EFFICACY OF TALAROMYCES FLAVUS MICROCAPSULE IN CONTROLLING COTTON IMPORTANT FUNGAL DISEASES

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ABSTRACT

Purpose: Evaluation of efficacy of Talaromyces flavus microcapsule in controlling cotton important fungal diseases and introducing Talaromyces flavus for using in the cotton fields.

Methods: In this research focused on the application and efficiency evaluation of two new formulations of T. flavus microcapsules (TF-Co-G-1: Isolate 1 obtained from the Gorgan cotton farm) in suspension and powder forms, which were compared with the fungicide Talaromin® in farmers’ fields. In this research, two cotton farms (Karkandeh and Hashemabad) with a history of infection with VW and DO were selected in two different regions of Golestan province. Each of the fields with an area of 1000 m² was divided into seven equal parts. In each part, the treatments were applied separately, including microcapsule suspension with soil application, microcapsule suspension as seed impregnation, microcapsule powder with soil application, microcapsule suspension as seed impregnation, Talaromin® fungicide with soil application, Talaromin® as seed impregnation, and a control. The efficiencies of different treatments in terms of controlling the studied diseases and strengthening different growth indicators were evaluated by comparing the means of nine measured traits, including three traits for disease indicators (the percentage healthy seedlings for DO and the incidence rate and severity percentage of VW). To this end, the mixed analysis of variance (ANOVA) was first performed for two regions using the MS TAT C software, and the ANOVA of means was performed for each region separately with the significant effect of treatment × location.

Results and conclusion: The results showed that among the studied formulations, powder microcapsules with seed impregnation by 32% and Talaromin® with soil application by 59% were respectively the most effective treatments for a significant increase in the percentage of healthy seedlings and a significant decrease in the severity of VW. Therefore, the obtained results indicate that the technical knowledge to produce T. flavus microcapsule formulations with two forms of suspension and powder can be transferred to manufacturing companies to carry out the commercialization and mass production process.

Originality/value: Production of Talaromyces flavus microcapsule.

Keywords: Microcapsule Formulation, Talaromyces Flavus, Biological Control, Plant Diseases.

EFICÁCIA DA MICROCÁPSULA TALAROMYCES FLAVUS FLAVUS NO CONTROLE DE DOENÇAS FÚNGICAS IMPORTANTES DO ALGODÃO

RESUMO

Objetivo: Avaliação da eficácia da microcápsula de Talaromyces flavus no controle de enfermidades fúngicas importantes do algodão e introdução de Talaromyces flavus para seu uso nos campos de algodão.

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Métodos: Nesta pesquisa, foi focado na aplicação e avaliação da eficiência de duas novas formulações de microcápsulas de T. flavus (TF-Co-G-1: Isolado 1 obtido da fazenda de algodão Gorgan) em suspensão e em pó, que foram comparadas com o fungicida Talaromyces flavus® nos campos de agricultores. Nesta pesquisa, duas fazendas de algodão (Karkandeh e Hashemabad) com histórico de infecção com VW e DO foram selecionadas em duas regiões diferentes da província de Golestan. Cada um dos campos com uma área de 1000 m² foi dividido em sete partes iguais. Em cada parte, os tratamentos foram aplicados separadamente, incluindo suspensão de microcápsulas com aplicação no solo, suspensão de microcápsulas como impregnação de sementes, suspenção de microcápsulas em pó com aplicação no solo, suspensão de microcápsulas como impregnação de sementes, fungicida Talaromin® com aplicação no solo, Talaromin® como impregnação de sementes, e um controle. A eficiência dos diferentes tratamentos em termos de controle das doenças estudadas e fortalecimento dos diferentes indicadores de crescimento foram avaliados comparando-se os meios de nove traços medidas, incluindo três traços para os indicadores de doenças (a porcentagem de mudas saudáveis para DO e a taxa de incidência e a porcentagem de gravidade de VW). Para este fim, a análise mista de variância (ANOVA) foi realizada inicialmente para duas regiões usando o software MS TAT C, e a ANOVA de médias foi realizada para cada região separadamente com o efeito significativo de tratamento x localização.

