

EFFECT OF FORMULATING OF *BEAUVERIA BASSIANA* CONIDIA ON THEIR VIABILITY AND PATHOGENICITY TO THE ONION THRIPS, *THRIPS TABACI* LIND. (THYSANOPTERA: THIRIPIDAE)

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Abstract: The wettable powder was prepared on the basis of aerial conidia of two isolates of the entomopathogenic fungus, *Beauveria bassiana*. Viability and pathogenicity of conidia products were evaluated against the second-instar larva of *Thrips tabaci* in four cases; Conidial-product Maintained in Refrigerator (CMR), Conidial-product Maintained in Laboratory (CML), New Formulated Conidia (NFC) and New Conidia without formulation (NC). Analysis of corrected seven-day total mortality data revealed that there were significant differences among these product-cases in their pathogenicity to thrips larvae. Recorded mortality rates for CMR, CML, NFC and NC showed that the pathogenicity of CML was lower compared to three other cases for both isolates. In the next step, inorganic salts ($MgCl_2$, NH_4PO_4 , KH_2PO_4 , $MgSO_4$ and NaCl) were added at a rate of 0.1 M into the both CMR and CML products. Bioassay results indicated that caused total mortality of thrips larvae increased with adding of salts. Our results showed that applied carriers and salts have positively effected preserving of conidia viability and pathogenicity to the second-instar larva of the onion thrips.

Key words: formulation, conidia, entomopathogenic fungus, *Beauveria bassiana*, wettable powder, *Thrips tabaci*

INTRODUCTION

To be of practical use, biological agents must be formulated as products capable of storage, distribution and application in agricultural market (Rhodes 1993). Formulation can be critical to commercial success of microbial biopesticides and has a potential to stabilize biological ingredient, improve its storage and field persistence, make product safe and easy to use and provide field efficacy. Formulation of fungal propagules may help to overcome unfavourable climatic conditions and may increase effectiveness of control (Luz *et al.* 1999). Conidia of entomopathogenic fungi are strongly hydrophobic and difficult to suspend in water. This feature prevents the suspension formation in water (Behle *et al.* 2006). Some research showed that the wettable powder formulation from fungal conidia by using wetting agent might solve this problem (Guijarro *et al.* 2007; Nagayama *et al.* 2007). Many of the currently available biological pesticide products are wettable powder containing conidia (Burgess 1998). Earlier investigations indicated that some major pests could be controlled by using of formulated conidia of entomopathogenic fungi based on WP formulation successfully. For example, WP formulation of conidia against *Leptinotarsa decemlineata* (Feng *et al.* 1994), *Tetranychus urticae* (Dresner *et al.* 1949), *Alphitobius diaperinus* (Steinkraus *et al.* 1991). The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (*Ascomycota: Hypocreales*) is able to infect over 700 species of arthropods, especially insects

(Tanada and Kaya 1993). Entomopathogenic fungi produce two types of propagules: conidia in solid medium and blastospore in liquid culture, so both of these propagules could be used for formulating of *B. bassiana* as well as many other fungal entomopathogens. It is important to determine which form might be the best suited for using in commercial formulation (Jiang *et al.* 2007).

The onion thrips, *Thrips tabaci* Lind. (Thys., Thripidae), is a polyphagous species which occurs world-wide (Lewis 1997). It causes damage directly through feeding and indirectly through transmission of lethal plant viruses (Hardy and Teakle 1992). The pest is difficult to control with insecticides due to its small size and cryptic habits (Herron and James 2005). Because of that there is a need of an alternative control method such as the use of entomopathogenic fungi (Ugine *et al.* 2005).

One of the most important factors for formulation of fungi is the adaptable additive materials to keep the biological ingredients during the storage time especially at a room temperature. The selection of formulation ingredients (e.g., oil, water or with solid carriers) may facilitate biopesticide delivery to target insects in the field or grain storage, using ultra-low droplet application equipment (Bateman 1992). Some additives were shown to enhance the storage potential for a longer period and to overcome restrictions caused by humidity and temperature (Alves *et al.* 1987). The relationship between the stability of dried-microorganisms at storage and storage

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temperature (Lievens and Van't Riet 1994), addition of supplements (Beker and Rapoport 1987) and package atmosphere (Champagne *et al.* 1991; Costa *et al.* 2001) were well documented.

