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Preparation of Talaromyces flavus liquid and microcapsule formulations and their application in commercial tomato greenhouses to increase yield

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Abstract

With the recent advances in the application of biotechnology in various sciences, the preparation of liquid bio-formulations and microcapsule suspension/powder from the important antagonistic fungal agent Talaromyces flavus has been investigated in recent years. In the first year of this research, a type of liquid bioformulation, a type of microcapsule suspension, and a type of microcapsule powder were prepared for use in tomato greenhouses. During one year, commercial greenhouses were investigated in tomato greenhouses in two regions of Yazd and Isfahan with a history of Fusarium wilt disease. The treatments in each study of the commercial greenhouse were 1-3) each of the liquid formulations, microcapsule suspension, and microcapsule powder by soil application, 4-6) each of the liquid formulations, microcapsule suspension, and microcapsule powder with the tomato seedling root dip, 7) Talaromin fungicide by soil application, and 8) a control (without any formulation and fungicide application). The results indicated that all three formulations in both application methods (soil application or seedling root dip and seed impregnation for tomatoes and cucumbers, respectively) significantly increased the yield of tomatoes compared to the control. Altogether, microcapsule powder with seed impregnation and liquid formulation with soil application were the most effective treatments with approximately 50% and 60% increases in yield compared to the control, respectively, for the management of tomato Fusarium wilt disease. According to the obtained results, the production method of these formulations is considered technical knowledge, and it is possible to carry out their commercialization steps.

Keywords: Biological Control, Cucumber, Fusarium wilt, Talaromyces flavus, Tomato.

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Introduction

According to the latest statistics provided by the Ministry of Agriculture Jihad, the cultivation area of greenhouse crops in Iran has reached 25,000 hectares in 2022. The latest reports indicate that the area under cucumber cultivation is 6432.70 hectares with a production of 1639172 tons and a yield of 254.81 tons/hectare, and that of tomato is 713.80 hectares with a production of 192775.30 tons and a yield of 270.06 tons/hectare.

Fusarium wilt and *Pythium* wilt have been introduced as the important soil-borne fungal diseases of the aforementioned greenhouse crops in the main areas of their cultivation, and significant annual damage is caused by these diseases. To manage such soil-borne diseases of plants, an effective developable method is biological control using products containing useful fungal and bacterial antagonistic factors. Such products have recently been developed inside and outside Iran for their commercialization and mass production; hence, it is of paramount importance to develop and optimize their formulations. For the production of biological formulations in the world, new technologies include using fermentation technology with a fermenter to produce liquid formulations and microtechnology to produce microcapsule formulations (containing antagonistic fungal spores in micro size with a capsule network of microparticles).

Recently, the results of previous research in Iran on the production of microcapsules containing *Talaromyces flavus*, their sporulation, stability, and efficiency in controlling *Verticillium* wilt of cotton and *Fusarium* wilt of tomato/cucumber in greenhouse conditions have shown that these microcapsules have been significantly effective in reducing these diseases (Naraghi et al., 2018a); (Naraghi et al., 2018b); (Naraghi et al., 2019a) and (Naraghi et al., 2019b).

Therefore, such studies should be complemented through the commercialization process of *T. flavus*-containing microcapsules. The current research project examines the efficiency of different doses of microcapsules in different application methods (seed impregnation and soil application) to investigate the control of studied diseases. The results can be used to employ the most effective dosages of microcapsules in cotton fields to determine their efficiency in reducing disease and increasing yield.

The favorable results of the antagonistic fungus *T. flavus* based on the control of some important soil pathogens, such as *Verticillium dahliae*, *V. albo-atrum*, *Fusarium oxysporum*, and *Rhizoctonia solani*, in several crops (cotton, sugar beet, potato, tomato, and greenhouse cucumber) have been proven in studies conducted in Iran.