Resultados e conclusão: Os resultados mostram que entre as fórmulas estudadas, as microcápsulas em pó com impregnação de sementes em um 32% e Talaromin® com aplicação em um 59% de foram, respectivamente, os tratamentos mais efetivos para um aumento significativo na porcentagem de plântulas sanas e uma diminuição significativa na severidade das plântulas. VW. Por tanto, os resultados obtidos indicam que o conhecimento técnico para produzir fórmulas de microcápsulas de T. flavus com as formas de suspensão e pó pode ser transferido para as empresas fabricantes para levar a cabo o processo de comercialização e produção em massa.

Originalidade/valor: Produção de microcápsulas de Talaromyces flavus.

Palavras-chave: Formulação de Microcápsulas, Talaromyces Flavus, Controle Biológico, Doenças de Plantas.

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1 INTRODUCTION

Verticillium wilt (VW) and damping-off (DO) account for the most important cotton diseases, and biological control using fungal and bacterial biocontrol agents has been introduced as an appropriate strategy to control these diseases (Mathivanan et al., 2000, Nagaratnam et al., 2016; Sehnem et al., 2016; Tapia et al., 2019).

In Iran, the results of previous research on producing microcapsules containing Talaromyces flavus, investigating their sporulation and stability, and determining their effectiveness in controlling cotton VW and Fusarium wilt of tomatoes and greenhouse cucumber in greenhouse conditions showed that these microcapsules could significantly reduce the abovementioned diseases (Naraghi and Negahban, 2018 a,b; Naraghi et al., 2018 a,b; Naraghi and Negahban, 2019 a,b). To complete these studies and carry out the commercialization process of T. flavus-containing microcapsules, the efficiencies of different microcapsule formulations to control the studied diseases and increase the yield in different application methods (seed impregnation and soil application) should be investigated in the current research project.

The promising results of the antagonistic fungus T. flavus concerning the control of some important soil pathogens, such as Verticillium dahliae, V. albo-atrum, Fusarium oxysporum, and Rhizoctonia solani, in several crops including cotton, sugar beet, potatoes, tomatoes, and greenhouse cucumbers, were proven in studies conducted in Iran (Naraghi et al., 2010a; Naraghi et al., 2010b; Naraghi et al., 2010c).

In recent decades, microtechnology has expanded dramatically in various fields of pharmaceutical chemistry, medicine, and agricultural chemical pesticides. The phenomenon of pest resistance to pesticides is an issue that necessitates research and development in the field.
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of micro-pesticides. Therefore, the introduction of micro-pesticides to researchers will improve research and development in this relatively new field. Research and development in the field of micro-pesticides can be raised as a necessity considering the environmental problems and costs caused by the high use of common pesticides and the problems resulting from the resistance of pests to these pesticides.

The use of biodegradable polymers in the production of highly efficient microemulsions and microcapsules made of natural and biodegradable materials can be an effective step in this field. Encapsulation formulation seems to be the best option to increase efficiency and reduce environmental risks. Therefore, the production of micro-encapsulated bio-formulations leads to controllability, increases strength and stability, and protects active ingredients in adverse environmental conditions such as light and humidity. Moreover, the application of micro-encapsulated formulation considerably helps pesticide dosage reduction, economic efficiency, environmental protection, and reduction of its environmental risks, as well as better product export (Martin et al., 2010). Another advantage of micrometric particles is that they do not stimulate the immune system of humans and animals and are quickly excreted from the body (Guan et al., 2008).

The technology of microcapsules containing fungicide or pesticide molecules with a micro scale is a production method for pesticide formulations that remove pests easier and faster (Guan et al., 2008). An emulsion is a heterogeneous system consisting of two immiscible liquids, one of which is dispersed as drops in the other. Emulsions with about micrometer droplet sizes, typically in the range of 20-200 µm, are called microemulsions (Ostertag et al., 2012). Compared to conventional emulsions, the unique structure and properties of microemulsions have created advantages for their use in many industries. Some applications of microemulsion systems in industries include their role in encapsulation and controlled release of functional compounds such as essential oils, vitamins, etc. (Kah and Hofmann, 2014).

In this research, the efficiencies of T. flavus microcapsules in suspension and powder with each application method (soil application and seed impregnation) were evaluated in terms of reducing the indicators of the studied diseases (VW and DO) and increases of some growth indicators such as yield. Besides, the efficiency of these bioproducts based on a microparticle substrate was compared with Talaromin® fungicide based on rice bran substrate regarding the mentioned aspects.

