Ekoloji 27(106): 103-112 (2018)

Growth Inhibition of Fusarium oxysporum f. sp. lycopercisi, the Causal Agent of Tomato Fusarium Wilt Disease by Nanoformulations Containing Talaromyces Flavus

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Abstract

Remarkable researches in Iran have shown the importance of the Talaromyces flavus antagonist fungus to inhibit the growth of some of the important plant pathogens such as Rhizoctonia solani, Verticillium dahliae and Fusarium oxysporum. According to the results obtained from the previous researches, the commercialization of the bioformulations of this fungus is of particular importance. Since the marketing is considered to be important factor in order to commercialization, consideration of the type of bioformulation with easy application can greatly affect the attraction of relevant consumers and a successful marketing. Therefore, regarding the recent advances in the application of nanotechnology in different sciences, it seems necessary to study different nanoformulations of the mentioned biological agents with an emphasis on the ease of application and their efficacy in biological control of plant diseases. In this study, the preparation of nanoformulations including two types of nanocapsules (F1 and F3), a nano-emulsion (F2) and a powder form (F4) from T. flavus fungus was carried out. Three months after production, experimental evaluations of the effect of different nanoformulations and the formulation prepared based on former technical knowledge (rice bran and T. flavus) on sporulation, active population and efficiency in inhibiting colony growth of some important terrigenous disease agents including Verticillium dahliae, Fusarium oxysporum f. sp lycopersici and Fusarium oxysporum f. sp cucumerinum in a completely randomized design were conducted. These evaluations began three months after the production of nanoformulations and continued at intervals of three months for six months after production.

Keywords: growth inhibition, fusarium oxysporum, nanoformulation

Naraghi L, Negahban M, Heydari A, Razavi M, Afshari-Azad H (2018) Growth Inhibition of Fusarium oxysporum f. sp. lycopercisi, the Causal Agent of Tomato Fusarium Wilt Disease by Nanoformulations Containing Talaromyces Flavus. Ekoloji 27(106): 103-112.

INTRODUCTION

In the studies conducted in Iran, the satisfactory effects of Talaromyces flavus antagonist fungus on controlling some of the most important pathogens such as Verticillium dahliae, Verticillium albo-atrum, Fusarium oxysporum and Rhizoctonia solani in several crops including cotton, sugar beet, potatoes, tomatoes and greenhouse cucumber have been proven. Also, application of proliferated form of this fungus in farms in solid fermentation on plant debris or their mixture with pit soil reduced the incidence of disease and increased yield in the mentioned products; so that in cotton plant, 50 % reduction in the verticillin wilt disease, 37% reduction in the seedling death and 30% increase in yield; in potato, 40% reduction in disease and 17% increase in yield (Naraghi et al. 2014) ; In sugar beet, 93% increase in healthy seedlings and 50% increase in yield; in tomato plants, 27% decrease in disease severity and 23% increase in yields Farhang Niy et al. (2015) and in greenhouse cucumber, 30% reduction in disease severity and 7% increase in yield were obtained. Since in the mass production and commercialization of biological agents, marketing and attracting the attention of relevant consumers are important issues (Alimi et al. 2009, Husen et al. 2006, Kaewchai et al. 2009), the commercialization of the biological agent T. flavus and the importance of producing its various bioformulations, including nanoformulations seemed necessary. In recent decades, nanotechnology have shown dramatic developments in in various fields of pharmaceutical chemistry, medicine, and chemical pesticides. The issue that necessitates

 Received: 02 Jun 2018 / Accepted: 3 Sep2018

research and development in the field of nanotechnology is the pest resistance to pesticides; therefore, the introduction of nanopesticides to researchers will boost research and development in this relatively new field. Considering the environmental problems and costs associated with the consumption of large quantities of pesticides, as well as the problems caused by the resistance of pests to these pesticides, research and development in the field of nano pesticides can be considered as a necessity.

The use of biodegradable polymers in producing nano-emulsions and high performance nanocapsules made of natural and biodegradable materials can be an effective step in this field. In order to increase efficiency and reduce environmental hazards, encapsulated formulation is the best option (Maji et al. 2014). Therefore, the production of bio-formulation in nano and micro scales provides a controlled ability, increased strength and stability, and protects the active ingredient in adverse environmental conditions such as light and moisture. Also, the application of nano-encapsulated formulations can help reduce pesticide and economic efficiency, protect the environment and reduce its environmental hazards, as well as increased product exports. Nanoparticles have a larger surface to volume ratio than microparticles, which increases their active level and causes their controlled release. Also, the other advantages of nano size of particles are that these compounds don't stimulate the human and animal body and quickly expel from the body (Guan et al. 2008).