The use of this fungus in the fields in the propagated form of solid fermentation on plant residues or their mixture with peat soil could reduce the incidence of disease and increase the yield of the mentioned crops. It led to reduced *Verticillium* wilt and seedling death of cotton by 50% and 37%, respectively, and increased yield by 30%, a 40% reduction in disease percentage, and a 17% increase in the yield of potato plants, a

93% increase in the number of healthy seedlings and a 50% increase in the yield of sugar beet plants (Wada et al., 2014), a 27% reduction in disease severity and 23% increase in the yield of tomato plants (Farhang Niya et al., 2015), and a 30% reduction in disease severity and 7% increase in the yield of greenhouse cucumber plants (Naraghi et al., 2017). The issue of marketing and attracting the opinions of relevant consumers is considered important in the mass production and commercialization of biological agents (Husen et al., 2007); (Alimi et al., 2006); (Kaewchai et al., 2009) and (Pereira et al., 2009). Thus, it is currently necessary to commercialize the *T. flavus* biological agent and to produce its various bioformulations, including microcapsules. It is noteworthy that *T. flavus* microcapsules were prepared using microtechnology, and all the particles have micro size (except the fungus) in the present research.

In recent decades, microtechnology has expanded dramatically in various fields of pharmaceutical chemistry, medicine, and agricultural chemical pesticides. An issue that necessitates research and development in the field of micro-pesticides is the pest resistance phenomenon to pesticides. Therefore, the introduction of micro-pesticides to researchers will expand research and development in this relatively new field. Given the environmental problems and costs caused by the excessive consumption of conventional pesticides, as well as the problems caused by pest resistance to these pesticides, research and development in the field of micro-pesticides can be raised as a necessity.

The use of biodegradable polymers in the production of highly efficient microemulsions and microcapsules produced from natural and biodegradable materials can be an effective step in this context. Encapsulation formulation seems to be the best option to increase efficiency and reduce environmental risks (Maji et al., 2014). Therefore, the production of micro bio-formulations leads to the ability to control, increase strength and stability, and protect the active ingredient in adverse environmental conditions such as light and humidity. Moreover, the application of micro-encapsulated formulation helps in pesticide dose reduction, economic efficiency, environmental protection, and environmental risk reduction, as well as better crop export (Martin et al., 2010). A higher surface area of microparticles than microparticles increases their active surface and controlled release. Another advantage of the micrometric particles (< 1 mm in size) is that they do not stimulate the immune system of humans and animals and are quickly excreted from the body (Guan et al., 2008).

Microcapsule technology, which contains fungicide or pesticide molecules with a micro-scale size, is a production method for pesticide formulations that eliminates pests easier and faster (Guan et al., 2008). The emulsion is a heterogeneous system consisting of two immiscible liquids, one of which is dispersed as drops in the other. Emulsions with droplet sizes around micrometers (on the micron scale) and typically in the range of 20-200 µm are called microemulsions (Ostertag et al., 2012). Compared to normal emulsions, the unique structure and properties of microemulsions

have created advantages for their use in many industries. The applications of microemulsion systems in industries include their role in the encapsulation and controlled release of functional compounds such as essential oils, vitamins, etc. (Kah & Hofmann, 2014).

This research was carried out to evaluate the efficiency of *T. flavus* suspension and powdered microcapsules and liquid formulation of *T. flavus* with each application method (soil application and seedling root dip for tomatoes in the greenhouse and seed impregnation for cucumbers in the greenhouse in the planting tray) in reducing the occurrence of the studied diseases (*Fusarium* wilt of tomato and *Pythium* wilt of cucumber). Furthermore, the efficiency of these biological products was compared based on microparticles and liquid culture media with Talaromin fungicide on rice bran substrate.

Methods and Materials

Preparation of *T. flavus* liquid formulation using a fermenter Based on the results of previous research (Naraghi et al., 2020), the most effective treatment among the eight studied treatments in terms of increasing sporulation, stability, and weight of the mycelial mass and reducing the infection level (treatment 8 at 25 °C, Potato Dextrose Broth culture medium, and dicycloserine stabilizer) was used for the production of liquid formulations. *T. flavus* liquid formulation was prepared using a 4-L fermenter available in the biological control research department of the Iranian Plant Protection Research Institute based on the modified method of (Naraghi et al., 2020). Two parts of the culture medium, including starter medium and fermentation medium, were prepared in previously arranged jar-like autoclavable propylene containers with a volume of 4 L and an opening diameter of 4 cm. The antagonist fungus was inoculated in the starter culture medium, and finally, this medium was used for inoculation to the fermentation culture medium.

According to the mentioned method, 400 ml and 3 ml were respectively determined for the starter and fermentation culture mediums for the volume of 4 L. The same type of liquid culture medium was used for both types of culture medium, and 30 g of molasses and 5 g of yeast were used for each liter.

