Evaluation the efficiency of packing cucumber seeds with alginic acid loaded on Trichoderma koningii spores for time periods against pathogenic fungus Rhizoctonia solani

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Abstract. This study was conducted at University of Agriculture and Veterinary Medicine in Bucharest laboratories, , the concentration of 3.6*10^6 of T.koningii spores loaded on alginic acid was used to pack cucumber seeds at room temperature and for 6, 12 and 18 months against pathogenic fungus R.solani. Effectiveness of germination and seedling was evaluated every 6 months. The results of antagonism tests show that the bio fungus T.koningii have a high antagonism ability against the pathogen fungus R.solani, the antagonistic ratio 2 according to the scale of Bell. Also the results showed the effectiveness of germination of cucumber seeds packed with alginic acid loaded on T.koningii and contaminated with R.solani for 18 months of storage under room temperature, the rate of germination and seedling death 70.00% and 13.39%, respectively compared with non-loaded and contaminated seeds with R.solani which the rate reached to 30.00% and 55.55 % respectively. The best results of germination and seedling death were in cucumber seeds unloaded with alginic acid on T.koningii and contaminated with R.solani after 6 months which reached to 96.33% and 13.79 % respectively in comparison with cucumber seeds packed with alginic acid loaded on T.koningii and contaminated with R.solani which reached to 66.66% and 40.00% respectively.

Keywords: Trichoderma koningii, Rhizoctonia solani, cucumber.

1. Introduction

Trichoderma spp. are fungal species in a certain natural suppressive soil prevents the plant from infectious diseases caused by soil-borne pathogens. Among these soils borne pathogen, the fungus R. solani causes serious damages to economically significant crops and trees. The control strategies such as breeding for resistant cultivars, crop rotations, and application of fungicides are insufficient to manage diseases caused by R.solani because it persists in soil by producing sclerotia which is a hard-resistant structure. Moreover, fungicides are now unacceptable as they are not environment-friendly. The Trichoderma spp. are the potential biocontrol agents which inhibit R.solani by direct confrontation through mycoparasitic or antibiosis or competition as well as inducing plant defense responses (Abbas et al., 2017). The use of Trichoderma on seeds is an alternative method of introducing fungi into soil instead of directly applied to soil in the different formations. This method requires a small amount of fungus preparation, measured in quantities placed in soil or in the form of small piles of the preparations of the fungus, if measured by quantities placed in the soil or in the form of small piles (Radhi, 2018). Also the applied field bio-resistance was started with the use of
Trichoderma fungi by (Wells et al., 1972) using preparations of Trichoderma growing on a solid environment (grain, rye or wheat) for field resistance of the Sclerotium Rolfsii on tomato plant. Cucumber is one of many susceptible crops to damping-off and root rot disease caused by R.solani. Sclerotia of R. solani can be remain viable in soil for several years as important source of primary infection (Safaa et al., 2013). The most substantial input for crop production is seed. For desirable plant populations and a good harvest, the pathogen free healthy seeds are necessary. About 16% annual crop losses because of diseases of plant, 10% loss happened because of seed-borne diseases. Severe fungal diseases which include seed-borne pathogens caused by diverse factors which are responsible of the crop low yield. Seeds transferred the seeds borne fungal diseases which the fungi can outrun as mycelia or conidia on the coat or surface of the seed. The using of synthetic chemicals managements of seed-borne fungi are efficient and effective (Farrag and Moharam, 2012). In this study we tried to evaluate the efficiency of packing cucumber seeds with alginic acid loaded on Trichoderma koningii spores for 6, 12 and 18 months against pathogenic fungus R.solani and their effect on the rate of germination and seedling death in cucumber plant.

2. Materials and methods

2.1. Study design. The experiment was held at the University of Agricultural Sciences and Veterinary Medicine in Bucharest on 03-01-2017. R.solani: DSM 63002 was obtained from institute of research and development of plant protection, Bucharest. While the bio-fungus T.koningii were diagnosed at the University of Basra in Iraq and the diagnosis was confirmed at the national institute for chemical and pharmaceutical research and development (ICCF) in Romania. The cucumber seeds were used from class Trilogy. The antagonism test between pathogenic fungus R.solani and bio fungus T.koningii was adopted according to the method of (Bell et al., 1982). The vitality germination percentage of cucumber seed was tested by taking 20 seeds and placing them in a dish with a piece of cotton saturated with water to provide the moisture needed for the seeds germination (Wesam, 2006). Cucumber seeds were packaged with alginic acid with bio-fungus T.koningii, where the seeds of cucumber were sterilized with 10% hydroxide and washed with sterile distilled water and dried on filter paper Whatman no.1.

