. Kudret Erzurum for her help in identifying *A. melleus*, and Dr. Aziz Karakaya for proofreading the manuscript. The author is also thankful to Dr. H. Nirenberg for identification of Fusarium species.

References

Anonymous, 1998. Agricultural structure and production. State Institue of Statistics Prime Ministry Rebublic of Turkey, Ankara. 591 pp.

Dolar, F. S. 1996. Survey of chickpea diseases in Ankara, Turkey. International Chickpea and Pigeonpea, 3, 33-34.

Domsch, K. H., W. Gams and T. Anderson, 1980. Compendium of Soil Fungi. Vol 1. Academic Press. London, pp 859.

Ellis, M. A., M. B., Ilyas and J. B. Sinclair, 1974. Effect of cultivar and growing region on internally seedborne fungi and *Aspergillus melleus* pathogenicity in soybean. Plant Disease Reporter, 58, 332-334.

Gazikhodzhaeva, M. A. and Z. E. Bekker, 1968. Adaptation of antagonistic fungi in the rhizosphere of cotton and their influence on yield and verticillium wilt. Review of Plant Pathology, 47, No. 3447.

Gokulapalan, C. and M. C. Nair, 1984. Antagonism of a few fungi and bacteria against *Rhizoctonia solani* Kuhn. Indian Journal of Microbiology, 24, 57-58.

Haral, A. K. and B. K. Konde, 1986. Microbial antagonism to fungal soil borne plant pathogens of chickpea. Journal of the Maharashtra Agricultural Universities, 11, 236-238.

Haware, M. P., Y. L. Nene and S. B. Mathur, 1986. Seed-borne diseases of chickpea. Technical Bulletin no.1. Copenhagen, Denmark: Danish Government Institute of Seed Pathology for Developing Countries, pp 32.

Joffe, A. Z. and S. Y. Borut, 1966. Soil and kernel mycoflora of groundnut fields in Israel. Mycologia, 58, 629-640.

Joffe, A. Z. 1968. Mycoflora of surface-sterilized groundnut kernels. Plant Disease Reporter, 52, 608-611.

Kaiser, W. J. and R. M. Hannan, 1984. Biological control of seed rot and preemergence damping-off of chickpea with *Penicillium oxalicum*. Plant Disease, 68, 806-811.

Khan, M. A. A. and D. Prakash, 1982. Rhizosphere and rhizoplane mycoflora of gram as affected by plant growth. Indian Phytopathology, 35, 717-718.

Maden, S. 1987. Seed-borne fungal diseases of chickpea in Turkey. Journal of Turkish Phytopathology, 16, 1-8.

Mathur, S. and S. K. Chauhan, 1972. A comparative study of rhizosphere of gram (*Cicer arietinum* L.) in relation to its wilt diseases caused by different fungi. Journal of the Indian Botanical Society, 51, 267-274.

Nene, Y. L. and M. P. Haware, 1980. Screening chickpea for resistance to wilt. Plant Disease, 64, 379-380.

Nene, Y. L. and M. V. Reddy, 1987. Chickpea diseases and their control. In: The Chickpea (Ed. Saxena, M.C. and Singh, K.B.). CAB International, Aberystwyth, pp.233-270.

Parakhia, A. M. and M. U. Vaishnav, 1986. Bio-control of *Rhizoctonia bataticola*. Indian Phytopathology, 39, 439-440.

Satyaprasad, K. 1982. Rhizosphere and rhizoplane mycoflora of wilt resistant and susceptible chickpea. Indian Phytopathology, 35, 153-155.

Soran, H. 1977. The fungus disease situation of edible legumes in Turkey. Journal of Turkish Phytopathology, 6, 1-7.