In this study, we prepared conidial WP formulations from *B. bassiana* and maintained these preparations under different conditions and evaluated the viability of conidia in these products during the storage time and finally we assayed pathogenicity of formulated and unformulated conidia to the onion thrips larvae.

MATERIALS AND METHODS

Insect rearing and fungal isolates

A colony of *T. tabaci* was established from greenhouse-collected specimens on cucumber plants in Karaj and reared on cucumber (*Cucumis sativus* var. *Sultan*) leaves in polygonal plastic dishes at 25±1°C, 60±5% R.H. and 16:8 h L:D. These ventilated plastic dishes, 11 cm in diameter, were filled with a 1cm- thick layer of 1% water agar. Freshly excised cucumber leaf discs, 8 cm in diameter were placed upside down onto the water agar and 25–30 *T. tabaci* females were picked up from the stock culture and placed on the leaf discs for egg laying at 12 hours. Two isolates of *B. bassiana* EUT105 and *B. bassiana* EUT116 (provided by the laboratory of Biocontrol and Biocoenology, University of Tehran) were used in this study. These isolates were cultured on Sabouraud's dextrose agar (Merck, Darmstadt, Germany) with 1% yeast extract (SDAY) plates in several Petri dishes (9 cm in diameter), and were grown for 2–3 weeks at 25±1°C, under a 16:8 h (L:D) photoperiod and 60±5% RH.

Preparing of WP formulation of conidia and storage conditions

In this step, we provided dry powder of pure conidia for preparing of WP formulation. For this purpose, conidia were harvested from 18-days old cultures. At first, we selected the Petri dishes with well grown and sporulated fungus on SDAY medium, then collected the pure conidia of each Petri dish by scraping off the sporulated fungus using sterile scalpels and maintained at a low temperature (4°C). For accession of proportion of carries that should be used with conidia, we carried out experiments based on adding some proportions of supplements to conidia and stored them for 30 days (unpublished data). In 2.5 g of fresh conidial powder, the concentration was equal and amounted to 2×10¹⁰ conidia ml⁻¹. Conidia were mixed with silica gel powder in 2.5:1 weight ratio. Then, other selected materials or carriers such as stickers, stabilizers, UV-protectant and wetting agents (Table 1) were added to the mixture of conidia and silica gel into the sterile cabinet. Approximately 12 g of dry powder was obtained for each case and poured in to 50-ml falcon plastic tubes. These tubes were covered with aluminum foil and stored under different conditions: in laboratory at a temperature ranging from 23–26°C and in a refrigerator at a temperature of 4±2°C for 30 days. Because of that they were named as CML and CMR, respectively. For examination of the effect of storage time and carriers on formulation efficacy, spores viability and their pathoge-

nicity, the NFC (New Formulated Conidia) and NC (New Conidia) were prepared 24 h before bioassay conducting. We used some mineral salts such as MgCl₂, NH₄PO₄, KH₂PO₄, MgSO₄ and NaCl (0.1 M of each compound) with formulated conidia for estimating the effect of these salts on survival, efficacy and pathogenicity of conidia in formulation during the storage period at room temperature and under refrigerator condition.

Table 1. Amounts of carriers which were added into 2.5 g fresh conidia powder of *Beauveria bassiana*

Rate of substances per weight or volume unit	Carriers
20% volume of conidia and silica gel powder mixture	Paraffin Liquid*
20% volume of conidia and silica gel powder mixture	Sucrose monohydrate*
2 % volume of conidia and silica gel powder mixture	Sodium Glutamate*
2 % volume of conidia and silica gel powder mixture	Sodium Alginate*
2.5% volume of conidia and silica gel powder mixture	Cereal flour
20% volume of conidia and silica gel powder mixture	Starch
20% volume of conidia and silica gel powder mixture	Casein
0.1 ml	0.02% Tween 80*
with ratio of 2.5: 1 (conidia: SMP)	Skimmed Milk Powder*
X**	Kaolin Powder

*these products belonging to Merck, Darmstadt, Germany Other materials were obtained in Biocontrol laboratory

**the amount of kaolin which added in formulation depends on humidity of prepared powder

Viability test

The viability of conidia in all cases was evaluated twice; the first time exactly before storage of formulated conidia and the second one, before bioassay of formulated and unformulated conidia. This test was conducted by applying 1ml of 1×10⁸ conidia ml⁻¹ concentration from each case with potter spray tower (Burkard, UK) on potato dextrose agar (PDA) plates 90 mm in diameter. Then five cover glass slips were fixed in five parts of each plate and incubated at 25±1°C for 18 h. The viability of formulated and unformulated conidia was estimated by using Zeiss (Oberkochen, Germany) light microscope. Only spores with germ tubes longer than their width were considered to have germinated.

