Preparin of granual inoculant of the fungus Trichoderma
harzanium and Evaluation activity

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Abstract. This study was carried out in order to determine the possibility of using invalid wheat
and rice grindery. Also, using the starch and reed to prepare an active formulation of the bio-
control agent (Trichoderma hazanium) as well as to evaluate the efficiency of this formulations
to control Fusarium wilt disease on cucumber plant Cucumis sativus ) caused by Fusarium
solani, after one month of storage in room degree. The results showed the efficiency of using
Reed and Starch powder as a nutritive substrate to act as a carrier for the biological agent
Trichoderma harzanium in a form of pellets where the values of flowers number, chloroplyle
content, leaves number and plant height were (1.6, 9.2, 4, 17.5)/plant respectively for the reed
carrier treatment. Also values were( 4.3, 9.9, 4, 21.1)/plant respectively for starch carrier
treatment in compare to the control .Viability to control Fusarium wilt disease after storage for
a month under room temperature, reached maximum value with the reed carrier were the average
of the healthy plants reached(8.6) followed by starch carrier(8.3), in compare to the Fusarium
solani (4.6).

Keywords. Alginate granules, Trichoderma spp., Fusarium wilt disease, Reed.

1. Introduction
Recently, the biological control techniques have acquired the ability to resist many diseases of the field
crop and became a promising alternative solutions for the harmful chemical pesticides. Moreover, it is
less cost, safer for environment, efficient and more effective as resistance programs for dangerous plant
diseases [1, 2]. Many studies recorded the efficiency of using the fungal bio-control agents like species
of Trichoderma sp. For controlling soil habituated pathogens which are caused by Fusarium spp. [3, 4]
Phytophthora spp., Pythium spp., & Rhizoctonia spp. [5].
Fungi that belong to Trichoderma Genus, considered as one of the most abundant fungi, theses
isolates can be found in most types of soils [6]. Some of them show an antagonistic react to the soil
habitatuated microorganisms and plant pathogen fungi [4], this is because of the different mechanisms
they have, which enable them to parasitize on these pathogens. Those mechanisms can be as; parasite
hypha, secreted enzymes and antibiotics, and many other mechanisms that make them an active factor
for biological control [7, 8]. Loading the fungal biomass within the alginate pellets with or without the existence of a nutritive substrate had become one of the studies that earned a noteworthy attention [9].

This technique considered one of the recent techniques which utilized to reach for the fungal antagonism in the soil, where the fungal hypha grows from the alginate pellets then approach directly to the targeted fungal aggregations' to parasitize on the target. It has been found that the distance of the fungal hypha growth from alginate pellets towards these aggregations and the distance of pathogenic fungi to infect the plant roots are the same [10].

The technique of bio-control agent preparation method may affect intensively in the success of the resistance process [10]. Also, it can enhance and elongate the shelf life of the bio-control agent. Moreover, it is viable to continue in the soil [11]. It has been conducted in some studies that many fungi like Trichoderma spp. and Clioocladium spp. require a subsist of the nutritive substrate as a carrier and nutritive source to fungus at the same time [12].

This study was carried out to examine the use of some agricultural materials to upload Trichoderma harzanium into alginate granules and test the viability of these pellets in wilt disease resistance after one month of storage.

2. Materials and Methods

2.1. Trichoderma harzanium Preparation
A high effective isolation of Trichoderma harzanium has been activated on Potato Dextrose Broth media (PDB) for five flasks, each flask contains 250 ml autoclaved (PDB) which was incubated with 7mm in diameter disk from the activated fungal growth T. harzanium for 2 weeks under 26±2˚C. [13].

2.2. Alginate Liquid Preparation
The mix was prepared by dissolving 1 gm of (Alginic acid) in (100ml) of autoclaved distilled water. Then, adding the fungal biomass to homogenous alginate solution. After that, adding 2gm of Reed powder to the mix and rotated intensively. All these steps were repeated three times for adding each of the other substrates (spoiled rice, wheat, and starch powder) [14].

2.3. Fungal Inoculums Preparations
The granule formula has been prepared by adding Alginate biomass mixture into 2% calcium chloride Ca C12 gradually. As a result, the formulated alginate pellets contain the fungal growth with a number up to 500 pellets for the whole of each treatment. Those pellets were left to dry at the room temperature.[12].

2.4. Green House Experiment
A test for examines the fungal granules viability was prepared after a month of storage under room temperature. The experiment consisted of 6 treatments;
*control = only the plant.
* Fusarium spp.= only the pathogen.
* T. harzanium (R) = T. harzanium within granules from reed.
* T.harzanium (R) +Fusarium spp.= T.harzanium (Reed granules)+ F. solani.
* T.harzanium(S) = T. harzanium within granules from Starch.
* T. harzanium(S)+ Fusarium spp.= T.harzanium (Starch granules)+F. solani.

The form of the inoculums carried on wheat and rice powder was eliminated, since the substrate has been consumed in a very short period of time which means a short shelf time and it is not efficient for long period of time for storage. Moreover, a quite numbers of spores have been formed and this can result in respiratory system risks when dealing with the inoculums.
1 gm/kg of the granule of each treatment was added to each pot. 25 ml of 107 spore Fusarium spp. was added to 5 days or each pot as well. After 2 days Cucumber Seeds were cultivated to the pots and data were collected each 3 days.

