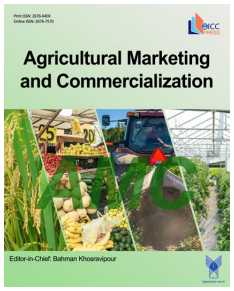

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Comparison of Reduction of Verticillium Wilt Disease by Using *Talaromyces Flavus* Microcapsule with the Conventional Method of Farmers in Potato Fields in Hamedan Province

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Review Article

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Abstract:

Verticillium wilt is one of the most important potato diseases. Due to the soil-borne nature of the agents of this disease (*Verticillium dahliae* and *Verticillium albo-atrum*), its control by chemical methods is not easily possible. The use of soil-based inhibitors can be considered a suitable method. During the implementation of a previous research project on the possibility of reducing the incidence of important fungal diseases of cotton with agents *Verticillium dahliae* and *Rhizoctonia solani* by using biological products *Talaromyces flavus* (liquid formulations, suspension microcapsules, powder microcapsules and Talaromin), it was found that suspension microcapsules were successful in reducing the incidence of the studied diseases and enhancing vegetative traits, including increasing yield, and its application method was easier than the other two types of products. With regard to the implementation of this project, a preliminary study was first conducted using the four aforementioned formulations in potato crops, and the most effective formulations in terms of reducing disease and increasing yield were liquid and microcapsule powder formulations, both of which were used in the research-promotion project. In this project, with the cooperation of the Extension Coordination Management of the Agricultural Jihad Organization of Hamadan Province, a two-hectare farm in Hamadan County with a history of infection with Verticillium wilt disease was selected to apply two effective formulations (powder microcapsule and liquid formulation) and compare them with the condition without the use of the formulation. The comparison of the two treatments with the use of the effective formulations of powder microcapsule and liquid formulation with the control treatment (without the use of the formulation) was carried out with the t test in terms of the percentage of disease occurrence and the percentage of severity of Verticillium wilt disease, and finally the yield was estimated. An economic comparison was made between the method of application of the biological product *Talaromyces flavus* with the conventional method of the operators. In this comparison, it was found that the effective liquid and microcapsule powder formulations, applied by seed impregnation, reduced disease severity by 73.33 and 66.66 percent, respectively, and increased yield by 25.96 and 27.08 percent, compared to the control.

Keywords: Soil-borne disease, Crop, Formulation, Biological control

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INTRODUCTION

Fungal wilt disease causes significant damage to potatoes, with more than 30% of the damage being caused by the pathogens *V. dahliae*, *V. albo-atrum*, and *Fusarium oxysporum* (Saremi & Amiri, 2010). In addition to endangering environmental health, the use of chemical pesticides is not recommended and is not very effective due to the emergence of pathogen resistance. One of the methods for controlling soil-borne pathogens is the use of antagonistic microorganisms. The results of the research project entitled "Possibility of biological control of potato verticillium wilt using the antagonistic fungus *Talaromyces flavus* under field conditions" showed that the efficiency of the rootstock affected by the *T. flavus* isolate (TF-Po-V-52) was effective in reducing the severity and incidence of the disease by 18.04 and 40.31 percent, respectively, and in increasing yield by 17.48 percent. Therefore, it is necessary to transfer such results to farmers through the implementation of a research-promotion project in their fields in order to be able to utilize the aforementioned rootstock as a fungicide and biological fertilizer in potato fields.

Considering the production of new formulations of *Talaromyces flavus* (liquid formulations, suspension microcapsules and powder microcapsules) using fermentors and nanotechnology, it is important and necessary to determine the most effective method of application and the amount of consumption of each of them in terms of reducing the incidence of the studied diseases in agricultural and greenhouse crops. In recent years, based on research, the most effective method of application and the amount of consumption for the previous formulation of T. flavus (registered biological fungicide Talaromin) was determined to reduce the incidence of some important fungal diseases such as seedling death, verticillium wilt and fusarium wilt in sugar beet, cotton, potato and tomato crops.