Bioassay

For conducting bioassay, at first 12 g of each formulated conidia from CML and CMR (30 days-old storage) and NFC were picked up, diluted with 50 ml of distilled water and vortexed for 5 min. The concentration of spore was approximately 2–4×10¹⁰ conidia/ml in each treatment that was called as stock solution. We used $C_1V_1 = C_2V_2$ formula for preparing of the latest concentration (1×10⁸ conidia/ml) from stock solution. Finally, the other concentrations (10³, 10⁴, 10⁵, 10⁶, 10⁷ conidia/ml) were prepared by serial dilution from 1×10⁸ conidia/ml. For bioassay of

larvae with conidial formulations, the bioassay chamber consisted of common hyaline canisters for storage of 35 mm photography film, 5 cm high and 3 cm in diameter (Pourian *et al.* 2008). Briefly, we reduced the height to 3 cm by cutting off the top 2 cm and made a hole, 1 cm in diameter in the lid and covered it with polyester net in size of 200 μm meshes. These small chambers were filled with a 0.5 cm-thick layer of 1% water agar and cucumber leaf disks, 2.5 cm in diameter, were placed upside down onto the water agar. The freshness of cucumber leaf discs was preserved for at least 3 days. Fifteen second instar larvae of onion thrips were then transferred onto the leaf disks in each canister for bioassay experiment. Prepared concentrations of each formulation were applied using potter spray tower with a nozzle 0.25 mm diameter and 0.7 kg/cm² pressure. Each replication was performed by spraying 1 ml of the conidial suspension onto the larvae. In the case of New Conidia, sterile distilled water with 0.02% Tween 80 and for the formulated conidia; only carriers were used as control. The lids of cups were then sealed with Parafilm[®]. Inoculated larvae were maintained at 25 \pm 1 $^{\circ}$ C under 16:8 h (L:D) photoperiod and 60 \pm 5% R.H. in the incubator. After 24 hours, mortality data was daily recorded for 7 days. Each concentration was performed in four replications with 15 larvae/each replication. This study was carried out as factorial experiments based on Completely Randomized Design (CRD).

Statistical analysis

Computations for this experiment were made using the statistical software package SYSTAT version 10 (SYSTAT, 2000). Treatment of mortality was corrected for the control mortality using Schneider-Orelli's formula (Püntener 1981). Total mortality data were analyzed using PROC General Linear Model. Tukey HSD test was used for pairwise comparisons of means among treatments. The LC₅₀ values were estimated for formulated and unformulated conidia using probit analysis, carried out by the statistical program POLO-PC (Leora Software 1987). We used t-test for comparison of means between two treatments.

RESULTS

Viability tests

The results of viability test showed that there were significant differences among CMR, CML, NFC and NC in spore germination for both isolates; *B. bassiana* EUT105 [$F_{3,8} = 54.99$; $p < 0.01$] and *B. bassiana* EUT116 [$F_{3,8} = 20.22$; $p < 0.01$]. Preserving viability and germination potential of spores during the storage time is important for enhancement of their efficacy. The effect of some mineral salts on formulations was evaluated and analysis of data showed that when salts were added to formulated conidia, their viability was preserved over 30 days comparing to the treatments without salts. Comparing CMR and CMR-S, we observed a significant difference between both isolates [*B. bassiana* EUT105 ($t = -8.027$, $df = 4$, $p = 0.001$) and *B. bassiana* EUT116 ($t = -6.971$, $df = 4$, $p = 0.002$)] and similarly between CML and CML-S [*B. bassiana* EUT105 ($t = -6.799$, $df = 4$, $p = 0.002$) and *B. bassiana* EUT116 ($t = -3.110$, $df = 4$, $p = 0.036$)]. Our results revealed that CML and CML-S in comparison with the other treatments showed less germination. The viability of formulated and unformulated conidia of those treatments ranged 90–95%, whereas the viability of CML and CML-S was 70–75% after 18 h.

