ABSTRACT
The B. bassiana is a fungus of many arthropods, including more than 200 species of insects and acaridae. When spores of the fungus come into contact with the body of an insect host, they germinate, enter the body, and grow inside, eventually killing the insect.

Two local strains, including B. bassiana-G07, which was isolated from grasshopper Oedaleus asiaticus, died on natural infection, and B. bassiana-G10, which was isolated from grasshopper Caliptomus abbreviates, died of soil borne infection, were detected and it was identified as species B. bassiana by PCR. SCAR primers OPB9 F/R677 and OPA15 F/R441 was specific to of B. bassiana.

The highest infection rate by B. bassiana-G07 and mortality was observed in variants of both concentrations 2.1 x 10^8 conidia/ml, 2.1 x 10^9 conidia/ml; where mortality reached 86.3-100%.

KEY WORDS: Beauveria bassiana, fungi, grasshopper, PCR

INTRODUCTION
The origins of the microbial pest control date back to the early nineteenth century, when the Italian scientist Agostino Bassi spent more than 30 years studying white muscardine disease in silkworms (BombyxmoriL.). He identified Beauveria bassiana (Bals.-Criv). Vuill., named in his honour, as the cause of the disease. The B. bassiana is a parasite of many arthropods, including more than 200 species of insects and acaridae. The disease caused by the fungus is called white muscardine disease [Fargues.J1997]. When spores of the fungus come into contact with the body of an insect host, they germinate, enter the body, and grow inside, eventually killing the insect.

Of approximately 750 species of fungi, two species such as Beauveria bassiana (B. bassiana) and Metarhizium anisopliae are mostly used for controlling harmful insects and as of 2007 a total of 58 biological preparations were produced by using fungus B. bassiana, and are being broadly used for controlling harmful insects in rangelands, forests, crop fields and greenhouses [Marcos.R.de Faria. 2007].

Fungus B. bassiana has not been studied and biological preparation of this fungus is not used in our country, it has been important to investigate the possibility of isolation of this fungus species and applicability of such preparation for controlling harmful insects in our country.
MATERIALS AND METHODS

Fungal strains

Two local strains (Table 1) of *B. bassiana* stored at the Microbiological laboratory in the Plant protection research institute were taken into this study. Strains of *B. bassiana* isolated from infected grasshoppers collected in 2007 and in 2010 from pasture.

<table>
<thead>
<tr>
<th>№</th>
<th>Strains</th>
<th>Insect host</th>
<th>Family</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B.b-G07</td>
<td><em>Oedaleus asiaticus</em></td>
<td>Acrididae</td>
<td>Mongolia</td>
</tr>
<tr>
<td>2</td>
<td>B.b-G10</td>
<td><em>Caliptamus abbreviates</em></td>
<td>Acrididae</td>
<td>Mongolia</td>
</tr>
</tbody>
</table>

Culturing the fungus;

From stocks, the culture was revived using the yeast extract peptone glucose agar (YPGA – with 2% glucose, 1% peptone, 1% yeast extract and 1.5% agar) slants. They were maintained in a chamber at 25 ± 0.5°C. The suspension of the conidia was swilled (solution 0.1% Tween-80) from 14 day old cultures for the experiments.

Insect assays;

Grasshoppers /Angaracris/ were collected from pasture outside Ulaanbaatar city, 5 day prior to testing and held in plastic vented containers (20 x 15 x 10 cm) containing fresh quitch leaves. There were ten grasshoppers per container (15 containers). Four concentration assays (at 2.1 x 10^6 conidia/ml, 2.1 x 10^7 conidia/ml, 2.1 x 10^8 conidia/ml, 2.1 x 10^9 conidia/ml 0.1% Tween-80 ). A control, 0.1% Tween-80 suspension, was included in each assay. The containers were kept in the laboratory at room temperature /22±3/ under natural light conditions. Mortality counts were taken every day for 14 days post treatment. Dead insect were removed daily and stored at cold storage /- 20°C/. Also Dead insect placed in Petri dishes covered with wet filtering paper for fungal emergence. Conidia from dead insects of each isolate were transferred separately to YPGA medium in a sterile condition. Light microscopic studies and colony form proved that recovered fungus is the same as inoculated fungus *B. bassiana*. Used Abbott's formula for control mortality in bioassays.

DNA extraction

From culture, 200μl of harvested culture should be placed in a 1.5 ml micro centrifuge tube and resuspended in 200 μl of TE buffer

From grasshopper;

30 mg of grasshopper tissue should be pulverized, placed in a 1.5 ml microcentrifuge tube and resuspended in 200 μl of TE buffer. Mix 200 μl of sample with 400 μl of lysis solution and incubate at 65°C for 5 min. Immediately add 600 μl of chloroform, gently emulsify by inversion (3-5 times) and centrifuge the sample at 10,000 rpm for 2 min.

Prepare precipitation solution by mixing 720 μl of sterile deionized water with 80 μl of supplied 10X concentrated Precipitation Solution. Transfer the upper aqueous phase containing DNA to a new tube and add 800 μl of freshly prepared precipitation solution, mix gently by several inversions at room temperature for 1-2 min and centrifuge at 10,000 rpm (~9400 x g) for 2 min.Remove supernatant completely (do not dry) and dissolve DNA pellet in 100 μl of sterile deionized water by gentle vortexing.

