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A mycoinsecticide from Metarhizium anisopliae (Ascomycota: Hypocreales) based on a solid-state fermentation method against some aphid species in Türkiye

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Abstract

The research aims to propose an appropriate and efficient mycoinsecticide from a local *Metarhizium anisopliae* (Ascomycota: Hypocreales) strain (KTU-51) against several aphid species (Hemiptera: Aphididae; *Myzus persicae* Sulz., *Aphis fabae* Scop., *Brevicoryne brassicae* L. and *Macrosiphum rosae* L.), which are the most important agricultural pests. *M. anisopliae* produced spores in large quantities utilizing solid-state fermentation (SSF) with rice as a substrate. An oil-based mycoinsecticide called AFIDISIDAL-OD Met-TR61 was improved by incorporating spores harvested from the sporulated biomass. The product provided a more deathful effect than commercial products against all the aphis species under laboratory conditions. *Met*-TR61 with 10^8 spores/ml concentration yielded 78.3% mortality in the leaf disc experiment and 79% in the pot experiment on *Myzus persicae*. Other aphids were also found to be extremely sensitive to the product. In this particular study, an oil-based mycoinsecticide was developed and its efficacy for biological control of aphid species was assessed. The study's findings indicate that the mycoinsecticide has the potential to be a workable and effective alternative to conventional chemical insecticides for controlling aphid populations.

Introduction

Aphids (Hemiptera: Aphididae) are among the most important pest groups in agriculture and horticulture all over the world. They both cause direct damage by absorbing plant sap and are effective in spreading plant pathogens from one plant to another. By causing the formation of honeydew deposits on the surface of the plant leaves, they also cause the photosynthetic surface of the leaves to decrease (Blackman and Eastop 2007; Hogenhout et al. 2008). It is known that 15 aphid species are very important pests for agricultural areas worldwide (Van Emden and Harrington 2017). Among these species, is *Myzus persicae* Sulz. and *Aphis fabae* Scop. are emphasized as essential pests. *Brevicoryne brassicae* L., which primarily feeds on the above-ground parts of its host plants, has been known to cause significant direct and indirect damage, ultimately resulting in production losses ranging from 35–80%, according to Ramanujam et al. (2017). The rose aphid, *Macrosiphum rosae* L., is an important aphid species that infests and damages roses (Karlik and Tjosvold 2003).

According to Barbosa (1998), a variety of pest management strategies have been used including agricultural practices, mechanical, physical, chemical and biological control. Aphids are primarily managed by insecticides at the moment (Foster et al. 2007). The uncontrolled use of insecticides has resulted in issues such as pesticide resistance, pest comeback, negative impacts on biocontrol agents, environmental contamination, and hazardous residue buildup in the natural ecosystem (Silva et al. 2012). Biological control is an alternative method of pest control. Aphids have many natural enemies that can be used for biological control, such as predators, parasites, and pathogens (Bloemhard and Ramakers 2008).

In recent years, a large number of studies have been conducted on the effectiveness of microbial pathogens including viruses, bacteria, and fungi an important agricultural pests (Ince et al. 2007; Secil et al. 2012; Sevim et al. 2012; Kocacevik et al. 2015; Eski et al. 2018; Gencer et al. 2020, Usta 2022). Many biological control products developed from these microorganisms are used as pesticides in integrated pest management (IPM) programs against various pests, including aphids (Vega et al. 2008). Nearly 1000 entomopathogenic fungi, known to be effective in maintaining the natural balance of insect populations,