Pathogenicity of formulated versus non-formulated conidia to thrips larvae

Bioassay of formulated and unformulated conidia was done on the second-instar larvae of onion thrips. The pathogenicity of New Conidia showed that there was no difference between two isolates ($t = -0.811$; $df = 54$; $p = 0.421$) and both isolates showed equal effect on larvae. The pathogenicity of formulated and New conidia to the larvae indicated that there were some significant differences among four treatments in both isolates; *B. bassiana* EUT105 [$F_{3,72} = 18.9$; $p < 0.01$] and *B. bassiana* EUT116 [$F_{3,72} = 23.3$; $p < 0.01$]. The pathogenicity of the CML formulation was lower than CMR, NFC and NC. (Fig. 1). The range of maximum and minimum corrected mortality data was determined for CMR, CML, NFC and New conidia

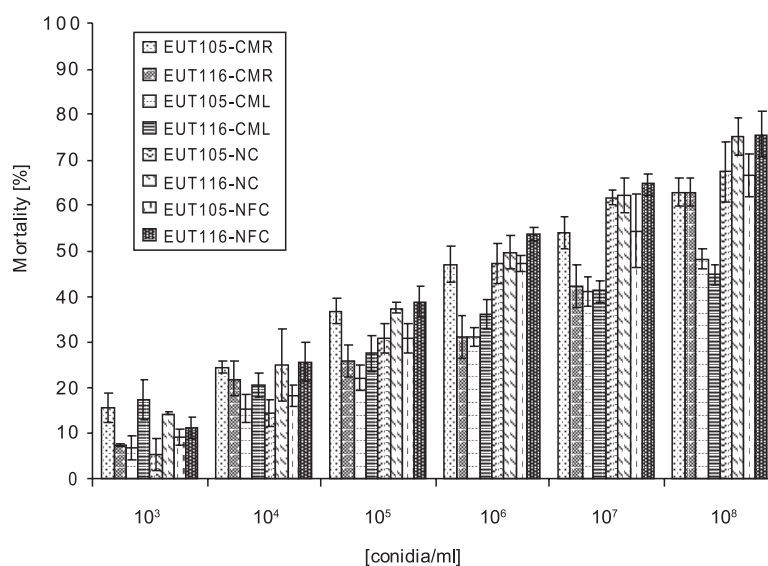


Fig. 1. Mortality of the second-instar larvae of *T. tabaci* caused by conidial preparations of *B. bassiana* EUT105 and *B. bassiana* EUT116 at different concentrations

for both isolates (Table 2). Pair-wise comparisons among formulated and unformulated conidia showed that there were some significant differences among treatments (Tukey HSD, $p < 0.05$). For all treatments, the LC_{50} value ranges were estimated (Table 3). The LC_{50} value in CMR formulation was almost 10-fold fewer than CML in both isolates. In fact, CMR was more pathogenic formulation and caused higher mortality than CML against the onion thrips larvae. The LC_{50} values of two other cases, NC and NFC, were close to CMR and both of them were pathogenic against larvae.

Table 2. Mortality percentage of formulated and unformulated conidia at the lowest and highest concentrations

	EUT105 Mortality [%]		EUT116 Mortality [%]	
	1×10^3 co.ml ⁻¹	1×10^8 co.ml ⁻¹	1×10^3 co.ml ⁻¹	1×10^8 co.ml ⁻¹
NC	5.5	67.5	14	75
NFC	9	66.7	11	75.7
CMR	15.6	63	7.5	63
CML	6.8	48	17	45