Based on this method, the use of *T. flavus*-containing liquid culture medium in the fermenter to multiply *T. flavus* required using 400 ml of the starter medium (liquid culture medium containing the antagonistic fungus) with a *T. flavus* colony-forming concentration of 10^9 units for a 4-L fermenter and 3 L of the fermentation medium (liquid culture medium without the antagonistic fungus). The starter culture medium with a concentration of 10^9 *T. flavus* colony-forming unit (CFU) per ml was first prepared by making a suspension with a concentration of 4×10^{11} per ml, and 1 ml of the suspension was made to 200 ml with the liquid culture medium. Therefore, a liquid culture medium was obtained with a *T. flavus* concentration of 2×10^9 CFU/ml. Then, 400 ml of the starting culture medium with a concentration of 10^9 was obtained by doubling the 200-ml liquid culture medium with a concentration of 2×10^9 *T. flavus* colony-making units per ml

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with the liquid culture medium and making a volume of 400 ml. Finally, the starter culture medium with a volume of 400 ml was inoculated into the fermentation culture medium with a volume of 3 L and placed in a 4-L fermenter to produce liquid bioformulation. It is worth noting that the term CFU means *T. flavus* spores in this research. (Figure 1) illustrates the fermenter and the resulting liquid formulation.

Figure 1. Photos of the fermenter (right) and the obtained liquid formulation (left)

The most suitable fermenter time and rotation speed for the CFU/ml were determined using the results obtained from previous studies. The pH or acidity for the fermentation environment was set to 8 based on the most optimal pH for the mass production of *T. flavus*. Anti-foam additives were also used based on previous investigations (Lee et al., 2008).

Preparation of the T. flavus microcapsule suspension

Microcapsules are produced through a combination of the polymerization and cross-linking method, which was carried out based on the method of (Negahban et al., 2011) by making modifications (in the polymer amount or type, surfactants, oils, fatty acids, the shaker rpm, and temperature). In the polymerization process, the organic phase consisted of vegetable oil with a mixture of *T. flavus* antagonistic fungus, which was added to the aqueous phase consisting of a mixture of hydrophilic polymers including polyurea formaldehyde, starch, and chitosan. Then, a cross-linker (e.g. calcium chloride), surfactants, accompanying materials, and fatty acid oils were added to the total of two phases and homogenized at 35 °C in a homogenizer at 10,000 rpm. Finally, cross-linked polymer particles were formed as capsules around the *T. flavus* antagonist fungus particles. The *T. flavus* microcapsule suspension is shown in (Figure 2).

Preparation of T. flavus microcapsule powder

In the *T. flavus* microcapsule powder, the suspension containing the antagonist fungus spores was spread in the aqueous phase containing maltodextrin, xanthan gum, fatty acids, ethanol amide, and oleic acid. It was then completely turned into a powder in a shaker at 10,000 rpm at 25 °C. *T. flavus* microcapsule powder is shown in (Figure 2).

Figure 2. *T. flavus* microcapsule formulation in two suspension (right) and powder (left) forms

Efficacy of microcapsule biological products and *T. flavus* liquid formulation in controlling *Fusarium* wilt disease of tomatoes in commercial greenhouses

Investigations of commercial greenhouses were carried out in tomato greenhouses in Yazd and Isfahan regions with a history of *Fusarium* wilt disease for one year. Each study was carried out as a statistical randomized complete block design with eight treatments in four replications. The treatments in each investigation of the commercial greenhouse were 1-3) a 5/1000 dose from each of the liquid formulations, microcapsule suspension, and microcapsule powder by soil application, 4-6) each of the liquid formulations, microcapsule suspension, and microcapsule powder at 10^7 CFU with the tomato seedling root dip, 7) Talaromin fungicide by soil application, and 8) a control (without formulation and fungicide application). Treatments were evaluated by determining the percentage of occurrence and severity of *Fusarium* wilt disease. Statistical data analysis and mean comparisons were done using Duncan's multi-range test with MS TAT C software.

For the soil application treatment for each of the *T. flavus* microcapsule and liquid formulations, the soil surface was covered with the suspension prepared from each of the formulations according to the treatment based on the application method of liquid biofertilizers (Seefeldt et al., 2001). Treatments were implemented at the same time as planting tomato seedlings.