On the other hand, the difference in the application rate of the fungicide Talaromin (25 kg/ha in the soil application method and 300 g to 1 kg for the treatment of sugar beet, tomato and cotton seeds depending on the seed size and 60 kg for the treatment of potato seed tubers consumed in one hectare, equivalent to four tons of seed for potatoes) with the application rate of the new formulations (concentration of five per thousand of the liquid and microcapsule suspension formulation and concentration of half per thousand of the powder microcapsule formulation that is being tested for tomatoes and cucumbers), required that the aforementioned concentrations of the new formulations be investigated at the field level for potatoes and its effectiveness be compared with the fungicide Talaromin at the corresponding rate (60 kg for the treatment of potato seed tubers consumed in one hectare, equivalent to four tons).

By conducting this research, the most effective formulation among the registered and new formulations (Talaromin fungicide, liquid formulation, microcapsule formulation with two forms of suspension and powder) was determined with its consumption rate for seed tuber impregnation to manage verticillium wilt disease in potato fields. Considering the positive results obtained from this research (results included in this report), it is possible to register new formulations and subsequently transfer technical knowledge of each of them.

METHODOLOGY

Land preparation method

A field with an area of 1.2 hectares was selected for preliminary investigation and was divided into five equal parts for applying five treatments including Talaromin, suspension formulation, powder formulation, liquid formulation and control. In each part, 6 planting lines with a length of about 360 meters were located, and the distance between two planting lines was 60 cm.

The goal was to select a common and cultivable variety in the farmers fields. seed tubers of the Bamba variety were prepared for preliminary investigation and in 2025, seed tubers of the Fabula variety were prepared by the respected farmers for supplementary investigation.

How to use and amount of chemical fertilizers

In these studies, urea fertilizer was used two to three weeks after planting during the initial growth of the plant at a rate of 10 to 20 kilograms per hectare.

Farm management in the field in terms of controlling weeds, pests and diseases

For weed management, the herbicide Metribuzin (Senkor WP 70%) was used after planting and before the emergence of potatoes and weeds, and finally until the stage of two to four leaves of weeds at a rate of one kilogram per hectare. For disease management in the framework of this project, biological formulations and chemical fungicide Celeste were used.

How to use and the amount of each biological formulation and chemical fungicide Celeste

To use each of the biological formulations including Talaromin, liquid formulations, suspension microcapsules and powder microcapsules, the seed impregnation method was used. The amount of Talaromin used for seed tubers consumed in one hectare (four tons) was 60 kg, which was impregnated with this mixture after moistening the tubers (Naraghi, 2024a).

For the impregnation of seed tubers used in one hectare, a volume of 15 liters of water was used, and based on the aforementioned volume, a concentration of five per thousand was prepared for each of the liquid and microcapsule suspension formulations and a concentration of half a thousand for the powder formulation, and then, the impregnation of seed tubers with each of the aforementioned formulations was carried out according to the application in the experimental units (Naraghi et al., 2024). To impregnate each ton of seed tubers with Celeste fungicide, according to the instructions inside the fungicide package, the tubers were first moistened and after 24 hours, they were impregnated with 250 ml of fludioxonil fungicide (Celeste FS 2.5%) manufactured by the Swiss company Syngenta.