Effect of mineral salts on formulated conidia

The effect of some salts as osmotic regulators on biological agent was evaluated as viability and pathogenicity potential of formulated conidia. Pathogenicity efficacy of formulated conidia accompanied by salts (CMR-S) is better than CML-S and there is significant differences between two cases in both isolates (Fig. 2); *B. bassiana* EUT105 ($t = -3.774$, $df = 46$, $p < 0.01$) and *B. bassiana* EUT116 ($t = -3.096$, $df = 46$, $p = 0.003$). The differences between salty formulations and formulations without salt indicated that the additive salts have not side-effect on conidia viability during the storage time in both formulations (CMR-S, CML-S). The result of salts effect on conidia maintaining in CML-S formulation than CML demonstrated that salts could preserve the conidia viability in same maintaining condition and was caused high mortality in comparison with CML [*B. bassiana* EUT105 ($t = -2.588$, $df = 46$, $p < 0.05$) and *B. bassiana* EUT116 ($t = -3.075$, $df = 46$, $p = 0.004$)] (Fig. 3). Similarly, these results are occurred for CMR-S with CMR in both isolates [*B. bassiana* EUT105 ($t = -3.462$, $df = 46$, $p < 0.01$) and *B. bassiana* EUT116 ($t = -4.856$, $df = 46$, $p < 0.01$)] (Fig. 4). In general, the above mentioned results indicated that salts caused positive effect on CML-S. The mineral salts could enhance the preserving time of conid-

Table 3. Results of probit analysis for estimating of LC_{50} values ($\pm SE$) of treatments

Isolates	Treatments	n	Slope $\pm SE$	χ^2	df	Upper limit 95%	Lower limit 95%	LC_{50}	Lower limit 99%	Upper limit 99%
EUT105	CML	360	0.268 \pm 0.051	0.05347	4	9.5×10^6	1.5×10^7	8.3×10^7	1.7×10^9	8.8×10^9
	CMR	360	0.262 \pm 0.043	0.5408	4	4.4×10^5	7.3×10^5	3.2×10^6	2×10^7	4.6×10^7
	NC	360	0.386 \pm 0.056	2.0489	4	5.3×10^5	8.1×10^6	2.6×10^7	8.8×10^6	1.3×10^7
	NFC	360	0.323 \pm 0.054	1.2021	4	6.3×10^5	1×10^6	4.2×10^6	2×10^7	3.2×10^7
EUT116	CML	360	0.173 \pm 0.045	0.8341	4	-	1.7×10^7	2.7×10^8	-	-
	CMR	360	0.317 \pm 0.059	0.9691	4	1.4×10^6	2.4×10^6	1.1×10^7	8×10^7	1.8×10^8
	NC	360	0.364 \pm 0.051	0.6289	4	2.2×10^5	3.3×10^5	1.1×10^6	3.5×10^6	5.4×10^6
	NFC	360	0.054 \pm 0.359	5.1128	4	1.1×10^5	1.9×10^5	7.6×10^5	2.6×10^6	4.4×10^6

Table 4. Result of probit analysis for estimating of LC_{50} values ($\pm SE$) of treatments with salts

Isolates	Treatments	n	Slope $\pm SE$	χ^2	df	Upper limit 95%	Lower limit 95%	LC_{50}	Lower limit 99%	Upper limit 99%
EUT105	CMR-S	360	0.393 \pm 0.054	2.4226	4	4.7×10^3	8.8×10^3	3.9×10^4	1.2×10^5	1.7×10^5
	CML-S	360	0.202 \pm 0.043	1.4398	4	6.9×10^5	1.4×10^6	1.3×10^7	2.6×10^8	1.2×10^9
EUT116	CMR-S	360	0.342 \pm 0.047	2.1723	4	1×10^4	1.8×10^4	7.3×10^4	2.2×10^5	3.1×10^5
	CML-S	360	0.212 \pm 0.047	0.1746	4	9.4×10^4	2.2×10^5	2×10^6	2.5×10^7	8.6×10^7

ia in CMR and CML. The CMR formulation has shown suitable viability and high pathogenicity against larvae (as mentioned before). The pathogenicity of CML-S and CMR against larvae were assessed and result showed that there are no significant differences between them in both isolates [*B. bassiana* EUT105 ($t = -0.217$, $df = 46$, $p = 0.829$)

and *B. bassiana* EUT116 ($t = -2.449$, $df = 46$, $p < 0.05$)] (Fig. 5). These recent cases are different as maintaining condition and carriers. The LC_{50} value of all cases with salts have shown lower amount than treatments without salts (Table 4).