2 RESEARCH BACKGROUND

The fabrication of highly efficient microparticles with more environmental degradability has recently received considerable attention. Therefore, the use of biodegradable polymers in the production of highly efficient microemulsions and microcapsules made of natural and biodegradable materials, such as the essential oils of medicinal seedlings, can effectively contribute to this field. The application, production, and environmental considerations of micro-pesticides show that research and development can effectively reduce the phenomenon of pest resistance to pesticides. The micro-insecticide formulation of Primiphos has caused good storage and more stability of this formulation in the dark (Wan et al., 2010).

A formulation of micro-extracts extracted from plants could have more than five times stronger pesticide effect against pests resistant to conventional pesticides (Rajakumara and Rahuman, 2011). A formulation of micro-permethrin prepared by the evaporation of oil solvent in water could show far better effects in the control of Colex mosquito larvae (Anjali et al., 2010). The lethal properties of Bacillus thuringiensis were investigated using micronized chitosan polymer particles on Anopheles mosquito larvae (Zhang et al., 2015). Moreover, a biopesticide containing Beauveria, Metarhizium, and Paecilomyces was prepared against Hypothenemus hampei using dextrose polymers and gelatin (Niranjana, 2004). The B. bassiana mushroom was used as a larvicide in a formulation containing silver microparticles (AgMPs)
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Prabakaran et al., 2016. A study on the disinfection effects of barley and sunflower seeds with fungicide-containing AgMPs on mycorrhizal symbiosis revealed that the treatment of seeds with micro-fungicide significantly increased the absorption rate of minerals by the root and, consequently, a significant increase in vegetative traits compared to a conventional fungicide. For encapsulation, coating materials include gum, starch, gelatin, and polymers, as well as chitosan and phospholipids in recent years. According to recent investigations, it seems that using the microencapsulation technique and its components in the control of storage pests can play an important role in increasing their efficiency and durability (Prasad et al., 2014).

For the preparation of a micro-fungicide from the fungal genus (Penicillium) studied in this research, two reports are so far available in the fields of medicine and agriculture (Khan and Jameel, 2016). In the field of medicine, a micro-fungicide against the pathogenic fungus Candida albicans was prepared using the live fungus Penicillium fellutanum. In agriculture, a micro-fungicide was prepared against some plant pathogens using T. flavus (the teleomorph of P. dangeardii) fungal extract.

3 METHODS

Preparation of T. flavus microcapsules as a suspension

Microcapsule production is a combination of polymerization and cross-linking methods, which was implemented by making changes tailored to the conditions of fungal growth (changes in the amount or type of polymers, surfactants, oils, fatty acids, the stirrer speed, and temperature). In the polymerization process, the organic phase consisted of vegetable oil along with a suspension of an antagonistic fungus, which was added to the aqueous phase consisting of hydrophilic polymers such as a mixture of one of two polymers, poly (urea formaldehyde) or alginate, starch, and chitosan. Then, cross-linkers (e.g., calcium chloride), surfactants, accompanying materials, and fatty acid oils were added to the sum of two phases homogenized at 35 °C in a homogenizer at 10,000 rpm. Finally, cross-linked polymer particles were formed as capsules around the antagonistic fungal spores. A view of the T. flavus microcapsule in a suspension is presented in Figure 1.

![Figure 1. The Talaromyces flavus microcapsule in a suspension](image)

4 RESULTS

4.1 Preparation of T. flavus powdered microcapsules

To prepare powdered microcapsules, 10-day T. flavus cultures were first removed from Petri dishes for drying and placed in the laminar flow hood chamber (Fig. 2) and then powdered.
completely with an electric grinder. The obtained powder containing *T. flavus* spores was spread in an aqueous phase including maltodextrine, xanthan gum, fatty acids, ethanol amide, and oleic acid. It was then completely powdered again in a homogenizer at 12000 rpm and 25 °C. Figure 3 depicts *T. flavus* microcapsule packaging in powder form.