2.2. Loading T.koningii on alginic acid. Firstly T.koningii was cultured in PDA medium in petri dish for one week after that, it was taken to the laboratory and washed with sterilized distilled water. The spores were then collected and counted by the hemocytometer and then were used to prepare T.koningii which loaded on alginic acid. Where the T.koningii spores were 3.6 × 10^5 spore/mL. Fifty milliliters of T.koningii suspension which have spores was aseptically mixed with sodium alginate solution (2%, w/v) and stirred gently for 1 h in shaking incubator. The mixture was vigorously stirred to allow a homogenous dissolution of alginate. Then the mixture was extruded through sterile syringe into gently stirred, sterilized 0.1 M CaCl2 at room temperature (Minaxi, 2011). The cucumber seeds were then soaked for 1 minute and after they placed in hydroxide until they were consolidated and washed with distilled water and dried on filter paper and placed at 10 °C for a day, after that, a part of the seeds was contaminated with R.solani and the other part was left untouched, the control treatment was used a sterile seeds, not loaded with alginic acid, some of which were contaminated with R.solani and the other was left untouched these treatments were as triplicates as shown in (table 1.), and were stored for 18 months at room temperature and tested the effectiveness and vitality of the seeds every 6 months of preparation by planting of 10 seeds for each pot in the laboratory.

| Cucumber seeds loaded on alginic acid | Cucumber seeds unloaded on alginic acid |
|--------------------------------------|----------------------------------------|
| Non-contaminated with R.solani | contaminated with R.solani |
| Non-contaminated with R.solani | contaminated with R.solani |

Table 1. Cucumber seeds treatments
2.3. The studied growth indicators. The percentage of germination after 10 days and the ratio of seedling death calculated after three weeks of germination according to the following equations (Mickenny, 1923; AL-Waily, 1988).

\[
\text{% germination} = \frac{N_{a\,gt\,s}}{N_{a\,gt\,s}} \times 100
\]

\[
\text{% seedlings death} = \frac{N_{a\,gt\,s}}{N_{a\,gt\,s}} \times 100
\]

To know about the vitality of \textit{T.koningii} and \textit{R.solani}, they were grown in PDA medium every 6 months of preparation.

3. Results and discussion.

The results of antagonism tests show that the bio fungi \textit{T.koningii} have a high antagonism ability against the pathogen fungus \textit{R.solani}, the antagonistic ratio against \textit{R.solani} the antagonistic ratio reached to 2 according to the scale of (Bell et al., 1982) which \textit{T.koningii} covered 3/2 from the petri dish. \textit{Trichoderma} \textit{spp.} have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which chiefly contribute to antagonism of these fungi. Mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites (Mausam et al., 2007). Tran (2010), found that \textit{Trichoderma} strains could reduce significant diseases caused by fungal pathogens including: \textit{Phytophthora palmivora}, \textit{R.solani}, \textit{Fusarium} \textit{spp.}, \textit{Sclerotium rolfsii} and \textit{Pythium} \textit{spp.}