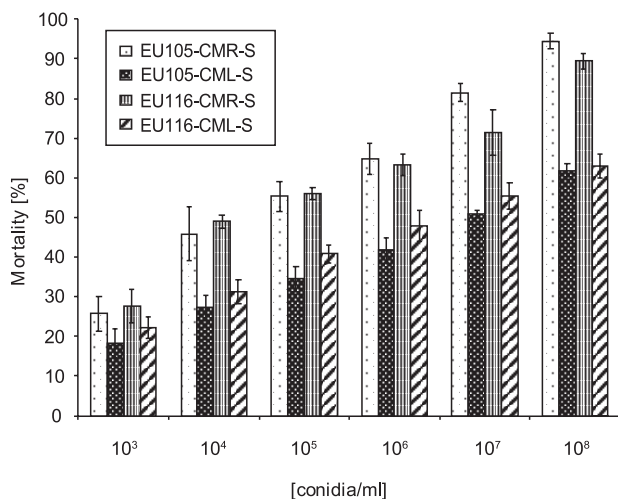


Fig. 2. Mortality of the second instar larvae of *T. tabaci* caused by conidial preparations of CMR-S and CML-S from *B. bassiana* EUT105 and *B. bassiana* EUT116 at different concentrations

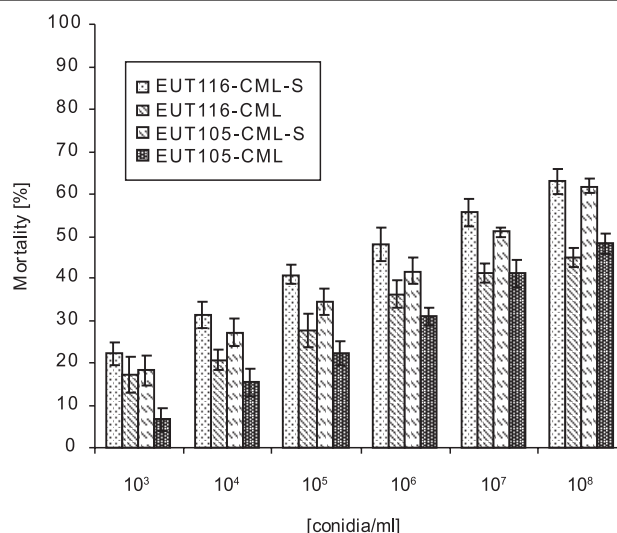


Fig. 3. Mortality of the second instar larvae of *T. tabaci* caused by conidial preparations of CML-S and CML from *B. bassiana* EUT105 and *B. bassiana* EUT116 at different concentrations

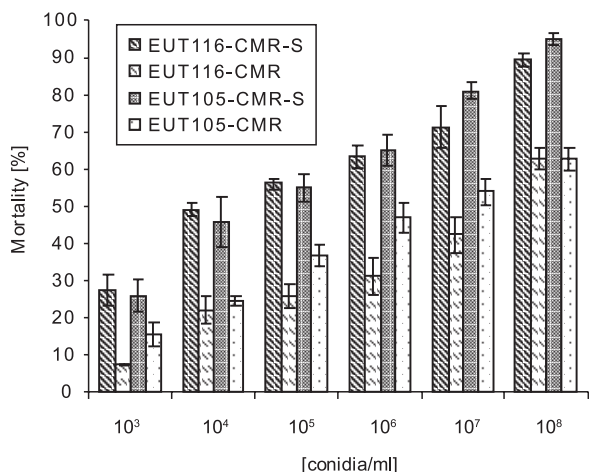


Fig. 4. Mortality of the second instar larvae of *T. tabaci* caused by conidial preparations of CMR-S and CMR from *B. bassiana* EUT105 and *B. bassiana* EUT116 at different concentrations

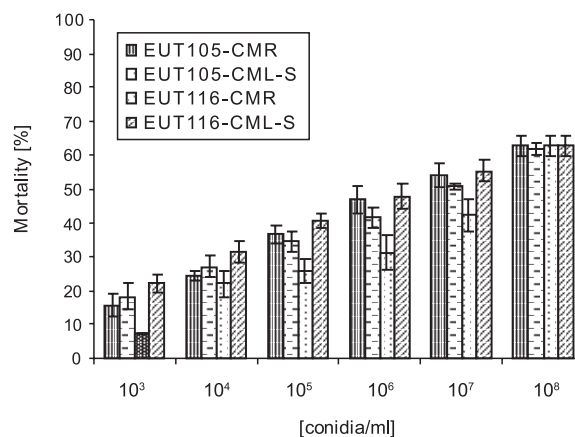


Fig. 5. Mortality of the second instar larvae of *T. tabaci* caused by conidial preparations of CML-S and CMR from *B. bassiana* EUT105 and *B. bassiana* EUT116 at different concentrations