RESULTS

The results of the study in the form of disease incidence and disease severity percentage for different treatments including Talaromin, liquid formulation, powder microcapsule formulation and suspension microcapsule formulation and control are listed in Table 1. These results show that the lowest disease incidence percentage (24%) belonged to Talaromin and suspension microcapsule, while the disease severity was calculated as 2.7% for Talaromin treatment and 4.14% for suspension microcapsule treatment. Obviously, the definitive introduction to introduce the most desirable product will be based on the yield results. If the highest yield also belongs to the two mentioned products, the product that will result in a lower consumption will be selected. The consumption amount of Talaromin and water required for impregnating potato seed tubers consumed in one hectare was 4 tons, 60 kg and 60 liters, respectively; Whereas, considering the required concentration of microcapsule suspension (5/1000) and the required volume of water for soaking the seed tubers used in one hectare (60 liters), only 300 ml of microcapsule suspension will be required to prepare a concentration of 5/1000 by bringing its volume to 60 liters. A view of the field with separate treatments using the formulations is shown in Figure 3 (Figure 1). Also, the assessment of the percentage of occurrence and severity of the disease was carried out two and a half months after planting and the occurrence

Table 1. Percentage of occurrence and percentage of disease severity in different field treatments including Talaromin, suspension microcapsule, powder microcapsule and liquid formulation

Treatment	Disease incidence percent	Disease severity percent
Control	60.00	72.00
Talaromin	24.00	7.20
Liquid formulation	60.00	24.00
Suspension microcapsule	24.00	14.40
Powder microcapsule	60.00	28.80

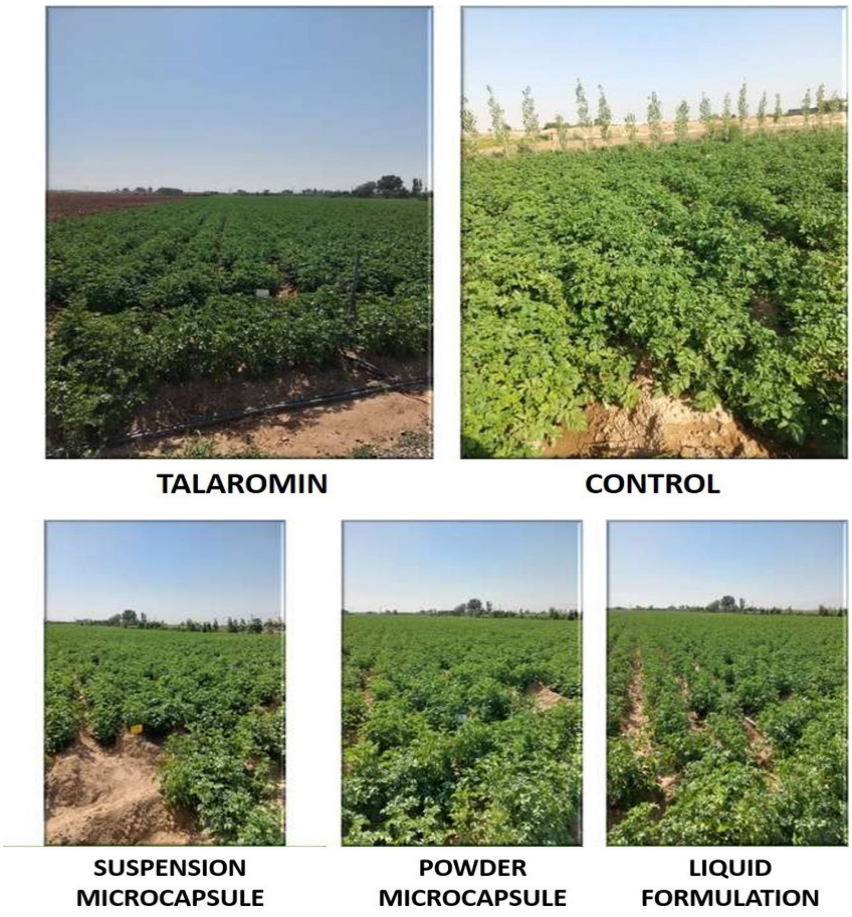

Figure 1. View of the field with treatments containing formulations

Figure 2. Occurrence of symptoms of Verticillium wilt disease on plants in the control section

Figure 3. Appearance of white fungal filaments during isolation of the pathogen *V. dahliae* from a stem fragment of an infected plant