Nanocapsul technology that contains nano-sized nanoscale fungicides or pesticides molecules is one of the methods of producing pesticide formulations, which eliminates pests more easily and quickly (Guan et al. 2008). An emulsion is a heterogeneous system composed of two non-mixing liquids, one of which is dispersed in droplets in another. Emulsions with nanoscale droplet size and usually in the range of 20 to 200 nm are called nanoemulsion. The unique structure and properties of nano-emulsions have created many advantages in many industries compared with conventional emulsions. Some of the applications of nanoemulsion systems in different industries are their role in encapsulating and controlling the release of functional compounds, such as essential oils, vitamins, etc.

LITERATURE REVIEW

Recently, the tendency to produce nanoparticles that are more biodegradable and have high efficiency has attracted enormous interest. Therefore, the use of biodegradable polymers in the production of high-performance nano-emulsions and nano-capsules made of natural and biodegradable materials such as essential oil of medicinal plants can be an effective step in this field. The use of nano pesticides, production, application and their environmental considerations indicate that research and development can be effective in reducing pest resistance phenomena to pesticides. The nano formulations prepared by microorganisms, such as bacteria and fungi, are used in the biological struggle against insects and facilitate the entry of agents to insect's body. The formulation of the primus nano-insecticide makes the formulation well-kept in the dark and more stable. The nanoformulation of plants extracts had more than five times stronger effect against pests compared to conventional pesticides. The formulation of nano-permethrin by evaporation of oil solvent in water showed a better effect on controlling the of the pipiens larvae (Anjali et al. 2010). Killing effects of Bacillus thuringiensis using nanoparticles of chitosan polymer on Anopheles mosquito larvae have been investigated. Also, using dextrose and gelatin biopesticide containing Beauveria, Metharhisium and Paecilomyces against Hypothenemus hampei was prepared. Also from the Beauveria bassiana fungus, the formulations containing silver nanoparticles were used as larvicide. A study on the disinfecting effects of barley and sunflower seeds with silver nanoparticles containing fungicides on mycorrhizal symbiosis showed that, in comparison with conventional fungicide, in seed treatment with nanofungicide, a significant increase in the absorption of mineral elements by the root and consequently a significant increase in vegetative traits have been observed. Coating materials for encapsulation include gum, starch, gelatin and polymers. Recently chitosans and phospholipids are also used. According to recent research, the use of nano encapsulation techniques and their components in controlling storage pests can play an important role in increasing their efficiency and durability.

A large number of insecticidal plant compounds are highly evaporated and susceptible to degradation. Loading the plant compounds into nanoparticles leads to their under control release, delay in degradation and evaporation. There are limited articles about the effect of nano emulsion insecticides and nanocapsules loaded with pesticide-based compounds against insect pests (Margulis-Goshen and Magdassi 2013). There are several methods for the production of nanoparticles or nano-capsules, including the polymerization method, which is the fastest method and the continuous aqueous

phase mixes with organic phase. It is also coacervation method is ion gel formation using biodegradable hydrophilic polymers such as chitosan, gelatin, sodium alginate, which is in fact a mixture of two aqueous phase that is in a polymeric phase such as chitosan and sodium alginate, and in this method positive charge in the polymer integrate with a negative charge of crosse linkers, such as sodium triphosphate or calcium chloride, and form capsules Ebrahimnejad et al. (2011) Until now, there have been two reports in the field of medicine and agriculture for the preparation of the nanofugicide (Penicillium) (Khan and Jameel 2016). In the medicine, for the preparation of nanofugicide against the pathogen fungus of Candida albicans, the fungus of Penicillium fellutanum has been used. In the agriculture, for the preparation of nanofugicide against some plant pathogens, the extract of the fungus Talaromyces flavus (Penicillium dangeardii telomorph) has been used.

In the last 20 years, there have been significant reports on the preparation of bioformulations containing antagonistic fungi using solid and liquid fermentation and their optimization in various stages of production (Budge and Whipps 2001, Caramez et al. 2012, Kakvan et al. 2013, Pascual et al. 1999). Pascual et al. (1999), for example, succeeded in producing solid bioformulations by Epicoccum nigrum fungi on wheat, and after analyzing alcoholic solutions containing glycerol, mannitol and arbitol on sporulation of this fungus, the most significant increase was reported by glycerol. Also, Sargin et al. (2013) compared with the different methods of drying methods to increase the efficiency of the bioformulation containing Trichoderma harzianum EGE-K38. Results of a study have shown that the use of compounds containing minerals such as manganese, iron, zinc and phosphorus in the production of biological formulations containing antagonistic fungi has increased their stability. So far, outside of Iran bioformulations such as Ketomium containing Chaetomium globosum and Ch. Cupreum, Promote containing T. harzianum and T. Virus, Soil Gard containing Gliocladium virens; Trichodex containing T. harzianum, Pisolithus tinctonus and Glomus intraradices, Trichodermin containing T. harzianum and Prolust WG containing Talaromyces flavus have been commercially registered.