DISCUSSION

For the practical application of entomopathogenic fungi spores under the field conditions, their preservation in a liquid or as a dry powder forms is the most important step for the development of a fungal biopesticide. So far, various preservation methods have been tested, using conidia (Stathers *et al.* 1993; Moore *et al.* 1995). In this study, CML formulation showed low mortality percent in comparison to the other treatments. In spite of the fact that the kind and ratio of carrier materials was similar in all treatments, but the storage temperature of CML was different from the others. Therefore, differences between pathogenicity of CML and other cases especially CMR was related to a storage temperature. The temperature as an abiotic factor significantly effected spore survival and activity of fungi. This finding was confirmed by some other investigators (Sandoval-Coronado *et al.* 2001; Kucuk and Kivanc 2005; Jackson and Erhan 2006). Shelf-life of a biological

product refers to the period of time during which the propagules of the microbial agent are able to remain viable and effective (Elzein *et al.* 2004). When CML preserved at laboratory temperature for 30 days, spore germination was at a suitable level before testing, but in the bioassay, viability potential of conidia was reduced, that might be referred to some other factors such as temperature and kind of carriers in formulation. Storage temperature is the most important abiotic factor that affects the shelf-life of biological formulations (Connick *et al.* 1997) by maintaining them in a state of low metabolic activity (Elzein *et al.* 2004). In our study, preserving of conidia viability was correlated with biocontrol efficacy in CML. This was in accordance with De Cal *et al.* (2002) and Larena *et al.* (2002) who demonstrated that viability potential of *Penicillium frequentans* conidia was decreased within 30 days at room temperature and this factor influenced fungal efficacy. Chen *et al.* (2008) reported that the temperature was the most critical factor influencing conidial storage stability, among tested factors

affecting survival of *Lecanicillium lecanii* conidia stored at room temperature. They revealed that both conidial germination and infection of host decreased with storage the temperature ranging from 15 to 35°C. In CMR, cool maintaining condition caused that the metabolic activity of conidia was decreased during storage time and this agent could be justified by higher spore viability and pathogenicity of CMR comparing to CML after 30 days. The efficacy of NFC was similar to CMR although they were different in storage time. This showed that the carriers effect was not revealed early at the storage period and the same level of pathogenicity referred to freshness of conidia in first 24 h in NFC and it was not due to carriers within the formulation. Probably, the carriers' effect is gradually appeared with passing off time. Additives could induce a wide range of effects on different fungal species (from toxicity on viability of conidia to reduction on conidial production) (Burges 1998). Presumably, the main reason that led to preserve the viability of conidia in CMR and CML referred to a kind of additive which was selected for this experiment. The main additives used for formulation are given in table 1. We selected these carriers basing on their effect on viability maintainance of spores during formulation process. Some investigations were done on effect of these additives on characteristics of fungal spores. For example; silica gel preserves viability and germination of spores during the storage time (Teera-Arunsi et al. 2003), liquid paraffin enhances the germination and pathogenicity of *Metarhizium anisopliae* to *Boophilus microplus* relative to water (Polar et al. 2005), kaolin powder is a preservative agent (Bhattacharya and Basu 1982). Sucrose solution is an osmotic protector (Burges 1998) leading to preserving of spore survival and germination (Jackson and Erhan 2006; Shabana et al. 2003). Sodium glutamate as a stabilizer and osmotic protector (Guijarro et al. 2007; Larena et al. 2007) increased shelf life of fungal spores during drying period (Burges 1998). Sodium alginate prevented loss of viability of chlamydo spores during Pasta production and improved the mycoherbicidal efficacy, stability, and shelf-life comparing to the standard Pasta with no additives (Shabana et al. 2003). Skimmed milk powder has several effects on efficacy of spores such as; preserving of viability (Stephan and Zimmermann 1998; Abadias et al. 2001), UV-protectant (Edgington et al. 2000), the best particle source for fixing the spores due to its solubility in water and its highly porous nature (Tadayyon et al. 1997), a good sticker (Stephan and Zimmermann 1998), and increasing of suspensibility in powder formulation of *B. bassiana* (Teera-Arunsi et al. 2003). All of these researches indicated that carriers interfered in neither maintaining process nor pathogenicity of entomopathogenic fungi. Investigation on adding of some mineral salts on efficacy of CML and CMR showed that using of KH_2PO_4 , NaCl , MgSO_4 , NH_4SO_4 and MgCl_2 (0.1 M) in formulated conidia improved their shelf-life and efficacy. Probably, higher pathogenicity of CMR-S and CML-S formulations compared with CMR and CML to larvae was related to compatibility of the additive salts with other carriers and caused an increase of the pathogenicity of conidia by preserving their viability in formulation over 30 days. Basing on results of other studies, mineral salts showed different