have been reported (Sandhu et al. 2012). A significant number of these species appear to have a high potential to be developed as mycoinsecticide and mycoacaricide and used in the biological control of pests (de Faria and Wraight 2007; Khan et al. 2012). In recent studies, entomopathogenic fungi (EPF) have been shown to be among the best biocontrol agents for aphids. Especially entomopathogenic fungal pesticides have critical importance in IPM applications because these organisms cause diseases in insects and suppress their growth and rate of multiplication (Thomas and Read 2007). They have considerable advantages compared to conventional pesticides, notably cost-effectiveness, high yield, no negative impacts on beneficial organisms, no chemical residues in the environment, and increasing biodiversity in ecosystems (Vega et al. 2009; Lacey 2016). The most common species used against aphids are *Lecanicillium* spp., *Beauveria* spp., *Metarhizium* spp., and *Isaria fumosorosea* (formerly *Paecylomyces fumosoroseus*), which belong to the order Hypocreales of the division Ascomycota (de Faria and Wraight 2007; Khan et al. 2012). Various entomopathogenic fungi, such as, *Metarhizium anisopliae*, *Beauveria bassiana* and others, have been reported as substantially control agents of several aphid species such as *Myzus persicae* (Sulzer), *Aphis gossypii* (Glover), *Melanocallis caryaefoliae* (Davis), *Aulacorthum solani* (Kaltenbach) (Kim et al. 2007; Shapiro-Ilan et al. 2008; Mohammed et al. 2018).

Under unfavorable climatic circumstances, formulations can greatly enhance the field efficacy of entomopathogenic fungal products while also protecting their activity and persistence (Jackson et al. 2010). The approach recognized for avoiding abiotic factors is an oil-based formulation so that they can prevent conidia from imbibitional damage, the harmful impact of Uv irradiation, or chemical pesticides (Alves et al. 1998; Lopes et al. 2011). As a result, using oil formulations containing EPFs may enhance conidial stability and prolong their durability in the field, protecting fungi against heat stress, water loss, and, in importantly, UV radiation (Barreto et al. 2016). EPFs differ from other insect pathogens since they can infect through the hosts' integument. EPF infections are not limited to chewing insects, as they are not required to be ingested or eaten. They are unique in controlling insect pests that feed by sucking plant or animal juices (Lacey and Goettel 1995; Biryol et al. 2021; Eski et al. 2022). Despite all of the control methods, strategies and agents, the aphid species continue to be a major agricultural and horticultural pest, and their harm is gradually increasing. Thus, the objective of this study is to develop a local mycoinsecticide from *Metarhizum anisopliae* (KTU-51) that originated from soil samples against the different aphid species in Türkiye.

Materials and Methods

Providing and rearing the aphids

Myzus persicae colonies were obtained from the Central Plant Protection Research Institute (Ankara, Türkiye) and were maintained on pepper seedlings (*Capsicum annuum*). Pepper seedlings were grown in pots. Growing conditions and temperatures of 25 ± 2°C (L) and 18 ± 2°C (D) were maintained in a 65% RH plant growth chamber (Biryol et al. 2022).

Aphis fabae, *Brevicoryne brassicae* and *Macrosiphum rosae* were collected from infested areas in Trabzon, Türkiye and cultured on bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea capitata*), and rose (*Rosa* sp.) plant under laboratory conditions, respectively. These aphids were maintained on bean leaves, cabbage

leaves, and fresh rose shoots, respectively. Experiments were performed on the same materials. The aphid culture was maintained until all experiments were completed.

Fungal cultures

Metarhizium anisopliae (KTU-51) was obtained from the entomopathogenic culture collection of Karadeniz Technical University, Faculty of Science, Department of Biology, Microbiology Laboratory. Previously, Biryol et al. (2022) revealed that this isolate had an effect of over 80% on *M. persicae*. Considering its effectiveness, KTU-51 has the potential to be developed as a mycoinsecticide and used in the biological control of aphids.

Spore suspensions

To maintain stock cultures, sterile cryovials containing 10% glycerol (Sigma-Aldrich) in sterile 0.01% Tween 80 solution were utilized and stored at -80°C until they were required for experiments. A 10-day incubation period on Sabouraud dextrose agar with yeast (SDAY) at 25 ± 2°C was used to cultivate fungal cultures and promote sporulation. Conidia were harvested by gently scraping and suspending them in a 0.01% Tween 80 solution. The spore concentration was established and adjusted for test concentrations by filtering the conidial suspension with sterile muslin and homogenizing it by vortexing for 2 min. The conidium viability test was performed according to the method of Hywell-Jones and Gillespie (1990). After 24 hours, the ratio of germinated spores on each plate was evaluated using a microscope, with positive germination defined as germ tube length that is at least half the spore length. All isolates exhibited a viability rate of more than 90%.