In Iran, the results of greenhouse experiments in the field of biological control with potato, tomatoes and caviar verticillium wilt disease, caused by V. albo-atrum by bioformulations containing various T. flavus isolates indicated that these isolates were significantly effective in reducing the disease index and increasing vegetative traits such as root length, crown length, height, wet weight and dry weight of these plants. Also, farm studies were conducted on the possibility of biological control of verticillium wilt disease and cotton seedling death, sugar beet seedling death, potato verticillium wilt, tomato and greenhouse cucumber Fusarium wilt using T. flavus bioformulation; and the results showed that the use of this bioformulation, in addition to the significant reduction of the disease index, has also led to a significant increase in yield (Farhang Niya et al. 2015).

MATERIALS AND METHODS

Laboratory Tests

Production of nanocapsulated bioformulation containing Thalaromyces flavus fungus

The production of nanocapsule was done through a combination of the polymerization and networking process by making changes consistent with the growth conditions of the fungi (changes in the amount or type of polymer, surfactants and oils, fatty acid and the rate of stirring and temperature). In the polymerization process, the organic phase was consisted of vegetable oil with a mixture of fungi added in a water phase including hydrophilic polymer monomers such as a mixture of one of two polymers of ureaformaldehyde or alginate, starch and chitosan. Subsequently, in the mixture of two phases, the cross linkers such as calcium chloride, as well as surfactants and the related materials, and fatty acid oils were added, and the homogenization was done at 35 ° C in a homogenizer with stirring rate of 5,000 to 10,000 rpm. Finally, cross-linked polymer particles formed in the form of a capsule around the particles of the fungus.

Production of nano-eomulsion bioformulation containing the Talaromyces flavus fungus

Self-assemblage pattern was used to prepare nano-emulsion formulations containing T. flavus fungus. The final formulation was a nano emulsion which in, vegetable oil hydrophobic nano-particles are formulated in a biocompatible formulation of. The composition of this formulation was: the active ingredient of the fungus and vegetable oil, such as castor oil, tween surfactant, carboxymethylcellulose viscous material, coconut moisturizer, ethanol amide fatty acid, stabilizing agent such as poly vinyl alcohol and linker such as calcium chloride and biocompatible polymers such as ethylene glycol, starch and carrageenan. First, a homogeneous solution of biocompatible polymers was prepared, then surfactants such as tween and the related materials were added to the solution and a completely homogeneous

mixture of polymer and solvent was prepared with a homogenizer in 2000 to 12000 rpm at 25 ° C. Then, suspensions containing spores of biological fungus along with fatty acids of castor oil and coconut were added by dropping. In the next step, crosslinker (calcium chloride) was added to a total of two phases to form the nanoparticles around the spores of the biological fungus. Finally, the nanoparticles were coated by spores of the biological fungus.

Production of nanopowder bioformulation containing Thalaromyces flavus fungi

In powdered nanoformulations, a suspension containing biological fungal spores in the aqueous phase including maltodextrine, xanthan gum, fatty acids, ethanol amide and oleic acid was spread out and after placing in a homogenizer with stirring rate from 2000 to 12000 rpm at 25 ° C was completely powdered.

Preparation of T. flavus bioformulation based on former technical knowledge

To prepare solid formulation, an effective isolate of T. flavus from the collection of fungi in the useful microorganism research laboratory and a modified method by Naraghi et al. (2010) was used. Some rice bran was soaked in water (30-35°C) for 24 hours, then spread out on large filter papers and dried out. In the next step, 250 grams of rice bran were sterilized in cellophane bags in autoclave (1.5 atm, 121 ° C for 20 min). In the next step, a suspension containing 40 ml sterile distilled water and six 0.5 cm pieces of 10-day culture medium of T. flavus isolate was added to into cellophane bags. Then, one of the stabilizing compounds, such as dicycloserin or sodium nitrate, was added based on the amount of addition of the supplements to the culture media (10 ml of supplement solution with concentration of 20 g / l for 250 g of each bed) (Engelelkes et al. 1997). For the growth of T. flavus isolate, the cellophane bag was placed in the 30°C incubator for 1.5 to 2 months, and during this period, if the contents were dried, 20 ml of distilled water was added to make moisture. After this time, the contents of the cellophane bag spread out on filter paper to dry and were used as bioformulation based on former technical knowledge.