The percentage of germination of cucumber seeds used in the study was 100% because of their vitality. The results of (table 2.) showed the ability of seeds loaded with alginic acid and \textit{T.koningii} after 6 months of storage at room temperature and a good protection against pathogenic fungus \textit{R.solani} where germination rate and seedling death were 96.33 % and 13.79 % respectively in comparison with cucumber seeds unloaded on alginic acid and \textit{T.koningii} contaminated with \textit{R.solani} which germination rate and seedling death were 66.66% and 40.00 % respectively. For germination rate and seedling death in cucumber seeds loaded on alginic acid and \textit{T.koningii} non-contaminated with \textit{R.solani} were 100 % and 6.66 % respectively in comparison with cucumber seeds unloaded on alginic acid and \textit{T.koningii} non-contaminated with \textit{R.solani} which reached to 100% for germination ratio and 3.33% for seedling death. Also (table 2.) show the results of germination ratio and seedlings death after 12 months of seeds storage which show the ability of cucumber seeds loaded on alginic acid and \textit{T.koningii} contaminated with \textit{R.solani} on germination rate and seedlings death reached to 96.33 % and 17.24 % respectively compared with cucumber seeds unloaded on alginic acid and \textit{T.koningii} contaminated with \textit{R.solani} which were 50.00% for germination rate and 60.00 % for seedling death. Also the germination rate and seedlings death with cucumber seeds loaded on alginic acid and \textit{T.koningii} non-contaminated with \textit{R.solani} were 100% and 3.66% respectively in comparison with cucumber seeds unloaded on alginic acid and \textit{T.koningii} non-contaminated with \textit{R.solani} which reached to 96.66 % for germination rate and 0.00% for seedlings death. While the results after 18 months of storage were as the following, the germination ratio and dead seedlings for cucumber seeds loaded on alginic acid and \textit{T.koningii} contaminated with \textit{R.solani} were 70.00% and 13.79 % respectively compared with cucumber seeds unloaded on alginic acid and \textit{T.koningii} contaminated with \textit{R.solani} which reached to 30.00% and 55.55 % respectively. It has been found that the germination ratio and dead seedling for cucumber seeds loaded on alginic acid and \textit{T.koningii} non-contaminated with \textit{R.solani} were 76.66 % and 16.66 % respectively compared with cucumber seeds unloaded on alginic acid and \textit{T.koningii} non-contaminated with \textit{R.solani} which reached to 66.66 % and 10.00 % respectively. For the vitality of \textit{T.koningii} and \textit{R.solani}, the results show the vitality of them during the storage period.
Table 2. Germination ratio and seedlings death for cucumber plant after 6, 12 and 18 months of storage

| Storage period | Cucumber seeds loaded on alginic acid | Cucumber seeds unloaded on alginic acid |
|----------------|--------------------------------------|----------------------------------------|
|                | Non.contaminated with *R*.solani     | Contaminated with *R*.solani            | Non.contaminated with *R*.solani     | Contaminated with *R*.solani |
| 6 months       | Germination ratio                    | 100.00*                                | 96.33*                                | 100.00*                                | 66.66*                                |
|                | Seedlings death                      | 6.66                                   | 13.79                                 | 3.33                                   | 40.00                                 |
| 12 months      | Germination ratio                    | 100.00                                | 96.33                                 | 96.66                                 | 50.00                                 |
|                | Seedlings death                      | 3.66                                   | 17.24                                 | 0.00                                   | 60.00                                 |
| 18 months      | Germination ratio                    | 76.66                                 | 70.00                                 | 66.66                                 | 30.00                                 |
|                | Seedlings death                      | 16.66                                 | 13.79                                 | 10.00                                 | 55.55                                 |

*Triplicates average

L.S.D. 0.05 (Germination ratio) = 5.63, L.S.D. 0.05 (Seedlings death) = 6.85

*Trichoderma spp.* is widely used for biocontrol agent that enhance plant growth as well as inhibits phytopathogenic fungi. (Vandana and Priya, 2014) found higher seed germination and disease control against soil-borne pathogen with the seed bio priming of *Trichoderma spp.* (Pooja et al., 2003) treated the seeds of mung bean with *T. harzianum* and found maximum seed germination, root length, fresh weight and dry weight of seedlings and increased in yield due to seed treatment. *Trichoderma spp.* provide a full protection for seeds from pathogen before germination, in addition to this, the production of biochemical materials in the external environment stimulates and increases the germination ratio. These materials act to corrode the outer envelope of the seed and thus help to accelerate the germination. (Windham et al., 1986) who mentioned that the addition of *Trichoderma spp.* increases seed germination ratio. Good resistance was obtained for the sudden drop of pea and radish caused by *R*.solani and *Pythium* fungi by treating the seeds of both pea and radish with the total coliforms *T*.coli bacteria, and good resistance was obtained from the bio tube resulting from ultraviolet of fungi *T.harzianum* and *T.viride*. There was also a significant improvement in plant growth and increased production of soybeans grown in soil contaminated with Rhizoctonia when treated with *T.pseudokoningii*, as well as in the treatment of corn and soybeans with *T.harzianum* (Radhi, 2018).

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5. References

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