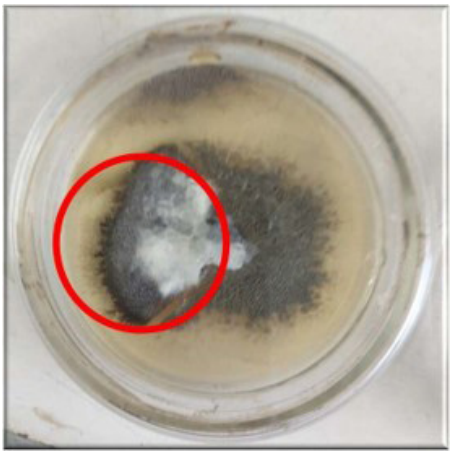

Figure 4. Observation of the colony of the pathogenic fungus *V. dahliae* with black microsclerotia during isolation of the pathogenic fungus from infected plant samples

of symptoms of Verticillium wilt on the plants in the control section can be seen in Figure 2.

During the investigation into the isolation of the causative fungus from infected plant samples, first the appearance of white filaments (Figure 3) and then the colony of the causative fungus *V. dahliae* with black microsclerotia was observed (Figure 4).

Also, while cultivating the field soil at a one-tenth dilution, a colony of the biocontrol agent fungus *T. flavus*, present in the biological products used in the field, was observed (Figure 5).

The results showed that the percentage increase in yield of the treatments of powder microcapsule with 61 tons per hectare, liquid formulation with 59 tons per hectare, Talaromin with 54 tons per hectare and suspension microcapsule with 53 tons per hectare compared to the control with 48 tons per hectare (traditional conditions of farmers) was 27.08, 22.92, 12.50 and 10.42 percent, respectively (Table 2). It should be noted that the average potato yield in the crop year of this experiment (2024) was reported to be 36 tons per hectare. A view of the field at the time of harvesting potato tubers in different field treatments using four biological products including Talaromin, liquid formulation, powder microcapsule, suspension microcapsule and control is shown (Figure 6). Also, a view of the small size, cracking, and malformation of potato tubers in the control section is shown (Figure 7).

During the implementation of a previous research project, the efficacy of the only biological product Talaromyces flavus was investigated in the laboratory sample production stage and before the registration stage in the Plant Protection Organization under the name Talaromin, in terms of reducing the incidence of Verticillium wilt disease and increasing potato yield in a Hamedan farm. The results of this study also showed a significant reduction in the incidence of the disease and a 17% increase in yield compared to the control (Naraghi, 2024b).

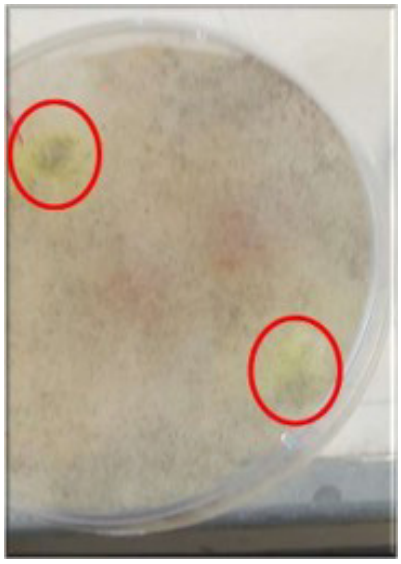

Figure 5. Isolation of *Talaromyces flavus* colonies present in biological products used in the field while cultivating the field soil at a one-tenth dilution

Table 2. Yield mean in tons per hectare in treatments and percentage increase in yield of treatments compared to the control

Treatment	Yield mean (tons per hectare)	Percentage increase in yield in sections containing biological formulation compared to control
Control	48.00	-
Talaromin	54.00	12.50
Liquid formulation	59.00	22.92
Suspension microcapsule	53.00	10.42
Powder microcapsule	61.00	27.08

