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Preliminary study of selected entomopathogenic fungi for corn leaf aphid, *Rhopalosiphum maidis*, control

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Abstract: *Rhopalosiphum maidis* Fitch. (Homoptera, Aphididae), corn leaf aphid, is a pest of maize (corn) and other related crops with worldwide distribution. This insect can infest all aboveground parts of the corn plant and cause serious damage to the yield. The aim of this study was to determine the pathogenicity of selected entomopathogenic fungi (*Beauveria bassiana* (Bals.-Criv.) Vuill., *Isaria fumosorosea* Wize and three different strains of *Metarhizium anisopliae* (Metschn.) Sorokin against *R. maidis* in the second nymphal stage under controlled conditions (23 °C temperature, 60% humidity). Maize local variety ‘Adjametis tetri’ was used for the assay. Maize seeds were grown in separate pots containing growth substrate mix. Two weeks old corn seedlings` stems and leaves were sprayed with suspension of entomopathogenic fungi at concentration 1.0×10^6 conidia/ml. Controls were sprayed with distilled water with 0.01% Tween 80 as a wetting agent. Ten minutes later, ten *R. maidis* aphids were confined on each corn seedling previously infested by fungal entomopathogens and pots were placed in micro-perforated polypropylene bags to avoid migration of the aphids from plant to plant. Aphid mortality and the progeny production were studied 7 days later. Four replicates have been done per treatment. Significantly high aphids’ mortality rate has been shown by *B. bassiana* and *I. fumosorosea* and relatively low influence was caused by *M. anisopliae* strains. Almost all studied fungi caused suppression in aphid progeny production, but in different extent. *B. bassiana* and strain MA2 caused highest suppression, while effect of strain MA3 on progeny production was not significantly different from the control sample. Number of aphids’ new generation after *I. fumosorosea* and MA1 treatment was almost the same.

Key words: biocontrol, *Beauveria bassiana*, *Isaria fumosorosea*, *Metarhizium anisopliae*

Introduction

Aphids are major pests of arable crops throughout the world. Usually, the majority of foliar insecticide applications to cereal crops are against aphids (Thomas *et al.*, 1996). The development of insecticide resistance in aphid populations (Foster *et al.*, 1996) and increasing public concern over deleterious effects of pesticide use on human health have led to investigations into alternative aphid control strategies.

Application of naturally occurring entomopathogenic fungi has the potential to enhance control of insect populations, including aphids. These strategies rely on the ability of the fungus to persist in host populations providing season-long control. The use of other methods, particularly inundation, has led to the development of entomopathogenic fungi as ‘mycoinsecticides’ for pest control (Charnley, 1989). Thus, the development of

entomopathogenic fungi as aphid biocontrol agents has received increasing interest as part of integrated pest control strategies (Milner, 1997).

Beauveria bassiana (Bals.) Vuill. with a broad host range of about 700 insect species is a registered biopesticide used for management of several crop insect pests (Butt *et al.*, 2001). The entomopathogenic fungus *Metarhizium anisopliae* has been reported to infect more than 100 insects (Krueger *et al.*, 1992; Bruck, 2005) and *Isaria fumosorosea* is a potential bioinsecticide for controlling various softbodied insects, including sweet potato whitefly (*Bemisia tabaci*), a severe pest causing direct damage to many economically important crops (Smith, 1993).

The aim of this study was to determine the pathogenicity of selected Georgian strains of entomopathogenic fungi – *B. bassiana* (Balsamo) Vuillemin, *I. fumosorosea* Wize, *M. anisopliae* (Metschnikoff) Sorokin – against *R. maidis* in the second nymphal stage under controlled conditions.

Material and methods

Fungal cultures

Fungal cultures were obtained from Georgian Agricultural University fungal culture collections: *Beauveria bassiana* was isolated from *Hishimonus sellatus* (Uhler), *Isaria fumosorosea* from *Hyphantria cunea* (Drury), and three different strains of *Metarhizium anisopliae* from soil by using *Anisoplia austriaca* (Herbst) larvi. For inocula production fungal strains were cultured on PDA Petri dishes from collection stored under mineral oil. Isolates were grown at ± 25 °C for 20 days. Conidia were harvested with sterile distilled water containing 0.01% Tween 80. Mycelia were removed by filtering conidia suspensions through sterile cheesecloth. Concentrations of the suspension were adjusted to 1.0×10^6 conidia/ml for each isolate by using Gorjaev haemocytometer.

Plant growth condition

Local Georgian maize variety ‘Adjametis tetri’ used for the study was obtained from the Genebank collections of Agricultural University of Georgia (AUG). Maize plants were grown in Terracult blue mix [Terracult GmbH, combination of TC3, TC6, TC7 with clay]. Seeds were planted ~ 1.5 cm deep in 7 × 7 cm pots and were placed in growth chamber at 23 °C temperature and 60% humidity. All experiments were conducted with two-weeks-old maize seedlings (V2-V3 growth stage), taking into account that the level of benzoxazinoids (defensive metabolites against insect feeding) (Cambier *et al.*, 2000), as well as defensive responses tend to decrease as the plant ages and most of the maize-aphids resistance studies are conducted on 2-3 weeks old seedlings (Betsiashvili *et al.*, 2015), when the plant is just getting more susceptible to insect feeding.

Aphid colony growth

R. maidis aphids were gathered in the maize field (Mukhrani Experimental Station, AUG) and the colony was maintained on ‘Adjametis tetri’, known susceptible variety to aphids (unpublished data) under controlled conditions (23 °C, 60% RH).