To use the liquid bioformulation and microformulations of *T. flavus* in root-dip assay treatment, tomato seedling roots were dipped for 10-20 min with the suspension of each formulation at a concentration of 10^7 CFU/g. Treatments were evaluated 4 months after planting (Ostertag et al., 2012). By determining the percentage of disease incidence and the percentage of disease severity. To determine the percentage of disease severity according to (Liu et al., 1995), the disease incidence was first determined by observing its symptoms using a six-point scale as follows:

0 = no disease symptoms

1 = leaf chlorosis and plant wilting less than 25%
2 = leaf chlorosis and plant wilting from 25 to 50%
3 = leaf chlorosis and plant wilting from 51 to 75%
4 = leaf chlorosis and plant wilting from 76 to 100%
5 = Dead or completely destroyed plant
Then, the percentage of disease severity for each treatment was calculated according to the formula mentioned for the pathogenicity proof test as follows:
Disease severity percentage = Σ (nivi)/NV × 100
n is the number of plants related to each point, v is the number of each point, N is the total number of plants; and V is the number of the highest infection level (5).
These investigations were conducted using the seeds of the German tomato variety (SV 4129 SH) belonging to Seminis Company, a subset of the Bayer Company.

Results

The efficiency of different T. flavus formulations in controlling Fusarium wilt of tomato in the commercial greenhouse of Yazd Evaluation of the total yield of the first four harvests

The test for the effects of different *T. flavus* formulations on the total yield of the first four harvests was significant at the 1% probability level. The results of grouping the means of this yield in different treatments showed that the mean values of different treatments were located in seven statistical groups (Table 1). In order of priority for the maximum yield of the first four harvests, the statistical groups were as follows. 1) Statistical group a with liquid formulation treatment by soil application. 2) Statistical group b with Talaromin and liquid formulation treatments by seed impregnation. 3) Statistical group c with liquid formulation treatments by seed impregnation, and microcapsule powder by soil application. 4) Statistical group d with microcapsule powder treatments by soil application and microcapsule suspension by soil application. 5) Statistical group e with microcapsule suspension treatments by soil application and microcapsule powder by seed impregnation. 6) Statistical group f with microcapsule powder treatments by seed impregnation and the control. 7) Statistical group g with control treatments and microcapsule suspension by seed impregnation (Table 1).
The results in (Table 1) show a significant increase in the total yield of the first four harvests in all treatments, except the microcapsule powder with the seed impregnation method and microcapsule suspension with the seed impregnation method, compared to the control. Among the treatments with a significant increase in the total yield of the first four harvests compared to the control, the liquid formulation treatments with soil application, Talaromin fungicide, the liquid formulation with seed impregnation, the microcapsule powder with soil application, and microcapsule suspension with soil application respectively led to the utmost significant increase in the total yield of the first four harvests compared to the control (Table 1).
The estimated percentage increase in the average yield of the first four harvests in different treatments compared to the control

revealed that, despite no significant differences between the microcapsule powder treatments with seed impregnation and microcapsule suspension with seed impregnation and the control, the microcapsule powder treatment with seed impregnation could increase the total yield of the first four harvests by 10.00% compared to the control (Table 1). Compared to the control, the increase percentages of the total average yield of the first four harvests were obtained for the other treatments, namely liquid formulation with soil application (55.83%), Talaromin fungicide (44.02%), liquid formulation with seed impregnation (33.05), the microcapsule powder with soil application (27.50%), and microcapsule suspension with soil application (18.33) (Table 2).

Table 1. Comparison of the average total yield of the first four harvests in different treatments of *T. flavus* formulations (microcapsule suspension, microcapsule powder, liquid formulation, and Talaromin fungicide) in the commercial tomato greenhouse of Yazd in 2021

Treatment	The average total yield of the first four harvests (kg/4 m^2)
T. flavus microcapsule suspension with soil application	**8.52de
T. flavus microcapsule suspension with seedling root dip	6.92g
T. flavus microcapsule powder with soil application	9.18cd
T. flavus microcapsule powder with seedling root dip	7.92ef
T. flavus liquid formation with soil application	11.22a
T. flavus liquid formation with seedling root dip	9.58bc
Talaromin fungicide	10.37b
Control	7.20fg

*Each experimental plot has an area of 4 m^2.
**There are no statistically significant differences between experimental treatments with similar letters at the 1% probability level.