Figure 6. View of potato tuber harvest in different field treatments using four biological products including Talaromin, liquid formulation, powder microcapsule, suspension microcapsule and control

Figure 7. View of the small size, cracking, and malformation of potato tubers in the control section

DISCUSSION AND CONCLUSION

Overall the results showed that it was necessary to conduct an experiment to select and use the most effective biological product among four products including Talaromin, liquid formulation, powder microcapsule and suspension microcapsule for comparison with the conventional conditions of farmers. Based on the guidelines of research-extension projects based on the use of only one biological product and its comparison with the conventional conditions of farmers and the lack of a definitive result for the most effective biological product to reduce the occurrence of Verticillium wilt and increase yield in potato crops, a research project in this regard was initially proposed for potato crops to the Scientific and Technical Committee of the National Plant Medicine Research Institute. However, due to the implementation of a research project with a similar topic (use of three biological products Talaromin, powder microcapsule and suspension microcapsule) for cotton crops, the proposed research project to determine the most effective product among the four products for use in potato fields was not approved.

According to the then committee of the National Plant Medical Research Institute, the selection of the most effective biological product for use in the framework of a research-extension project related to the potato crop was based on the results of a previous research-executive project on the use of microcapsule suspension and microcapsule powder formulations in cotton fields to reduce the incidence of Verticillium wilt diseases and seedling death (Naraghi & Nattaj, 2022). The results of this study showed that there was no statistically significant difference between the powder microcapsule and the suspension microcapsule in terms of effectiveness in reducing the incidence of Verticillium wilt diseases and seedling death of cotton. Therefore, given the lack of statistical difference in the results between these two products in terms of the above-mentioned traits and the easier use of the suspension microcapsule, the suspension microcapsule product was selected for the implementation of the research-extension project. However, the results of this preliminary study showed that for potato crop, despite the results obtained for cotton crop, the most effective biological products were powder microcapsules and liquid formulation, with a 27 and 22 percent increase in yield compared to the control, respectively (Table 2). It is worth noting that previous research has also reported the difference in the effectiveness of a biological product such as Talarumin for various crops including cotton, sugar beet, potatoes and tomatoes in reducing the incidence of soil-borne fungal diseases and increasing yield. Therefore, achieving such a result for the biological products of powder microcapsules and suspension microcapsules regarding their difference in efficiency in cotton and potato crops was not far from expected. According to the results obtained from this study, powder microcapsules were used as the most effective biological product for impregnating potato seed tubers in the implementation of the research-extension project in order to compare with the conventional conditions of farmers.

Considering that the liquid formulation also worked successfully in the study with a 22% increase in yield compared to the control, in coordination with the management of the Agricultural Jihad of Hamadan County, the Agricultural Research Center and Natural Resources, and the Extension Coordination Management of the Agricultural Jihad Organization of Hamadan Province, the liquid formulation was also considered due to its easier application method for comparison with the powder microcapsules and the conventional conditions of farmers. It is worth noting that in the use of the liquid formulation, the product is in a liquid state and after adding the required volume with water to prepare the consumption concentration (five per thousand), there is no need for time and it can be used immediately for impregnating seed tubers. Whereas, for the use of powder microcapsule formulation, due to the presence of the effective biocontrol agent (*T. flavus* fungus) in the capsules, after making up to the required volume with water to prepare the consumption concentration (half a thousand), a period of about one hour is allowed for the release of part of the *T. flavus* population from the capsules to occur (Negahban et al., 2011) and then they are used to impregnate potato seed tubers.

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.

Authors' Contributions
The first author (Laleh Naraghi) contributed to the development of the theoretical framework and literature review. The second author (Maryam Negahban) was responsible for statistical analyses. The third author (Mazdasht Giti) contributed to data collection, as well as the formulation of findings and recommendations. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials
The datasets generated or analyzed during the current study can be obtained from the corresponding author on a reasonable request.

Conflict of interest
The authors state that there is no conflict of interest.

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