Experimental design

Two-weeks-old plants stem and leaves were sprayed with a suspension of entomopathogenic fungi at a concentration of 1.0×10^6 conidia/ml. Control versions were sprayed with distilled water. Ten minutes later, ten *R. maidis* aphids were confined on each corn seedling using

micro-perforated polypropylene bags to prevent migration of aphids from plant to plant. Evidence of fungal entomopathogens on dead insect bodies was verified by microscopic inspection for the presence of conidiogenous cells. Three replicates have been done per treatment.

Statistical analysis: Mortality data were corrected using Abbott's Formula (Abbott, 1925). Data were analyzed using the software GRAPHPAD PRISM 5 (GraphPad Software, Inc.). Differences in mortality caused by different fungal strains in comparison with control were assessed using one-way ANOVA and Tukey's multicomparison test. Results were considered as significantly different when ($P \leq 0.05$).

Results and discussion

Pathogenicity of selected fungal strains on aphids survival and the progeny production were studied 7 days after plants treatment. In case of maize seedlings treated with distilled water no mortality of the aphids was observed. Treatment of plants by fungal suspensions at a concentration of 1.0×10^6 conidia/ml caused insects' death. The highest aphids' mortality rate was revealed after plant treatment by *B. bassiana* and *I. fumosorosea* ($P < 0.05$) and relatively low effect was caused by *M. anisopliae* strains ($P \geq 0.05$) (Figure 1).

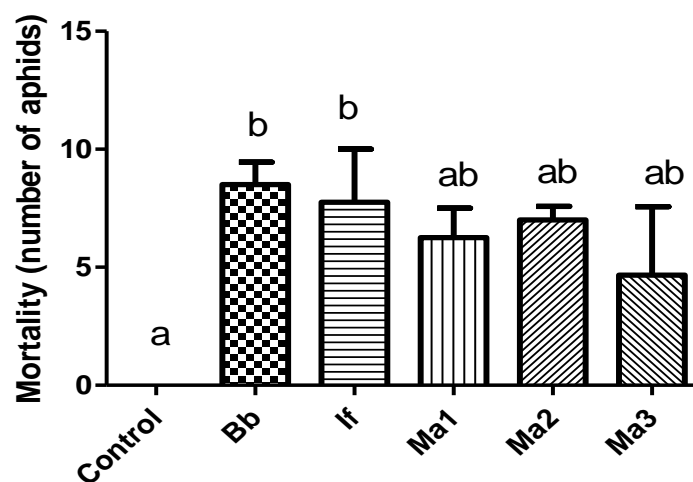


Figure 1. Mortality of *R. maidis* aphids caused by suspension of fungal entomopathogens [Bb – *B. bassiana*, If – *I. fumosorosea*, Ma1 – *M. anisopliae* (strain 1), Ma2 – *M. anisopliae* (strain 2), Ma 3 – *M. anisopliae* (strain 3)] at concentration 1.0×10^6 conidia/ml, 7 days after plant treatment.

Almost all studied fungi caused suppression in aphid progeny production – the highest was caused by *B. bassiana* and strain MA2 and lowest by strain MA3, but the result was not significantly different from the control sample ($P \geq 0.05$). The number of aphids' new generation after *I. fumosorosea* and MA1 treatment was almost identical ($P \geq 0.05$) (Figure 2).

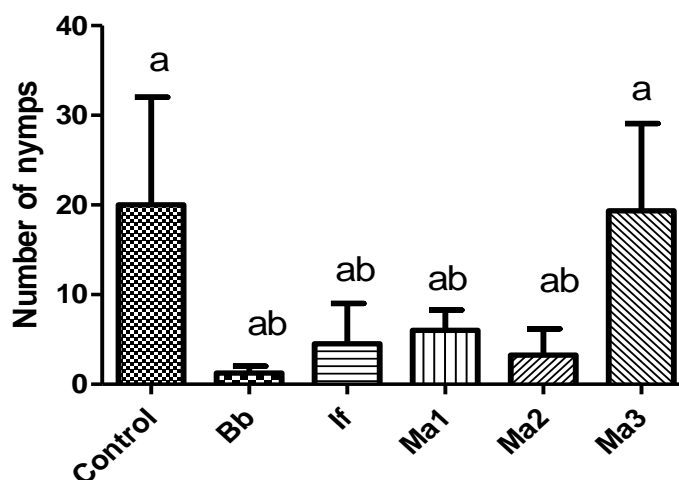


Figure. 2 *R. maidis* progeny production on corn seedlings after 7 days of treatment with different strains of fungal entomopathogens [Bb – *B. bassiana*, If – *I. fumosorosea*, Ma1 – *M. anisopliae* (strain 1), Ma2 – *M. anisopliae* (strain 2), Ma 3 – *M. anisopliae* (strain 3)] at concentration 1.0×10^6 conidia/ml.

In this study biocontrol potential of five Georgian strains of entomopathogenic fungi of three different species were evaluated. Results of our experiments showed that application of fungal entomopathogens on maize seedlings caused mortality of aphids and suppression of nymphs' production. *B. bassiana* and *I. fumosorosea* showed significant pathogenic effects on *R. maidis*, revealing adult aphids' highest mortality rate, while *B. bassiana* and strain MA2 caused highest suppression in aphids' progeny production.

On the contrary of our results, in some experiments (Moraes *et al.*, 2015), fungal entomopathogens didn't show promising effects for controlling *R. maidis* on genetically modified maize. However, other experiments (Khan *et al.*, 2012; Saruhan *et al.*, 2015) confirm that entomopathogenic fungi can be used with great success for both sap sucking pests, as well as pests with chewing mouth parts with no hazard effects on human health and the environment.

Ability of the fungal biocontrol agents to control aphid pest population on the early stage of maize development, before the insect spread become economically damaging is very important in current IPM context.

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