Development of the SSF

The mycoinsecticide was developed as an oil formulation using KTU-51 obtained from a solid substrate. The strain was first cultivated in a liquid medium and later transferred to a solid substrate. Afterward, spores were collected from the PDA medium as described above. The spore suspension was counted with a Neubauer hemocytometer, and the concentration was adjusted to 2.2 x 10^6 conidia/ml. To produce blastospores, a spore suspension of 1 ml was added to a liquid medium (containing 30 g glucose, 25 g casein hydrolysate, 20 g yeast extract, 4 g potassium hydrogen phosphate (K2HPO4) and 10 mg gentamicin per liter) in a ratio of 1:10. The resulting mixture was placed in a 500 ml flask, with 150 ml volume, and incubated at 28°C and 150 rpm for a period of 4 days, as reported by Seema et al. (2013). The pH of the medium was maintained at 5.6. Following inoculation, the liquid culture comprising blastopores was centrifuged for one minute at 10,000 rpm, and the spores were dissolved in sterile distilled water. The concentration was determined to be $1 \times 10^7 - 5 \times 10^8$ conidia/ml

The rice was used as a SS (solid substrate) to provide both a source of carbon and energy. Semi-cooked rice removed from its starch was distributed in growth bags had air holes of 150 gr per bag and autoclaved at 121°C at 1.1 atm pressure for 40 minutes (Seema et al. 2013). After the rice bags had cooled to room temperature, a 7.5 ml suspension of blastopores was inoculated into each bag, and fungal spores were brought into contact with rice grains by massaging. For growth, they were kept in an incubator at 25°C for 20–30 days. For regular and stable fungal growth during the incubation, the fungus growth bags were massaged every 5 days, and the rice-fungus mixtures became homogeneous.

The rice was covered with fungal bodies placed in Kraft paper bags for 10 days to pre-dry. The fungus spores were then removed from the rice surface with a sieve (45 µm $mesh^{-1}$) and dried in a vacuum desiccator until the humidity level was less than 5%. A suspension of the sieved conidia was obtained by adding 1 g of conidia to 50 ml of sterile distilled water. A 100 µl of the decimal dilutions of the solution was inoculated into Sabouraud Dextrose Agar and spread with a glass spreader. The resulting CFUs were counted at 72 h post-inoculation at 28°C. The calculation was performed according to dilution with the appropriate number of colonies (30–300 colonies). Finally, spores were individually packaged in 100 ml glass bottles, and stored in a refrigerator with low humidity capacity at + 4°C until used in oil formulations (Fig. 1).

To prolong the shelf life of the fungi before application, an oil-based formulation was developed according to Jackson et al. (1997). The oil-based formulation was prepared as described by Nian et al. (2015) and Biryol et al (2021) with minor modifications. The formulation was prepared by mixing naphthalene-2-sulfonic acid (3%), lecithin (12%), silvet L-77 (1%), ascorbic acid (0.1%), sodium alginate (1%), and vegetable oil (40%) with 10 gr powder spores (10^{10} spore/g) to protect its physical and chemical properties, to extend its permanence and shelf life in nature. The mixture was then diluted to 100 ml using sterile distilled water and stirred gently (at 50 rpm) for 5 minutes at a temperature below 10°C until it became homogenous, which took approximately 30 minutes. A local new mycoinsecticide was named AFIDISIDAL-OD *Met*-TR61 (Biryol et al. 2021; 2022) (Fig. 1).

Insecticidal effect of the mycoinsecticide on Myzus persicae

An oil-based mycoinsecticide (AFISIDAL-OD *Met*-TR61), developed from *Metarhizium anisopliae* acting on *Myzus persicae*, was tested against the aphid using both leaf disc and pot assays. A concentration of 1 × 10^8 conidia/ml was applied by diluting the products at a 1:10 ratio. A commercial product (MET52) including *M. anisopliae* was used as a positive control. Untreated groups used as control were also created as the blank product, 0.01% Tween 80 without fungal spores.