Comparison of ascospores production process (oscillation ability) in various formulations of T. flavus (new nanoformulations and former bioformulation)

Comparison of ascaspor production process was done in various formulations of T. flavus for six months. This comparison began three months after production, and continued up to six months after with 3 months intervals. For this purpose, from each of the prepared T. flavus formulations, a dilution of 1:1000 was prepared and 0.1 mL of this dilution was expanded on the homocytometer slide and the number of spores was counted. One gram of each formulation was then reached to a volume of 10 ml and, after preparing a suspension with dilution of 1:1000, the number of spores per milliliter of the suspension was calculated by homocytometer. This study was conducted in a completely randomized design with five treatments (four new nanoformulations and one bioformulation based on former technical knowledge) in three replicates. Data analysis and comparison of mean spores per gram of formulation was done with Duncan's multiple range test by MS TAT C software.

Comparison of active population (Sustainability) in different formulations of T. flavus (New nanoformulations and former bioformulation)

Investigation of active population in different formulations of T. flavus for 1.5 years will be done. This comparison began three months after production, and continued up to six months after with 3 months intervals. For this purpose, from each of the prepared T. flavus formulation, a suspension of 10 and 100 spores per milliliter was prepared and one milliliter of each of the concentrations was spread out on plates containing Potato Dextrose Agar (PDA) medium. After observation of spores in the media containing both concentrations, the number of colonies was counted in a concentration that was easier. After calculating the percentage of active spores, the active population in each formulation was estimated by multiplying the percentage of active spores in the number of spores per gram of formulation obtained from the previous stage. This study was conducted in a completely randomized design with five treatments (four new nanoformulations and one bioformulation based on former technical knowledge) in three replications. Data analysis and comparison of mean number of spores per gram of formulation was performed with Duncan's multiple range test by MS TAT C software.

Comparison of the efficiency of various T. flavus nanoformulations to inhibit the growth of Fusarium oxysporum f. sp Lycopersici (FOL), the cause of tomato fusarium wilt disease

This comparison began three months after production, and continued up to six months after with 3 months intervals. For analysis of each nanoformulation, a plate containing PDA was divided into half with a hypothetical line, in one half a 1mm piece of disease agent was placed on by Cork Borer and

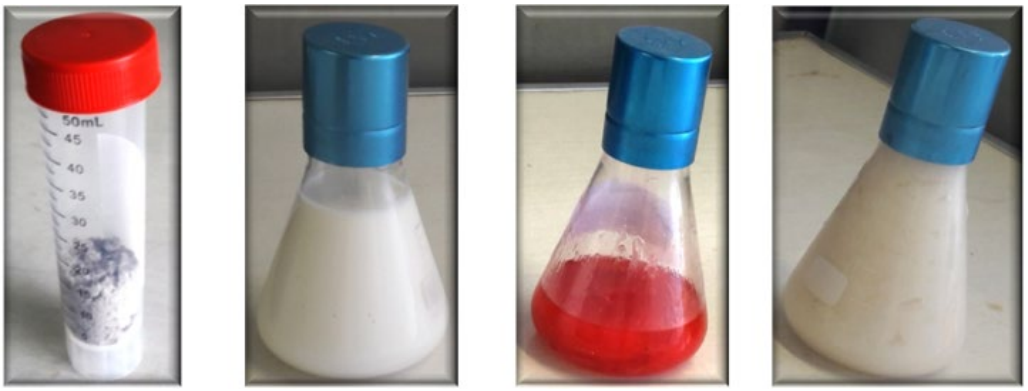

Fig. 1. Prepared nanoformulations: Right to left (nanocapsule 1 (F1), nanoemulsion (F2), nanocapsule 2 (F3) and nano powder (F4)

Table 1. Nanoformulations and the amount of compounds used per 100 g of each nanoformulation

nanocapsule 1 (F1)		nanoemulsion (F2)		nanocapsule 2 (F3)		nano powder (F4)	
Compound	**Amount (g)**	**Compound**	**Amount (g)**	**Compound**	**Amount (g)**	**Compound**	**Amount (g)**
Biological fungus*	19	Biological fungus*	35	Biological fungus*	53	Biological fungus*	5
Alginate 1%	8	Lauryl alcohol	17.5	Urea	9	Maltodextrin	41.5
Castor oil	16	Castor oil	9	Castor oil	4.5	Starch	33.5
Coconut fatty acid	32	Coconut fatty acid	17.5	Coconut fatty acid	4.5	Fatty acid (Ethanol amid, Oleic acid)	8.5
Sodium chloride 0.1%	2	Sodium chloride 0.1%	2	Sodium chloride 0.1%	2	Xanthan Gum	8.5
		Surfactant (tween)	4	Surfactant (tween)	5.5		
Polyethylene glycole	20	Polyethylene glycole	12	Formaldehyde	18.5	-	-
Butanol **	3	Butanol	3	Butanol	3	Butanol	3