It is noteworthy that no fungal contamination was observed in the preparation of microcapsules, and bacterial contamination was prevented using butanol. Regardless of the micro-scale antagonist fungus, the nanoscale of the capsule particles was tested in two suspension and powder forms by Avran Toubi Nanosabz Company, and its documents are attached to the final report file.

**Figure 2.** The drying stage of 10-day cultures of *Talaromyces flavus* to prepare *T. flavus* powder microcapsules

**Figure 3.** A view of *Talaromyces flavus* microcapsule packaging in powder form

### 4.2 Field studies

At this stage, two farms with a history of infection with VW and DO were selected in two regions of Golestan province. On each farm with an area of approximately 1000 m², treatments were applied in seven equal sections. Each treatment consisted of four plots or replications each with dimensions of 6 m long and 5 m wide and an area of 30 m². In each plot, the distances between two planting rows and between two plants on a row were 80 cm and 20 cm, respectively. Each plot included six planting lines, and the studied traits for disease and growth indicators were measured in the plants of the four middle lines of each plot. The experimental design as randomized complete blocks in Karkandeh and Hashemabad areas is presented in Figures 4 and 5.
Figure 4. The experimental design in the Karkandeh cotton farm area

Figure 5. The experimental design in the Hashemabad cotton farm area

The efficiencies of different treatments in the control of diseases under study and boosting different growth indicators were evaluated by comparing the mean values of nine measured traits, including three traits for disease indicators (percentage of healthy seedlings for DO and the incidence rate and severity percentage of VW). To this end, the mixed analysis of variance (ANOVA) was first performed for the two regions using the MS TAT-C software. The ANOVA of means was performed for each region separately with the significant effect of treatment × location.
The applied treatments were microcapsule suspension with soil application (T1), microcapsule suspension as seed impregnation (T2), microcapsule powder with soil application (T3), microcapsule suspension as seed impregnation (T4), Talaromin® fungicide as soil application (T5), Talaromin® as seed impregnation (T6), and a control (T7 without the application of Talaromin® and the powder or suspension microcapsule formulations).

In the soil application treatment for each T. flavus microcapsule formulation, the soil surface was covered with a suspension prepared from each of the formulations with a concentration of 5/1000 according to the treatment based on the liquid biofertilizer application method. The application time of the treatments was considered the same as sowing the seeds.

In the seed impregnation treatment with each of T. flavus microcapsule formulations, seeds were impregnated with the suspension of each formulation at a concentration equal to 10⁷ colony-forming units (CFU)/g according to the treatment. In the application of the Talaromin bioproduct in the soil application method, it was applied uniformly based on 25 kg/ha and the distribution of 25 g for each 10-m planting line up to the planting depth. To use Talaromin bioproduct in the seed impregnation method, cotton seeds were impregnated with the fungicide without using adhesive.

4.3 Evaluation of treatments

Treatments were evaluated in terms of three disease indicators by determining the percentage of healthy seedlings (green percentage) for DO 60 days after planting, and by determining the incidence rate and severity of the disease for VW 4 months after planting. The treatments were also evaluated in terms of six growth indicators, including plant height, the weight of 30 bolls, the number of bolls, yields of the first and the second cuttings, and total yield.

To determine the incidence rate of VW, four middle lines were selected from each plot, and the VW incidence rate in each plot was determined by calculating the number of plants with symptoms of VW. To determine the disease severity percentage, the severity of the disease was first determined by observing its symptoms using a five-point scale (Sanei and Razavi, 2017) as follows:

0 = no disease symptoms, 1= leaf chlorosis and wilting in a quarter of the plant, 2 = leaf chlorosis and wilting in half of the plant, 3 = leaf chlorosis and wilting in three-fourths of the plant, and 4 = yellow leaves and wilting (dead or completely destroyed plant) in the whole plant.

Then, the disease severity percentage for each treatment was calculated according to the mentioned formula for the pathogenicity proof test:

\[
\text{Disease severity percentage} = \left( \frac{\Sigma niv}{NV} \times 100 \right)
\]

where n is the number of plants corresponding to each grade, v is the number of each grade, N is the total number of plants, and V is the number of the highest level of infection (4).