The leaf disc experiment was conducted on pepper leaves on water agar in Petri dishes. A paintbrush transferred fifty *M. persicae* nymphs with five repetitions to the discs. The mycoinsecticide was applied to Petri dishes with a hand sprayer. Petri dishes were placed in Plexiglas cages (35 × 40 × 35 cm) and maintained at 25 ± 1°C, ≥ 65% RH, and a photoperiod of 12:12 (L:D) h. Mortality rates were recorded after 1, 3, 5, and 7 days post-infection. After surface-sterilizing cadavers with 0.2% sodium hypochlorite solution and washing them three times in sterile distilled water, fungal emergence and sporulation on the cadavers were observed by placing them in a moisture chamber to encourage sporulation outside the cadavers.

The pot experiment on *M. persicae* was carried out on healthy pepper seedlings according to Biryol et al. (2022). Selected seedlings were planted in pots with a 30 cm diameter and waited until the seedlings had at least seven leaves. Once the required growth was observed, the *M. persicae* nymphs were released into the pots, and populations were allowed to develop until there were about fifty per plant with five repetitions. The mycoinsecticide was sprayed on the seedlings as 1 ml with a concentration of 1 x 10^8 conidia/ ml. The pot experiment was conducted at 25 ± 1°C, 65 ± 5% relative humidity, and a light: dark cycle of 16:8 h in a climate

room. For 14 days, the application was monitored. Dead insects were recorded and tested for mycosis using a moisture cycle.

Host range of the mycoinsecticide

The leaf disc assay was used to determine the host distribution of the mycoinsecticide on three other aphids (*Aphis fabae*, *Brevicoryne brassicae* and *Macrosiphum rosae*) that caused significant economic losses on crops or plants. In the host range experiment, tests were conducted on bean leaves for *A. fabae*, cabbage leaves for *B. brassicae*, and rose leaves for *M. rosae* on water agar in Petri dishes. Fifty nymphs with five repetitions were transferred to the discs using a paintbrush. The mycoinsecticide was applied to Petri dishes with a hand sprayer. Experiments were carried out using conditions in leaf disc application. The mortality values of the insecticide on aphids and the mycosis rates of the cadavers were calculated at the end of the application.

Statistical analysis

Abbott's (1925) method was used to correct the mortality statistics, and the % mycosis results for the fungal bioassays were determined using mycelia growth outside the cadaver. To compare test isolates to each other and the control group in terms of mortality and mycosis (for the fungal bioassays), the results were submitted to ANOVA, followed by LSD multiple comparison tests ($p < 0.05$). SPSS 28.0 was used to compute all experiments, and GraphPad Prism was used to construct the drowning graph (version 6.0, GraphPad Software, USA).

Results

Laboratory and pot experiment of the Met-TR61 on M. persicae

The oil-based mycoinsecticide AFISIDAL-OD *Met*-TR61 was produced from powder spores containing 10^9 spores per ml as mycoinsecticides of *M. anisopliae* KTU-51 strain. The viability rate was determined to be 95%, fluid, and dark green in color (Fig. 1).

Insecticidal efficacy experiments showed that *Met*-TR61 had a significant lethal effect on *M. persicae* in both leaf disc and pot experiments ($p < 0.05$) (Table 1). In the leaf disc, it was determined that the deaths that started on the third day after the infection increased with the prolongation of the period (Fig. 2). *Met*-TR61 with 10^8 spores/ml concentration yielded 44.3, 66.3 and 78,3% mortality on 3rd, 5th and 7th post-infection, respectively ($F_{(6,24)}$) = 22.60, df = 3, $p < 0.05$). As a result of the humidity circle application, 92.6% of cadavers had mycosis ($p < 0.05$). The commercial product (MET52) produced lower mortality than *Met*-TR61 on the 3rd, 5th, and 7th days after infection when death numbers were recorded ($p < 0.05$, Table 1). Only 43.6% of the cadavers were found to have mycosis for commercial products ($p < 0.05$). Under the same conditions and time, the blank product genetated 19.6% of *M. persicae* ($p < 0.05$). Mortality rates in the control group were 17.6% of *M. persicae* after the 7th-day post-treatment ($p < 0.05$) (Fig. 2).