* For the preparation of biological fungi, suspension of Talaromyces flavus fungus at a concentration of 109 spores / ml was used.
** Due to the preparation of nanoformulation in non-sterile conditions, in order to prevent bacterial and fungal contamination, butanol was used to investigate the efficiency of nanoformulations in inhibiting the growth.

in the other half, 0.1 g of the nanoframulation was placed. For each of the agents of aforementioned diseases, a separate study was conducted in a completely randomized design with five treatments (four new nanoformulation and one control) in three replications. For the control plate, only in one half of the plate the agent of the disease was placed, and in the other half, the determined amount of nanoformulation was not placed. To determine the inhibitory percentage of each agent of the disease, seven days after the presence of the disease agent and formulation on the plate, the comparison of colony diameter of the disease agent in treatment and control sample was done. Based on the following formula, the growth inhibitory percentage of the colony of disease agent by nanoformulation was calculated. Inhibitory percentage =(diameter of the colony of disease agent in the control - diameter of the colony of disease agent in treatment containing nanoformulation)/ diameter of the colony of disease agent in the control Data analysis and comparison of growth inhibitory percentages of disease agent by nanoformulations was done with Duncan's multiple range test by MS TAT C software. Up to this stage of the study, the effectiveness of various nanoformulations in inhibiting the growth of some pathogenic agents of plants up to six months after the production was carried out. In this study, pathogenic agents of Verticillium dahlia, Fusarium oxysporum f. sp lycopercisi and Fusarium oxysporum f. sp the cucumerinum from the collection of the useful microorganism research laboratory at the National Institute of Plant Protection, Iran, which their pathogenesis was already proved, were used. Also, it should be noted that in this study, it was not possible to determine the efficacy of former bioformulation in inhibiting the growth of pathogenic agents. The main bed in the former bioformulation of T. flavus is the rice bran, which, upon placing on a medium in the plate, the bran particles containing the fungus was spread on the medium and very quickly filled the entire surface of the medium without any growth permits to each of the aforementioned pathogens. So measuring the colony diameter of the disease agent and calculating the growth inhibitory percentage of the disease agent disease by this formulation was not possible. Therefore, a comparison of the efficiency of various nanoformulations with bioformulation prepared based on former technical knowledge on controlling some important plant diseases in greenhouse conditions was performed.

RESULTS

Quantitative and qualitative introduction of compounds used in 100 g of nanoformulations prepared. In this research: four nanoformulations including two types of nanocapsules, one nanoemulsion, and one nanopowder were made (**Fig. 1**), which all of compounds used in 100 g, qualitatively and quantitatively are listed in **Table 1**.

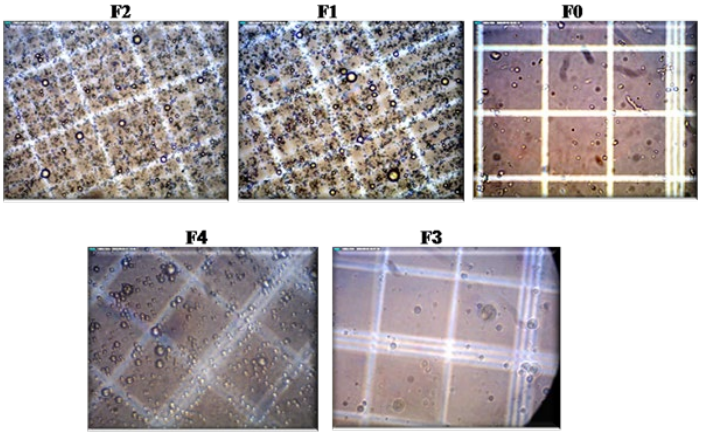

Fig. 2. Sporulation of various formulations in the first trimester after production: F0 (former formulation), F1 (nanocapsule 1), F2 (nanomulsion), F3 (nanocapsule 2) and F4 (nanopowder)