The investigated plants to determine the incidence rate and severity of VW disease in Karkandeh and Hashemabad regions are shown in Figures 6 and 7.
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In the field, the yield was determined during two stages including the first and second cuttings. At each stage, the crop was harvested and weighed separately for all the plots in each treatment. The measured growth indices, namely plant height, the weight of 30 bolls, the number of bolls, yields of the first and the second cuttings, and total yield, were calculated in the four middle lines of each plot.

5 DISCUSSION

The results of this research demonstrated that microcapsule treatments with two forms of suspension and powder were more successful than Talaromin treatment in reducing the incidence of important fungal diseases of cotton, including DO caused by *Rhizoctonia solani* and VW.

Based on previous studies in Iran and other countries, the biocontrol agent in the Talaromin (*T. flavus*) has a distinct feature to boost vegetative traits and increase yield, in addition to growth inhibition of pathogenic agents (Nagtzaam, 1998). Additionally, the results of these studies suggest that two important factors, i.e. the presence of an active population of
disease agents and favorable climatic conditions for their growth (an optimum temperature of 35 °C and a pH of 8) are necessary for the growth inhibition activity of pathogens by this agent, as with other inhibitory agents. Moreover, the boosting activity of vegetative traits and increasing the yield require maintaining a suitable population of CFU ($10^7 - 10^9$) for the biocontrol agent in each gram of soil until the end of the vegetative period and crop production. Therefore, the reduced activities of this biocontrol agent (growth inhibition of disease agents and boosting plant growth) with the fluctuations and changes of the mentioned conditions (changes in temperature and the absence of an active population of the disease) result in the destruction of the suitable population of the CFU of this biocontrol agent (Tut et al., 2021).

Given the more successful function of microcapsule formulations than Talaromin formulation in this research, the capsule coating for the biocontrol agent *T. flavus* in the microcapsule formulation and the gradual release of its spores during the vegetative period until the time of harvest play an important role in maintaining the desired population of the biocontrol agent CFU ($10^7 - 10^9$) per gram of soil from planting to harvesting times. Changes in the optimal conditions for the growth of *T. flavus* reduce the population of this agent in Talaromin, thereby reducing the activity of the biocontrol agent. However, it is deduced from the results that the negative effects of this phenomenon (changes in the optimal conditions for the growth and activity of the fungal biocontrol agent) are compensated by the capsule coating and its gradual release in microcapsule formulations in two suspension and powder forms.

It is noteworthy that after the delivery of technical knowledge of Talaromin production to the manufacturing company, the results of other studies on increasing the stability of the *T. flavus* bioproduct revealed the stability potential for this fungicide, and the adverse effects of fluctuations in optimal conditions for growth and the activity of the biocontrol agent in this fungicide could also be reduced by adding stabilizing compounds such as sodium nitrate or dicycloserine (Bahramian et al., 2016). To change the technical knowledge delivered to the manufacturing company regarding the use of stabilizing compounds, it is necessary to obtain the agreement of this company and accordingly apply the relevant changes in its registration file in the Plant Protection Organization.

To investigate the effects of the studied treatments (each of microcapsule suspension, microcapsule powder, and Talaromin bioproducts with two methods of soil application and seed impregnation with controls) on the measured traits including the percentage of healthy seedlings (the DO evaluation index) and reducing the incidence rate and severity of VW disease (VW evaluation indices), the results of this research are presented separately in the following.

### 5.1 The percentage of healthy seedlings (VW evaluation index)

The mixed ANOVA results for average percentages of healthy seedlings in different treatments, including the control and bioproducts (suspension microcapsules, powder microcapsules, and Talaromin) with two methods of soil application and seed impregnation, in Hashemabad and Karkandeh regions showed that the effect of treatment × location and the effect of treatments were significant at a probability level of 1%. Therefore, the ANOVA was performed separately as a randomized complete block design for the average percentages of healthy seedlings in two regions.

The results of ANOVA for the average percentage of healthy seedlings in different treatments in the Hashemabad region showed that this test was significant at the 5% probability level. The grouping of average percentages of healthy seedlings in different treatments indicated that all the averages were in two statistical groups. Except for the seed suspension microcapsule treatment, all the treatments and the control were assigned to the same statistical group (Table 1). The percentage (91%) of healthy seedlings in the control indicated that the DO disease was clearly absent in the Hashemabad region. As shown in the results of previous research (Whipp, 2001; Kohl et al. al., 2019; Lahlai et al., 2022) no statistically significant
differences were observed between the biological treatments and the control due to the inactivity of the dominant agent of the disease (*R. solani*) (Table 1).