Table 1

Susceptibility of *Myzus persicae* nymphs exposed at 1 × 10^8 conidia/ml of *Met*-TR61 in leaf disc and pot experiment under laboratory conditions.

Characters	The leaf disc experiments				The pot experiments			
	Mortality% ±SE	CI 95%	Mycosis %	CI 95%	Mortality% ±SE	CI 95%	Mycosis %	CI 95%
Met-TR61	78.33 ± 1.15 ^{a}	73.36–83.29	92.66 ± 1.27 ^{x}	89.03-110.96	79.00 ± 1.52^{A}	72.42–85.57	81.33 ± 1.33 ^{m}	75.59–87.07
MET52	75.00 ± 2.88^{b}	62.57–87.42	43.66 ± 1.56 ^{y}	35.54–61.92	72.33 ± 1.52 ^{B}	65.75–78.90	76.33 ± 1.15 ^{n}	71.03–80.96
Blank product	19.69 ± 1.15 ^{c}	14.72–24.65	NG	NS	16.33 ± 1.15 ^{C}	11.36–21.29	NG	NS
Control	18.32 ± 1.76 ^{d}	10.73–25.91	NG	NS	10.66 ± 0.66 ^{D}	7.79–13.53	NG	NS

Note: Mortality% ± SE within a column followed by the upper case letter indicates the differences between mortality and lower case indicates the difference in the rate of mycosis after mortality ($P < 0.05$) Tukey's LSD test, $P \leq 0.05$). NS: Nonsignificant, NG: Non-growth fungus

The pot experiment results revealed that plant vitality and flowering status were high and aphid populations were low in the seedlings where the *Met*-TR61 was applied (Fig. 3). On the contrary, plant viability and flowering status were incredibly low in the control group and the blank product, the seedlings died, and the number of aphids increased gradually in the first 3 days post-infection. *Met*-TR61 was determined as the most effective oil-based formulation in the pots experiment with 79% mortality ($F_{(3,8)}$) = 807.51, df = 3, $p < 0.05$) (Table 1). As a positive control, while MET52 caused 72,33% insecticidal effects on the pest, the blank product showed 16.3% mortality ($p < 0.05$). It was determined that commercial products on *M. persicae* nymphs were similar to each other in terms of mortality ($p < 0.05$). Mean aphid mortality in control was determined at 10.6% ($p < 0.05$). No fungal infection and mycosis were detected on aphids used in the blank product and control ($p < 0,05$) (Table 1).

Effect of Met-TR on different aphid species

Met-TR also produced remarkably high mortality values on other aphid species used in the study ($F_{(3,8)}$) = 1.28, df = 3, $p < 0.05$). Mortality rates were calculated as 79% on *A. fabae*, 84% on *B. brassicae* and 82% on *M. rosae* with 10^8 conidia/ml concentration on the 7th-day post-infection ($F_{(3,11)}$ = 4.003, $p < 0.05$), and mycosis rates of the cadavers were found as 84.0, 90.6 and 85.0%, respectively. The mortality values of the commercial product (MET52) on the same aphid species were determined to be 69% and 75% ($p < 0.05$). Also, the blank product was between 18% and 20% under the same conditions and time ($p < 0.059$ (Table 2, Fig. 4).

Table 2

Susceptibility of different aphid nymphs exposed at 1×10^8 conidia ml^{-1} of *Met*-TR61

Characters	*Brevicoryne brassicae*		*Aphis fabae*		*Macrosiphum rosae*	
	Mortality% ±SE	CI 95%	Mortality% ±SE	CI 95%	Mortality% ±SE	CI 95%
Met-TR61	84.00 ± 1.15 A	79.03–88.96	79.00 ± 1.73 B	71.54–86.45	82.00 ± 1.15 A	77.03–86.96
MET52	73.33 ± 1.76 C	65.74–80.92	69.33 ± 2.60 D	58.13–80.53	71.00 ± 1.15 CD	66.03–75.96
Blank product	18.33 ± 2.02 E	9.60 ± 27.05	18.00 ± 1.73 E	10.54–25.45	18.33 ± 2.02 E	9.60 ± 27.05
Control	11.33 ± 1.76 F	3.74 ± 18.92 H	8.33 ± 1.45 G	2.08–14.58	9.33 ± 1.45 G	3.08 ± 15.58

Note: Mortality% ± SE within a column followed by the letter indicates the differences in mortality ($P < 0.05$) Tukey's LSD test, $P \leq 0.05$.