Table 2. The percentage increase in total yield of the first four harvests in different treatments of *T. flavus* formulations (microcapsule suspension, microcapsule powder, liquid formulation, and Talaromin fungicide) compared to the control in the commercial tomato greenhouse of Yazd in 2021

Treatment	The percentage increase in the total yield of the first four harvests compared to the control (%)
T. flavus microcapsule suspension with soil application	18.23
T. flavus microcapsule suspension with seedling root dip	-
T. flavus microcapsule powder with soil application	27.50
T. flavus microcapsule powder with seedling root dip	10.00
T. flavus liquid formation with soil application	*55.83
T. flavus liquid formation with seedling root dip	33.05
Talaromin fungicide	44.02
Control	-

*The highest percentage increase in the total yield of the first four harvests in the treatment of *T. flavus* liquid formulation with the method of adding to the soil compared to the control.

According to previous studies and the principles of biocontrol, the activity of biocontrol agents and the production of volatile and non-volatile compounds inhibiting pathogenic agents depends on the presence of the disease agent (Kohl et al., 2019) and (Wang et al., 2022). Therefore, it can be argued that even the activity of Plant Growth Promoting Fungi (PGPF) to enhance plant growth through the production of compounds similar to plant hormones, including auxin, abscisic acid, cytokinin, ethylene, gibberellin, and brassinosteroid (Nicolletti et al., 2023) is positively correlated with the population size of the disease agent, in addition to the inhibitory activity of pathogenic agents (Manoch & Dethoup, 2011) and (Tian et al., 2021).

The biocontrol fungal agent *T. flavus* is not an exception to this rule, and the presence of a larger population of the disease agent further increases the activity of its plant-like hormones to enhance vegetative traits, including increasing yield. Similar to the results of the current research, evidence indicates that, despite the presence of a considerable population of the disease agent, compounds similar to plant hormones can preserve the properties of such biocontrol agents in enhancing vegetative traits, resulting in a significant increase in yield (Lymperopoulos et al., 2018).

The efficiency of different T. flavus formulations in controlling Fusarium wilt disease of tomato in Isfahan commercial greenhouse

The total yield of the first four harvests

The effects of different *T. flavus* formulations on the total yield of the first four harvests were significant at the 1% probability level. The results of grouping the average yield of the first four harvests in different treatments showed that the averages of different treatments were located in two statistical groups. In order of priority for the maximum yield of the first four harvests, the statistical groups were as follows. 1) Statistical group a with liquid formulation treatments by soil application, microcapsule suspension by soil application, liquid formulation by seed impregnation, microcapsule suspension with seed impregnation, microcapsule powder by soil application, and Talaromin. 2) Statistical group b with microcapsule powder treatments by soil application, Talaromin, and the control (Table 3).

The results in (Table 1) show significant increases in the total yields of the first four harvests in all treatments, except the microcapsule powder with soil application and Talaromin, compared to the control. Among the treatments with significant increases in the total yields of the first four harvests compared to the control, liquid formulation treatments with soil application, microcapsule suspension with soil application, the microcapsule powder with seed impregnation, the liquid formulation with seed impregnation, and microcapsule suspension with seed impregnation respectively resulted in the highest significant increase in the total yields of the first four harvests compared to the control (Table 3).

The estimated percentage increase in the average yields of the first four harvests in different treatments compared to the control

revealed that, despite no significant differences between the microcapsule powder with soil application, Talaromin, and control treatments, these treatments could increase the total yields of the first four harvests by 28.69% and 21.06, respectively, compared to the control (Table 4). Compared to the control, the increase percentages of the total average yields of the first four harvests were obtained for the other treatments, namely liquid formulation with soil application (56.58%), microcapsule suspension with soil application (50.01%), the microcapsule powder with seed impregnation (47.65%), liquid formulation with seed impregnation (47.65%), and microcapsule suspension with seed impregnation (43.93) (Table 4).

Table 3. Comparison of the average yields of the total first four harvests in different treatments of *T. flavus* formulations (microcapsule suspension, microcapsule powder, liquid formulation, and Talaromin fungicide) in the commercial tomato greenhouse of Isfahan in 2021

Treatment	The average total yield of the first four harvests (kg/4 m^2)
T. flavus microcapsule suspension with soil application	**16.15a
T. flavus microcapsule suspension with seedling root dip	15.50a
T. flavus microcapsule powder with soil application	13.86ab
T. flavus microcapsule powder with seedling root dip	15.90a
T. flavus liquid formation with soil application	16.86a
T. flavus liquid formation with seedling root dip	15.90a
Talaromin fungicide	13.03ab
Control	10.77 b

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

In the study of Isfahan commercial greenhouse, each experimental plot unit had an area of 35 m^2, and the unit was converted to an area of 4 m^2 to equalize it with the study in Yazd.