The results of ANOVA for the average percentage of healthy seedlings in different treatments in the Karkandeh area showed that this test was significant at a probability level of 1% (Table 3). According to the grouping of average percentages of healthy seedlings in different treatments, all the averages are in three statistical groups (Table 2). The percentage (73%) of healthy seedlings in the control suggested that the DO disease was present up to 30% in the Karkandeh area. As reported in previous research (Whipps, 2001; Kohl et al., 2019; Lahlai et al., 2022), statistically significant differences in terms of the percentage of healthy seedlings were observed between treatments due to the activity of the disease agent. The powder microcapsule treatment with the seed impregnation method was the best group in terms of the highest percentage (97.97%) of healthy seedlings (statistical group *a*) (Table 2). In statistical group *b*, except for Talaromin treatment with soil application, other biological treatments contained healthy seedlings in the range of 82.72-87.26% (Table 2). Besides, Talaromin treatment with soil application and the control with the percentage of healthy seedlings in the range of 60.44-73.40% were assigned to the statistical group *c* (Table 2).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average percentage of healthy seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcapsule suspension with soil application</td>
<td>76.81ab*</td>
</tr>
<tr>
<td>Microcapsule suspension with seed impregnation</td>
<td>65.67b</td>
</tr>
<tr>
<td>Microcapsule powder with soil application</td>
<td>81.81a</td>
</tr>
<tr>
<td>Microcapsule powder with seed impregnation</td>
<td>91.36a</td>
</tr>
<tr>
<td>Talaromin with soil application</td>
<td>82.95a</td>
</tr>
<tr>
<td>Talaromin with seed impregnation</td>
<td>88.40a</td>
</tr>
<tr>
<td>Control</td>
<td>91.13a</td>
</tr>
</tbody>
</table>

Table 1. Grouping of the average percentage of healthy seedlings in different treatments in the Hashemabad region

*There is no statistically significant difference between means with similar statistical letters at the 5% probability level.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average percentage of healthy seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcapsule suspension with soil application</td>
<td>82.72ab*</td>
</tr>
<tr>
<td>Microcapsule suspension with seed impregnation</td>
<td>86.58ab*</td>
</tr>
<tr>
<td>Microcapsule powder with soil application</td>
<td>86.13ab</td>
</tr>
<tr>
<td>Microcapsule powder with seed impregnation</td>
<td>97.27a</td>
</tr>
<tr>
<td>Talaromin with soil application</td>
<td>60.44c</td>
</tr>
<tr>
<td>Talaromin with seed impregnation</td>
<td>87.26ab</td>
</tr>
<tr>
<td>Control</td>
<td>73.40bc</td>
</tr>
</tbody>
</table>

Table 2. Grouping of the average percentage of healthy seedlings in different treatments in the Karkandeh region

*There are no statistically significant differences between means with similar statistical letters at the 1% probability level.

Based on the average number of healthy seedlings related to ANOVA results for the Karkandeh area, the percentage increase in the number of healthy seedlings in the biological treatments compared to the control in the Karkandeh area is shown in (Table 3). The highest percentage increase (32.52%) in the number of biologically healthy seedlings was observed in the powder microcapsule treatment with the seed impregnation method compared to the control (Table 3).
Table 3. Percentage increase in the number of healthy seedlings in different biological treatments compared to the control in the Karkandeh area

Note: The percentage increase in the number of healthy seedlings in the treatments compared to the control is shown in this table, and only the treatment with red color shows a significant increase compared to the control.

5.2 VW incidence rate (the VW index)

The results of mixed ANOVA showed that the effect of treatment × location and the treatment effect were not significant on the average percentages of VW in different treatments including, the control and bioproducts (microcapsule suspension, microcapsule powder, and Talaromin) with two methods of soil application and seed impregnation in Hashemabad and Karkandeh regions. Since the correction coefficient (C.V.) was higher than 30%, the data were transformed into the 8th root. The ANOVA obtained after data transformation also disclosed that the effect of treatment × location and the treatment effect were not significant. Therefore, the mixed ANOVA (Table 4) showed that the effect of treatment × location was not significant and there was no need to perform ANOVA for two locations separately, hence we referred to the status of the treatment effect in (Table 4). Accordingly, the effect of treatments alone was not significant, and no statistically significant differences were found between the treatments.