In particular, the effect of the new product on *B. brassicae* was found to be higher and significantly different than *A. fabae* ($p < 0.05$) (Table 2). It was determined that the results on *B. brassicae* and *M. rosea* were close and there was no significant difference ($p < 0.05$). When we compared the effect of commercial and blank products on aphids, it was determined that there was a similar mortality rate in all insects and no significant difference between them, but a significant difference in the control group ($p < 0.05$). Moreover, the aphid populations increased rapidly in the control and the blank product applications.

Discussion

The main objective of biocontrol research is to develop novel, useful, and readily applicable mycoformulations for use against target pests. The main goal of this study is to create a new oil-based mycoinsecticide from a strain of *Metarhizium anisopliae* that is native to Türkiye to control the *Myzus persicae* aphid species as well as a few other aphid species.

Fungi such as *Lecanicillium* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria* spp. are entomopathogenic fungi that play significant roles in regulating insect populations. Many entomopathogenic fungi that were effective against various insects in our country and all over the world were isolated from insect and soil samples, characterized according to their molecular and morphological features and their potential for use in the biological control of agricultural pests was determined (Biryol et al. 2020; Eski et al. 2022; Kocacevik et al. 2015; 2016; Gurulingappa et al. 2011). *M. anisopliae* is the second most widely exploited entomopathogenic fungi in biocontrol studies next to *B. bassiana*, and it is known to attack more than 200 insect species belonging to different orders (Jitendra et al. 2012; Chen et al. 2014). Entomopathogenic hyphomycetes-based mycoinsecticides have been tested against sucking insects such as aphids in recent years, and their effectiveness against such pests has been demonstrated (Milner, 1997; Vandenberg et al. 2001). The global demand for biopesticides is also increasing day by day. Most of these

mycoinsecticides are produced in America, Europe, and Asia (Faria and Wraight 2007). The mycoinsecticide AFIDISIDAL-OD *Met*-TR61 based on *M. anisopliae* developed in this study was produced using solid-substrate fermentation. Spore yield can also be increased with carbon and nitrogen sources such as rice, yeast extract, corn maceration liquid, or molasses, which are used as substrates or carriers (Talwar 2005). This method was preferred especially because of the high spore yield and some problems encountered in the production of strains of this species in a solid substrate. Spores obtained from production on a solid substrate can be used in different types of formulations, such as granular and spray (aqueous and oil-based) (Leland 2001). EPF-based biopesticides tend to be more effective when formulated in oil, as the conidia are evenly distributed on the insect cuticle and leaf surface. In contrast, aqueous spore formulations tend to remain as drops on the surface after application, which may reduce their efficacy (Inyang et al. 2000; Wraight et al. 2016). In bioassay studies, we determined that AFIDISIDAL-OD *Met*-TR61 remained active in host plants after application. The benefits of oil-based formulations for pest control, such as mortality and protection against adverse environmental effects, have been widely reported (Hedimbi et al. 2008; Lopes et al. 2011; Oliveira et al. 2018). It was determined that the aphids we used in the current study were sensitive to both the new product (AFIDISIDAL-OD *Met*-TR61) and the commercial product. *Met*-TR61 caused higher mortality than the commercial biopesticide products in our bioassay on the four aphid species. Oil-based carrier fungus conidia are more resilient to environmental stress factors, making them more resilient to environmental stress factors such as humidity, temperature, and UV radiation, according to research by Kaaya and Hassan (2000) and Batta (2003). This may extend the conidia's shelf life and vitality and raise the fungus's likelihood of spreading infections. In a study, Biryol et al. (2022) reported that the isolate used in the current study was resistant to environmental factors (UV-B and temperature) experiments and had high virulence. The results of the mortality experiments strongly support the development of biopesticides for the control of the four aphid species. In the study conducted by Mohammed and Hatcher (2016) on the 3rd stage *M. persicae* nymphs, it was determined that Mycotal caused 100% mortality with 1×10^{10} conidia/ml concentration in laboratory conditions on the 9th day. In the current study, *Met*-TR61 yielded a mortality rate of 79% in 7th days in a pot experiment of 1 × 108 conidia /ml concentration. The study by Lopes and Faria (2019) assessed the effect of oil-based formulation on the coffee beetle species *Hypothenemus hampei*. According to the findings of the study, the oil formulation had a 20% more considerable mortality rate after six days than the pure conidia suspension at a concentration of 2×10^{7} conidia/ml. According to the research findings, unformulated spore suspensions prepared in water, particularly oil-based formulations, are more effective when compared to the results of both laboratory and field applications of pests, and the reason for this effect is the spread and adhesion of the conidia on the hydrophobic cuticle of the host arthropod (David-Henriet et al. 1998). It has even been included in bioassays to enhance the mortality rate of *Spodoptera litura* and *Helicoverpa armigera* (Vimala Devi and Prasad 1996; Vimala Devi and Hari 2009). According to Albernaz et al. (2009), M. *anisopliae* formulations based on 10% sunflower oil showed increased ovicidal activity against *Aedes aegypti*. The oil has no impact on germination because of its low volatility and compatibility with environmental conditions (ULV). It has been demonstrated to improve *M. anisopliae* conidia efficacy (Albernaz et al. 2009; Bateman et al. 1998; Batta 2003).