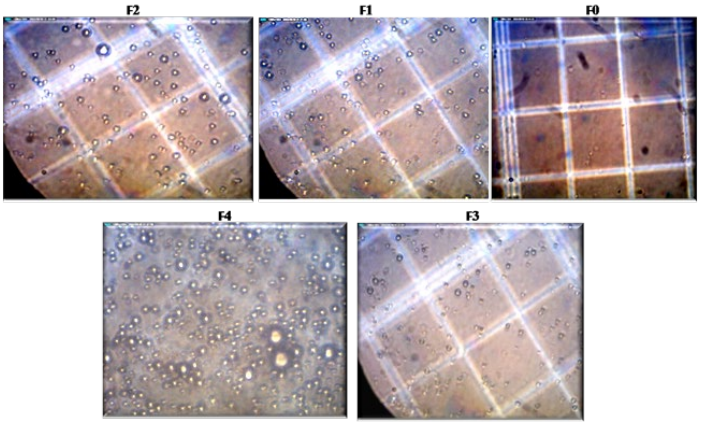

Fig. 3. Sporulation of various formulations in the second trimester after production: F0 (former formulation), F1 (nanocapsule 1), F2 (nanomulsion), F3 (nanocapsule 2) and F4 (nanopowder)

Table 2. Comparison of the average sporulation content of various formulations of Talaromyces flavus (new formulations based on nanotechnology and rice bran formulation based on former technical knowledge) (Log)

Formulation	Average sporulation content per gram			
	First trimester		Second trimester	
	Number of spores per gram (Log)	Number of spores per gram ($\times 10^9$)	Number of spores per gram (Log)	Number of spores per gram ($\times 10^9$)
F0 (Former biorformulation - rice bran)	9.17b*	1.50	9.25c*	1.80
F1 (Nano-capsule 1 polyalginate)	10.00a	10.00	10.00a	10.00
F2 (Nano-emulsion-polyethylene glycol)	10.00a	10.00	10.00a	10.00
F3 (Nanocapsule 2- Urea formaldehyde)	9.00c	1.00	9.47bc	3.00
F4 (Nano Powder - Maltodextrin, Xanthan gum)	8.00d	0.10	9.69ab	5.00

* There is no statistically significant difference between the means with the same letters in the 1% probability level.

Comparison of Process of Ascospore Production (Oscillation Ability) in Different Formulations of T. flavus (New Nanoformulations and Former Bioformulation)

In the first and second trimester after the production, study of sporulation in various formulations (**Figs. 2** and **3**) and the calculation of spores in one gram of each formulation showed that this amount was without change from the first trimester to the second trimester in nanocapsule formulation 1 and nanoemulsion, while in other formulations, especially in nanopowder, it increased (**Table 2**). The sporulation study in different formulations in the first trimester and second trimester after production was significant at the 1% probability level. In the first trimester after the production, the comparison of the average number of spores per gram of each formulation showed that the formulations were in four statistical groups, and the most effective ones in terms of the amount of spore were nanocapsule and nanoemulsion (**Table 2**). However, in the second trimester after the production, comparison of the average number of spores per gram of each formulation showed that formulations were in two statistical groups, and the most effective ones in terms of the amount of sporulation were nanocapsule 1, nanoemulsion and nano-powder (**Table 2**).

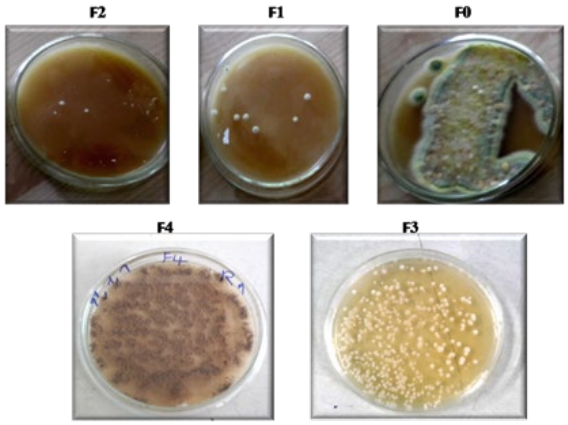

Fig. 4. Spores of Talaromyces flavus at a concentration of 100 spores/mL of different formulations on the PDA medium in the first trimester after production: F0 (Perevious formulation)
F1 (nanocapsule 1), F2 (nanoemulsion), F3 (nanocapsule 2) and F4 (nanopowder)