Table 4. Mixed ANOVA for the average percentage of VW in Hashemabad and Karkandeh regions of Gorgan city, Golestan province, in the crop year 2021 after data transformation into the 8th root

**The location effect alone is significant at the 1% probability level.
ns: treatment effect and the effect of treatment × location are not significant.

Based on the average percentages of VW from the results of mixed ANOVA, the percentage of reduction in the incidence rate of VW in different treatments compared to the control is shown in (Table 4), despite the absence of significant differences between the treatments and the control in terms of the VW incidence rate. Compared to the control, the highest reductions in the VW incidence rate were observed in Talaromin treatments with soil application (44.38%) and microcapsule powder with seed impregnation (42.35%) (Table 5).

As reported in the results of previous investigations, this biocontrol agent effectively produces a significant difference in the disease incidence rate between the treatments with this
agent and without it when the germplasm of the disease agent in the soil causes more than 30% of the disease incidence rate. In this regard, the cause of this issue is reported to be the activity of biocontrol agents in the presence of a considerable amount of the germplasm of the disease agent. Therefore, when less than 30% of the disease incidence rate is visible, the amount and activity of the germplasm of the disease are not sufficient and effective for the activity of the biocontrol agent to effectively produce a significant difference between the treatments with and without this agent (Wei et al., 2017; Miljakovic et al., 2020; Fernandez-San Millan et al., 2021).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Reduction of VW incidence rate compared to the control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcapsule suspension with soil application</td>
<td>18.60</td>
</tr>
<tr>
<td>Microcapsule suspension with seed impregnation</td>
<td>29.83</td>
</tr>
<tr>
<td>Microcapsule powder with soil application</td>
<td>23.95</td>
</tr>
<tr>
<td>Microcapsule powder with seed impregnation</td>
<td>42.35</td>
</tr>
<tr>
<td>Talaromin with soil application</td>
<td>44.38</td>
</tr>
<tr>
<td>Talaromin with seed impregnation</td>
<td>No reduction compared to the control</td>
</tr>
</tbody>
</table>

Table 5. Reduction of VW incidence rate in different biological treatments compared to the control

Note: This table represents the reduction of VW incidence rate in the treatments compared to the control, and no significant decrease occurred in the treatments compared to the control.

5.3 VW severity percentage (VW disease index)

The results of the mixed ANOVA for the average VW severity rates in different treatments, including the control and biological products (suspension microcapsules, powder microcapsules, and Talaromin), with two methods of soil application and seed impregnation in Hashemabad and Karkandeh regions showed that the effect of treatment × location was not significant but the treatment effect was significant. Since the C.V. was higher than 30%, the data were transformed into the square root. The ANOVA obtained after data transformation also showed that the effect of treatment × location was not significant but the treatment effect was significant (Table 6).

Therefore, the mixed ANOVA (Table 6) indicated that the effect of treatment × location was not significant and there was no need to perform ANOVA for two locations separately, therefore we referred to the status of the treatment effect in (Table 6). According to the results in (Table 7), the effect of treatments alone was significant at the 5% probability level.

Based on the mixed ANOVA, the grouping of the average VW severity percentages in different treatments showed no statistically significant differences between the biological treatments at the 5% probability level (Table 8). Among these treatments, a significant reduction (59.17%) of the VW severity percentage was observed only in the Talaromin biological treatment with the soil application method compared to the control (Table 7) and (Table 8).
Table 6. Mixed ANOVA for the average VW severity percentages in Hashemabad and Karkandeh regions of Gorgan city, Golestan province, in the crop year 2021 after data transformation into the square root

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-value</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of location</td>
<td>1</td>
<td>95,999</td>
<td>95,999</td>
<td>32.8462**</td>
<td>0.0012</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>17,536</td>
<td>2.923</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Effect of treatment</td>
<td>6</td>
<td>6,940</td>
<td>1.157</td>
<td>2.8592*</td>
<td>0.0221</td>
</tr>
<tr>
<td>Effect of treatment × location</td>
<td>36</td>
<td>14,536</td>
<td>0.405</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>138.157</td>
<td>—</td>
<td>28.61%</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 7. Grouping of the mean VW severity percentages in different treatments based on the mixed ANOVA