EPF-based formulations must be effective before the immature stages of these pests reach the reproductive age, which means that they are effective in a short time. The maturation period of *M. persicae* is 7.6 days,

according to a study (Hong et al. 2019), and when we transplanted it into our study, it was determined that the new mycopesticide was effective on this pest from the second day, and the pest population decreased.

Conclusion

Previous research on *Metarhizium anisopliae* KTU-51, including isolation, identification, and insecticidal activity studies, has revealed that KTU-51 holds great promise for biological pest control. The results of this study indicate that the local mycoinsecticide developed from this isolate is also effective against other aphids, particularly *M. persicae*, and can be used successfully for the biological control of these important agricultural pests. Studies on the commercialization of an effective, environmentally friendly, and ecologically compatible AFIDISIDAL-OD *Met*-TR61 must be conducted as soon as possible.

Declarations

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Author contributions

The study was designed by ID and SB, with SB carrying out the research. ID and SB contributed to the initial draft of the manuscript, with both authors involved in subsequent review and editing. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

All data generated and analyzed during this study are indicated in the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

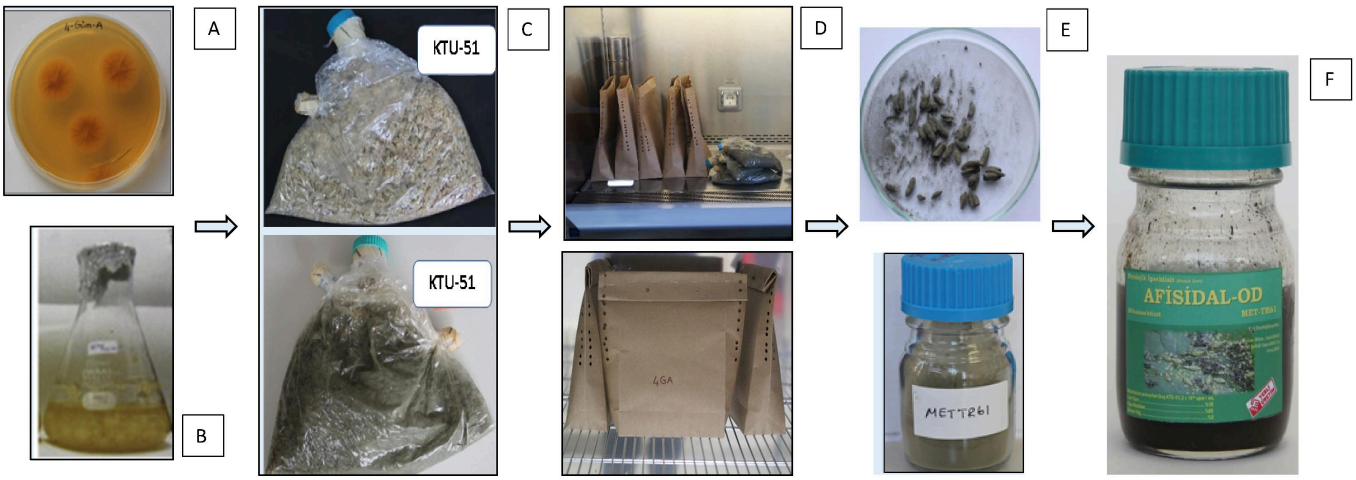

Fig.1 Oil-based formulation production. A) Growth of KTU-51isolate on PDAY B) Preparation of pre-cultures from the isolate C) İnoculation of spores from pre-culture onto the solid substrate and sporulation on rice D) Transferring solid substrate from fungus growth bags to kraft paper and removing excess moisture E) Collection of fungal spores by sieving from solid substrate surface F) Creation of oil-based formulation.

Figure 1

See image above for figure legend.

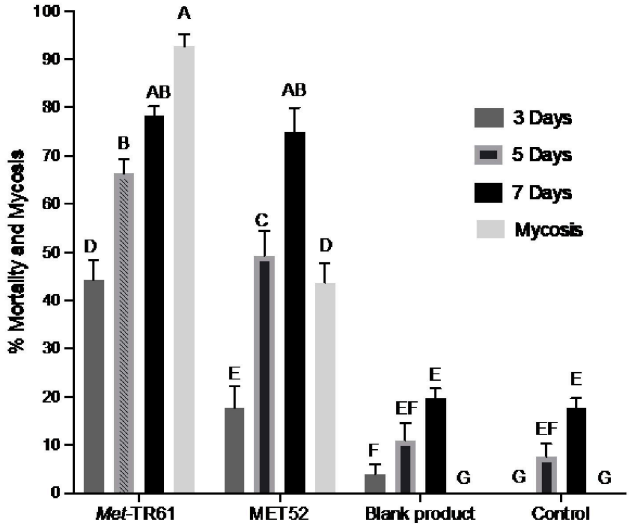

Fig 2. The effect of *Met*-TR61 on *Myzus persicae* nymphs in the leaf disc experiments at 10^8 conidia/ml concentration. Different case letters represent statistically significant differences among mortalities according to the least significant difference (LSD) multiple comparison test (p <0.01). Mortality indicates the mean of three replications. Bars show standard error.

Figure 2

See image above for figure legend.

Fig 3. The effects of *Met*-TR61 on *Myzus persicae* nymphs in the pot experiments. A) The pepper seedlings treated with *Met*-TR61, B) Treated blank product and C) Control

Figure 3

See image above for figure legend.

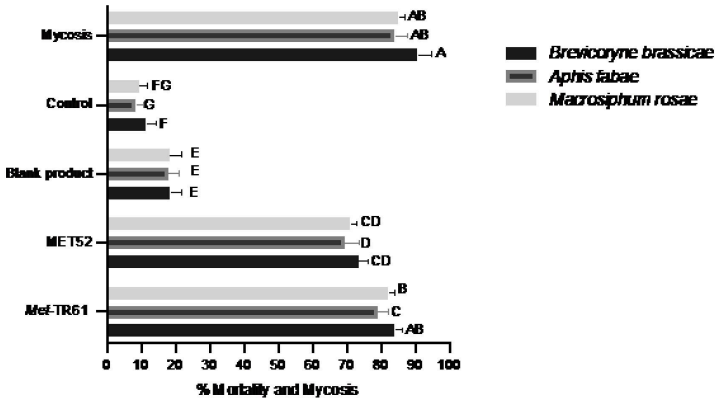

Fig 4. Mean percent mortalite of differ aphids after treatment with suspensions of Met-TR61 at 1×10^8 conidia/ml conditions under the laboratory. Mortality data were calculated based on Abbott's formula (1925). Bars show standard deviation.

Figure 4

See image above for figure legend.