Table 4. The percentage increases in total yields of the first four harvests in different treatments of *T. flavus* formulations (microcapsule suspension, microcapsule powder, liquid formulation, and Talaromin fungicide) compared to the control in Isfahan commercial tomato greenhouse in 2021

Treatment	The percentage increase in the total yield of the first four harvests compared to the control (%)
T. flavus microcapsule suspension with soil application	50.01
T. flavus microcapsule suspension with seedling root dip	43.93
T. flavus microcapsule powder with soil application	28.69
T. flavus microcapsule powder with seedling root dip	47.65
T. flavus liquid formation with soil application	*56.58
T. flavus liquid formation with seedling root dip	47.65
Talaromin fungicide	21.06
Control	-

*The highest percentage increase in the total yield of the first four harvests in the *T. flavus* liquid formulation treatment with soil application compared to the control.

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Discussion

In the present study, the use of microcapsule formulations led to significant increases in the yields of the first to fourth harvests and the total yield compared to the control. A similar result was obtained in the cotton fields of Karkandeh and Hashemabad in a project on the use of microcapsule formulations to control important fungal diseases of cotton, and a significant increase in the cotton crop yield was observed compared to the control (planter conditions) (Naraghi & Razi Nattaj, 2022).

In the current research, the liquid formulation application resulted in a significant reduction in the disease incidence and severity and a significant increase in yield. Regarding the application of *T. flavus* liquid formulation, a study on the efficiency of this formulation on sugar beet damping-off disease indicated a significant increase in the number of healthy seedlings in the treatment containing this formulation compared to the control treatment (Naraghi et al., 2019a).

In both the Isfahan and Yazd regions, the liquid formulation treatment by soil application (with more than a 50% increase in yield compared to the control) was identified as one of the effective treatments for increasing yield. The powder microcapsule by seed impregnation (with more than a 50% increase in yield) was an effective treatment in increasing yield in the Isfahan region, and Talaromin was determined with more than a 40% increase in yield in the Yazd region. To justify these results, the presence of the disease agent as a stimulating factor for the release of *T. flavus* spores led to the faster release process of the biocontrol agent spores in the powder microcapsule treatment in Isfahan than in the Yazd region with no disease incidence. This enabled the activity of *T. flavus* biocontrol agent spores to inhibit the disease agent and enhance the vegetative traits, thereby increasing the yield.

On the other hand, the soil acidity was acidic in the range of 4.5-6.5 in Isfahan during the research implementation year, which caused the activity of *Fusarium* and the disease incidence. This prevented the activity of Talaromin for a significant increase in yield due to the need for alkaline acidity (pH = 8) of the biocontrol agent present in the fungicide (Kim et al., 1990) and (Cruz et al., 2019).

Conclusion

The *T. flavus* liquid formulation treatment as soil application in both regions of Isfahan and Yazd with acidic with alkaline soils, respectively, acted as the most effective treatment in disease control and increasing yield. This result shows that the minimum population of *T. flavus* spores per gram of this formulation is preserved to carry out the fungal activities until the last harvest due to the use of the sodium nitrate stabilizer in this formulation, despite changes in soil acidity at the disease incidence time in Isfahan (from the fourth harvest time onwards). Due to *Fusarium* wilt disease in the Isfahan region, probably the soil acidity of this region was acidic in the studied crop year. Despite the corresponding acidity conditions with the optimal acidity (pH = 8) for the activity of the

T. flavus antagonistic agent, the liquid formulation containing this antagonistic agent could also effectively increase yield with alkaline acidity in the Isfahan region besides the Yazd region. This resulted from the presence of fixing and stabilizing compounds in the liquid formulation containing the antagonistic *T. flavus* despite the lack of optimal acidity conditions for its growth and activity.

Recommendations

According to the results obtained from this research, for the technical knowledge of liquid formulation production, *T. flavus* microcapsule suspension, and microcapsule powder, it is recommended to conclude a contract between the institute and the producing company. The steps of their registration in the Plant Protection Institute should be carried out by the producing company for the commercialization of the aforementioned technical knowledge.

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