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean VW severity percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcapsule suspension with soil application</td>
<td>7.64ab*</td>
</tr>
<tr>
<td>Microcapsule suspension with seed impregnation</td>
<td>6.44ab</td>
</tr>
<tr>
<td>Microcapsule powder with soil application</td>
<td>6.91ab</td>
</tr>
<tr>
<td>Microcapsule powder with seed impregnation</td>
<td>6.16ab</td>
</tr>
<tr>
<td>Talaromin with soil application</td>
<td>5.17b</td>
</tr>
<tr>
<td>Talaromin with seed impregnation</td>
<td>6.95ab</td>
</tr>
<tr>
<td>Control</td>
<td>12.68a</td>
</tr>
</tbody>
</table>

Based on the average percentage of VW from the results of mixed ANOVA, the reduction in the severity percentage of VW in different treatments compared to the control is shown in (Table 6), despite the absence of significant differences between the other treatments and the control in terms of the VW severity percentage. In addition to a significant decrease of 17.59% for the VW severity percentage in Talaromin treatment with soil application, a decreased VW severity percentage in the range of 39.73-51.41% was also recorded in the other biological treatments compared to the control. A decrease of 51.41% belonged to the powder microcapsule treatment with seed impregnation (Table 8).

As discussed for the disease incidence rate, this biocontrol agent effectively produces a significant difference in terms of disease indicators, i.e. the incidence rate or severity of the disease, between the treatments with and without this agent when the germplasm of the disease agent in the soil causes more than 30% of disease incidence or more than 20% of disease severity (Miljakovic et al., 2020; Fernandez-San Millan et al., 2021). Therefore, this result is expected to observe no statistically significant differences between the control and biological treatments having a maximum disease severity of 12.68% in the control.
Treatments | The percentage of reduction in the incidence rate of VW (%) |
---|---|
Microcapsule suspension with soil application | 39.73 |
Microcapsule suspension with seed impregnation | 47.63 |
Microcapsule powder with soil application | 45.50 |
Microcapsule powder with seed impregnation | 51.41 |
Talaromin with soil application | 59.17 |
Talaromin with seed impregnation | 45.18 |
Control | - |

Table 8. The percentage of reduction in the severity of VW in different biological treatments compared to the control.

Note: This table represents the percentage of reduction in the severity of VW in the treatments compared to the control, and significant reductions are seen in the treatments with red color compared to the control.

6 CONCLUSION

The overall results further confirm that the inhibitory activities of these agents against the growth of pathogenic agents, and consequently the disease control, are observable when the population of the disease germplasm is present in the soil close to the threshold level according to the type of disease based on the principles of the efficiency of living biocontrol agents.

From the results of this research, it can be concluded that in cotton fields, to manage Verticillium wilt and seedling damping-off diseases, biological control can be used using products prepared through nano and fermentor technologies. In this research, nanotechnology was used to prepare *T. flavus* microcapsules in two forms, suspension and powder, and fermentor was used to prepare *T. flavus* liquid formulation. The use of these formulations will be in the form of impregnating seeds and adding to the soil, and in the method of adding to the soil, the concentration used for each of the liquid, microcapsule suspension, and powder microcapsule formulations will be 5 per thousand, 5 per thousand, and half per thousand, respectively. Was. According to the amount of water calculated to cover one hectare (400 liters), the consumption amount for liquid and microcapsule suspension formulations will be 2 liters and for powder microcapsule formulation, 200 grams. These consumption amounts are much lower compared to the registered fungicide consumption of Talaromin (25 kg per hectare) and due to the mixing of these formulations with water and their application with a sprayer, their method of application is known to farmers.

Currently, one of the limitations of using these formulations is their lack of commercialization and their unavailability for users. Therefore, in order to apply these formulations, it is suggested that the technical knowledge of the production of the mentioned formulations for the purpose of mass production and commercialization be provided to the manufacturing company.

REFERENCES