The complete random design (C.R.D.) (general analysis variance) was used in all experiments to analyze the characteristics of (numbers of healthy plants, percent of infected plants, numbers of flowers per plant, chlorophyle content, number of leaves per plant, height of plant per plant, dry weight per plant, wet weight per plant). Results were analyzed by using SPSS program. Means were chosen by using the minimum significant difference under probability level (0.05).

3. Results and Discussions

3.1. The efficiency of T. harzanium inoculums' preparations on cucumber plant under greenhouse conditions

Results showed a disparity at the efficiency of reed and starch preparations of the bio-control agent (T. harzanium) about their resistance to the Fusarium wilt disease of the cucumber plants since they achieved a significant increase in the average of healthy number of plants (8.6, 8.3) plants respectively compared to the results of the pathogen treatment which were (4.6) plants with an infection percent up to (54%) for the pathogen and (14% and 17%) for reed and starch preparation respectively in compare to the pathogen, as in table(1). Also, alginate preparations exiled in number of leaves, chlorophyll content, number of flower, height of plant which recorded (4.3, 14.8 spad, 3.3, 21.5 cm) respectively. For the reed carrier treatment compared to the pathogen treatment which showed the following results for the same features (2, 6.1spad, 0, 9.5 cm). Finally, Starch carrier treatment's values were (4, 8.3spad, 4.3, 21.1cm) respectively, as in table (2).

Both preparations showed a significant increase in wet and dry weight (1.65, 0.743) gm/plant respectively for reed preparations and (1.34, 0.538) gm/plant respectively for starch preparations, compared with the pathogen treatment, which was (0.862, 0.316) gm/plant, as in table (3).

These results belong to the mechanisms which the fungus has in resisting the plant pathogenic fungi, like hormones producing as (ouxines and gibberellins) which can reduce Fusarium sp. Infections [15, 16]. Several types of Trichoderma spp. [6, 17] like T. harzanium and T. viride have uncanny ability to produce these hormones. These hormones can accelerate plants growth. Many studies and researches showed agreement with these results and point out to the uncanny potential of Trichoderma harzanium in enhancing plant growth.

The stabile viability of Trichoderma harzanium within the alginate granules, which contains Reed as a substrate after long period of storage, belongs to the nutrients components in Reed which can be utilized by the fungus to maintain its activity for the quite period of time by using the necessary enzymes for digestion (Carbohydrates 55.7%, fibers 21.9%, and 7.6% protein) [18]. Alginate granules preparations have been used for many biological resistance agents like Gliocladium spp. [19]. Also, [14] used wheat bran to be a substrate to form T. harzianum inoculums as alginate pellets [12] made a proliferation of T. koningii in alginate prills as well as, the granule inoculums of the fungus Talaromyces falvus for controlling Verticillium dahliae which cause Verticillium wilt disease [10].
Table 1. The effect of *T. harzanium* inoculants treatments on *Fusarium solani* treatment in compare to the control

| Treatments                | Numbers of healthy plants | infected plant % |
|---------------------------|---------------------------|------------------|
| Control                   | 10                        | 0                |
| *Fusarium solani*         | 4.6                       | 54               |
| *T. harzanium*(R)         | 10                        | 0                |
| *T. h(R)+F.solani*        | 8.6                       | 14               |
| *T. harzanium*(S)         | 10                        | 0                |
| *T. h(S)+ F.solani*       | 8.3                       | 17               |

LSD <= 5.33  LSD >= 15.5

The LSD values were obtained below the probability level of 0.05

Table 2. Results of several standards for the effect of *T. harzanium* inoculums preparations on Cucumber plants

| Treatments          | Number of flower/ plant | Chlorophyle content | Number of leaves/ plant | Height of plant (cm)/plant |
|---------------------|-------------------------|---------------------|-------------------------|---------------------------|
| Control             | 0                       | 11.5                | 3.6                     | 13.6                      |
| Fusarium            | 0                       | 6.1                 | 2                       | 9.5                       |
| *T. harzanium*(R)   | 1.6                     | 9.2                 | 4                       | 17.5                      |
| *T. h(R)+Fus*       | 3.3                     | 14.8                | 4.3                     | 21.5                      |
| *T. harzanium*(S)   | 4.3                     | 9.9                 | 4                       | 18.5                      |
| *T. h(S)+Fus*       | 4.3                     | 8.3                 | 4                       | 21.1                      |

LSD <= 1.5  LSD <= 3.4  LSD <= 1.4  LSD <= 4.3

The LSD values were obtained below the probability level of 0.05

Table 3. The effect of the fungal inoculums *T. harzanium* on the plant wet and dry weight

| Treatments          | Dry weight gm/plant | Wet weight gm/plant |
|---------------------|---------------------|---------------------|
| Control             | 6.09                | 18.94               |
| *Fusarium solani*   | 3.16                | 8.62                |
| *T. harzanium*(R)   | 4.33                | 20                  |
| *T. h(R)+Fus*       | 7.43                | 16.5                |
| *T. harzanium*(S)   | 6.64                | 14.5                |
| *T. h(S)+Fus*       | 5.38                | 13.4                |

LSD <= 0.2  LSD <= 3.39

The LSD values were obtained below the probability level of 0.05
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