effects on entomopathogenic fungi spores when added into formulation. Hong et al. (2005) demonstrated that adding of NaCl to oil formulated conidia of *B. bassiana* enhanced shelf-life of spores, whereas KCl decreased shelf-life of spores during the storage time. Daoust and Roberts (1983) indicated that shelf-life of conidia increased in salt solution of NaCl, Mg (NO_3)₂ and MgCl_2 . Increasing of conidia efficacy by adding of salts to the formulation showed that there was no negative interaction among salts and other carriers, so they could be used as effective additives in formulation process to prevent of biological ingredient from decaying and stress. A very low viability of an unformulated *B. bassiana* conidia (after a month) obtained here was similar to that found by other investigators under similar storage condition (Burges 1998; Batta 2004). This indicated that the carries played an important role in maintaining of conidia during storage in addition to temperature effect. An adequate shelf-life of the mycopesticidal product at room temperature is an essential requirement for their acceptance and commercialization (Jones and Burges 1998), so we attempted to produce the formulation of conidia that could be stable in temperature room. Our bioassay revealed that the mortality of larvae after application of CML was in an acceptable level compared to CMR. Our study and several other studies on storage of entomopathogenic fungi were focused on the temperature influence on shelf life and viability of the formulated conidia (Marques et al. 1999; Smith et al. 1999) but the effect of carriers should not be neglected in this field. The mortality percentages of CML and CML-S indicated that the carries used in this study were suitable for formulations especially after storing formulations for a month. In general, there is a need to improve formulations of other compatible carriers and increase of storage period especially at storage temperature (room temperature). However, the problem of developing a suitable formulation for entomopathogenic fungi which enhances their efficacy against target insect whilst maintaining of the fungus in a viable, virulent and stable state for a prolonged storage period still needs to be addressed and solved.

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POLISH SUMMARY

WPŁYW FORMULACJI KONIDIÓW GRZYBA *BEAUVERIA BASSIANA* NA ICH ŻYWOTNOŚĆ ORAZ PATOGENICZNOŚĆ DLA WCIORNASTKA TYTONIOWCA *THRIPS TABACI* LIND. (*THYSANOPTERA: THIRIPIDAE*)

Na bazie powietrznych zarodników konidialnych dwóch izolatów entomopatogenicznego grzyba *Beauveria bassiana* sporządzono proszek zawiesinowy. Żywotność i patogeniczność konidiów testowano na drugim stadium larwalnym *Thrips tabaci* w formie biopreparatu: przechowywanego w lodówce (CMR), w warunkach laboratoryjnych (CML), na bieżąco sporządzonej formy formacji konidiów (NFC) oraz samych świeżych zarodników (NC). Analiza statystyczna skorygowanych danych całkowitej śmiertelności wykazała istotne różnice pomiędzy zastosowanymi wariantami biopreparatu pod względem patogeniczności dla larw wciornastka. Porównanie uzyskanych wielkości śmiertelności larw dla wariantów CMR, CML, NFC oraz NC wykazało słabszą skuteczność wariantu CLM. W kolejnym etapie badań dodano sole nieorganiczne ($MgCl_2$, NH_4PO_4 , $MgSO_4$ i $NaCl$) rzędu 0,1 mola zarówno do wariantu CMR jak też CML. Wyniki testów biologicznych wykazały wzrost całkowitej śmiertelności larw wciornastka po dodaniu soli. Podsumowując wyniki przeprowadzonych badań należy stwierdzić, że dodatek soli nieorganicznych oraz nośników wpływał korzystnie na żywotność i patogeniczność zarodników konidialnych dla drugiego stadium larwalnego wciornastka tytoniowa.