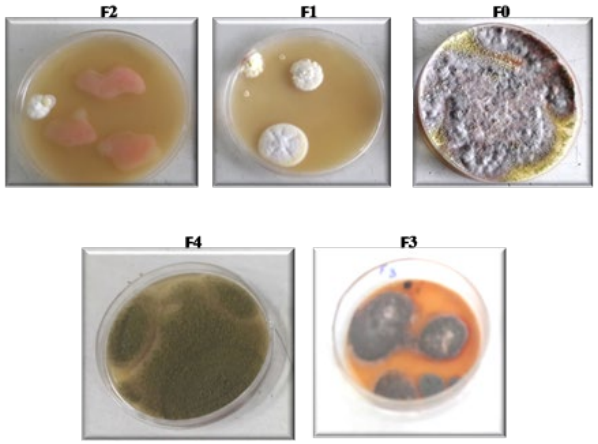

Fig. 5. Spores of Talaromyces flavus at a concentration of 10 spores/mL of different formulations on the PDA medium in the first trimester after production: F0 (former formulation)
F1 (nanocapsule 1), F2 (nanoemulsion), F3 (nanocapsule 2) and F4 (nanopowder)

Table 3. Comparison of the average active population per gram of different formulations of Talaromyces flavus (new formulations based on nanotechnology and rice bran formulation based on former technical knowledge) (Log)

Formulation	Average active population per gram			
	First trimester		Second trimester	
	Active population per gram (Log)	Active population per gram ($\times 10^9$)	Active population per gram (Log)	Active population per gram ($\times 10^9$)
F0 (former biorformulation - rice bran)	9.17a*	1.50	9.25c*	1.80
F1 (Nano-capsule 1 polyalginate)	8.95a	0.90	9.47b	3.00
F2 (Nano-emulsion-polyethylene glycol)	8.47b	0.3	9.30bc	2.00
F3 (Nanocapsule 2- Urea formaldehyde)	9.00a	1.00	9.07d	1.20
F4 (Nano Powder - Maltodextrin, Xanthan gum)	8.00c	0.10	9.69a	5.00

* There is no statistically significant difference between the means with the same letters in the 1% probability level.

Comparison of Active Population (Stability) in Different Formulations of T. flavus (New Nanoformulations and Former Bioformulation)

In the first and second trimesters after production, evaluation of the percentage of spores of **T. flavus** in different formulations (**Figs. 4** and **5**) and the calculation of the active population in one gram of each formulation showed that the amount of spores increased from the first trimester to the second trimester in different formulations (**Table 3**). Stability evaluation test in different formulation in the first and second trimester after production was significant at 1% probability level. In the first trimester after production, the comparison of active population per gram of each formulation showed that the formulations were in three statistical groups, and the most effective ones in terms of active population were former bioformulation, nanocapsele 1 and the nanocapsule 2 (**Table 3**). However, in the second trimester after the production, the comparison of the average of active population per gram of each formulation showed that the formulations were in four statistical groups and the most effective one in terms of active population was nano powder (**Table 3**).

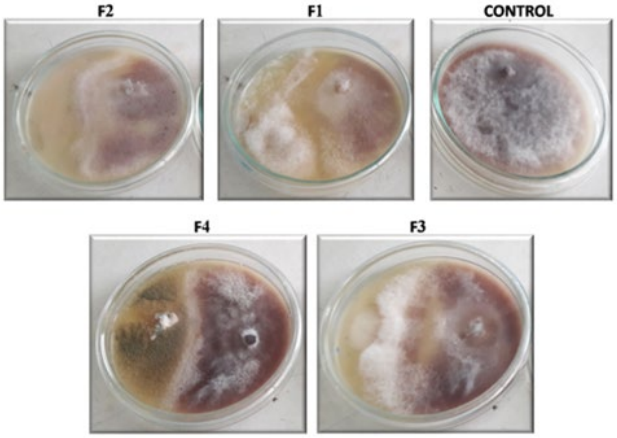

Fig. 6. Growth inhibition of Fusarium oxysporum f. sp Lycopersici colony by different nanoparticles on PDA medium in the first trimester after production: CONTROL, F1 (Nanocapsule treatment 1), F2 (Nanoemulsion treatment), F3 (Nanocapsule treatment 2) and F4 (Nanopowder treatment)

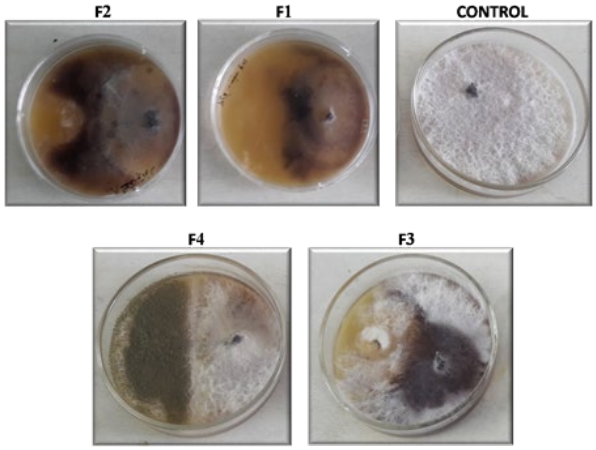

Fig. 7. Growth inhibition of Fusarium oxysporum f. sp Lycopersici colony by different nanoparticles on PDA medium in the first trimester after production: CONTROL, F1 (Nanocapsule treatment 1), F2 (Nanoemulsion treatment), F3 (Nanocapsule treatment 2) and F4 (Nanopowder treatment)

Table 4. Comparison of the averages of growth inhibition of colony of Fusarium oxysporum f. sp Lycopersici (FOL) in treatments containing different nanoformulations

Formulation	Growth inhibition of colony of FOL (%)	
	First trimester	Second trimester
F1 (Nano-capsule 1 polyalginate)	31.25a	10.0b
F2 (Nano-emulsion-polyethylene glycol)	26.12ab	13.33b
F3 (Nanocapsule 2- Urea formaldehyde)	20.00b	14.00b
F4 (Nano Powder - Maltodextrin, Xanthan gum)	27.77a	21.42a

***** There is no statistically significant difference between the means with the same letters in the 1% probability level

Comparison of the Efficiency of Different T. flavus Nanoformulations to Inhibit the Growth of Fusarium oxysporum f. sp Lycopersici (FOL), the Cause of Tomato Fusarium Wilt Disease

In the first and second trimester after production, the study of the efficiency of nanoformulations to inhibit the growth of the FOL colonies (**Figs. 6** and **7**) showed that the inhibitory rate decreased from the first trimester to the second trimester in all of nanoformulations (**Table 4**). The efficiency of nanoformulations to inhibit the growth of the FOL colonies in the first and second trimester after production was significant at 1% probability level. In the first trimester after production, the comparison of the inhibitory of growth of FOL colonies by each formulation showed that the formulations were in two statistical groups and the most effective ones in terms of inhibitory effect were nanocapsule, nanoemulsion and nanopowder (**Table 4**). In the second trimester, the comparison of the inhibitory of growth of FOL by each formulation also showed that formulation were in two statistical groups, and the most effective nanoformulation was the nanopowder in terms of efficiency to inhibit the growth of FOL colony (**Table 4**).

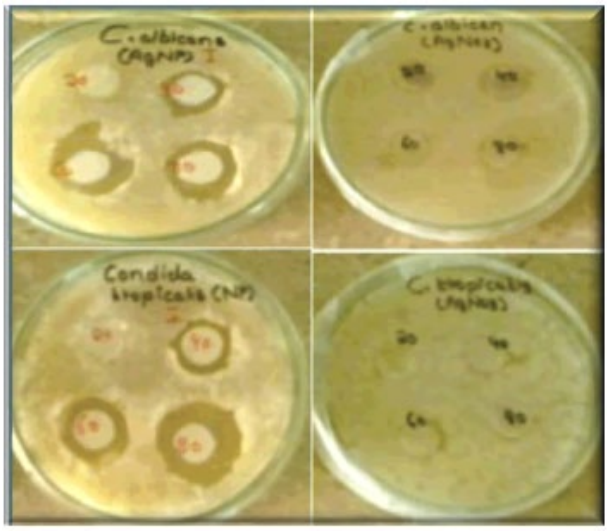

Fig. 8. Candida albicans growth inhibitory halos by pills containing penicillium loweratanum nanoformulations (Khan, Jameel 2016)

DISCUSSION

In this part of the study, it was shown that in different T. flavus nanoformulations, prepared as capsule, emulsion and powder, during the six months after production the amount of spores and the active population of the fungus was higher than 10^8 grams per gram of formulation. According to previous researches, the Fungal or bacterial CFU have been reported in a biological product between 10^7 and 10^9 (Hammoudi et al. 2012, Johnson and Dileone 1999). Also, the results obtained from the inhibitory effect of nanoformulations on the growth of some plant pathogens in the present study, were in consistent with the results of the research by Khan and Jameel (2016) on the inhibitory effect of nanoformulation containing Penicillium fallutanum on Candida albicans. As the halos associated with the lack of growth of pathogenic fungus around the nano-formulation tablets, in laboratory conditions have been shown in this study (**Fig. 8**).

ACKNOWLEDFGEMENTS

The authors would like to thank the support of Iranian national science foundation (INSF) for financial support for the implementation of this research.

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