Add 300 μl of cold ethanol, let the DNA precipitate (10 min at -20°C) and spin down (10,000 rpm (~9400 x g), 3-4 min). Remove the ethanol. Wash the pellet once with 70% cold ethanol and dissolve DNA in 100 μl of sterile deionized water by gentle vortexing.

Primers

Two SCAR primers (Table 2) were synthesized by the Sigma.
Table 2

| №  | SCAR markers | Primers       | Sequence (5' – 3')
|----|--------------|---------------|---------------------
| 1  | SCA15_{441}  | OPA15 F_{441} | TTC CGA ACC CGG TTA AGA GAS |
|    |              | OPA15 R_{441} | TTC CGA ACC CAT CAT CCT GC |
| 2  | SCB9_{677}   | OPB9 F_{677}  | TGG GGG ACT CGC AAA CAG |
|    |              | OPB9 R_{677}  | TGG GGG ACT CAC TCC ACG |

PCR mixture and conditions:
In total of 25 μl of mixture, containing 12.5 μl Dream tag (Fermentas, USA), 1 μl of each primer (forward and reverse primers), 3 μl template DNA, 7.5 μl ddH2O.
The amplification profile was 2 min initial denaturation at 94°C, 10 cycles of denaturation at 94°C for 15 s, annealing at 63°C for 30 s and elongation at 72°C for 45 s; followed by 15 cycles of denaturation at 94°C for 15 s, annealing at 63°C for 30 s and elongation at 72°C for 45 s, with an additional 5 s for each successive cycle; and a final elongation at 72°C for 7 min. PCR products were harvested in 1% agarose gels stained with ethidium bromide.

RESULTS AND DISCUSSION
Insect assays
150 Grasshoppers /Angaracris/ were collected from pasture outside Ulaanbaatar city for use in this experiment. The efficacy of the isolates of *B. bassiana* was examined and their virulence against grasshoppers tested (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Strains</th>
<th>Concentration (conidia/ml)</th>
<th>Grasshoppers in days (Mean of four replicates)</th>
<th>Mortality % (by Abbott)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>B.b-G07</td>
<td>2.1 x 10^6</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1 x 10^7</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1 x 10^8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1 x 10^9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>0.1%Tween-80</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

The experiments showed that isolated the local strain *B.b - G07* is high virulence for the Angaracris. The highest infection rate by fungi and mortality was observed in variants of both concentrations 2.1 x 10^6 conidia/ml, 2.1 x 10^9 conidia/ml; where mortality reached 86.3-100%. Comparison of grasshopper mortality generally demonstrated the superiority of four concentrations and no significant difference was observed (Figure 1).

Figure 1. Mortality of grasshopper in test days
Dead insect for test time placed in Petri dishes covered with wet filtering paper for fungal emergence. Dishes were incubated at 25 ±0.5°C in an incubator for 7 days.

![Picture 1. Emergence of B. bassiana from dead Angaracris](image1)

After host death and utilization of all its internal nutrients, fungus emerged from insect body and produced aerial mycelia and conidia on it (Picture 1).

**Results of PCR**

Using the above-described PCR screening protocol, PCR assays two strains of *B. bassiana* /B.b-G07, B.b-G10/, one isolate from infected grasshopper cadavers (pending experiment), soil and healthy grasshopper showed that SCAR primers OPB9 F/R\(_{677}\) and OPA15 F/R\(_{441}\) was specific to of *B. bassiana* (Picture 2, 3).

![Picture 2. PCR products of DNA samples detected with B. bassiana – specific 677bp gene fragment (SCAR primer OPB9 F/R\(_{677}\)). M- Standard marker (100bp); 1- Grasshopper; 2- Strain B. bassiana-G07; 3- Isolate from infected grasshopper cadavers pending experiment of laboratory (B.b-G07 treated); 4- Strain B. bassiana-G10; 5. Healthy grasshopper, 6. Soil](image2)

![Picture 3. PCR products of DNA samples detected with B. bassiana – specific 441bp gene fragment (SCAR primer OPA15 F/R\(_{441}\)). M- Standard marker (100bp); 1- Grasshopper; 2- soil; 3- Strain B. bassiana-G07; 4- Isolate from infected grasshopper cadavers pending experiment of laboratory (B.b-G07 treated); 5- Strain B. bassiana-G10; 6. Healthy grasshopper](image3)
OPA-14 F/R445, OPB9 F/R677 and OPA15 F/R441 SCAR primers were highly sensitive, capable of detecting 100pg B. bassiana genomic DNA, and thus could be used to detect varying levels of the fungus in the field (Castrillo L.A.2003).

**SUMMARY**

1. Two local strains, including *B. bassiana-*G07, which was isolated from grasshopper *Oedaleus asiaticus*, died of natural infection, and *B. bassiana*-G10, which was isolated from grasshopper *Caliptomus abbreviates*, died of soil borne infection, were detected and it was identified as species *B. bassiana* by PCR. SCAR primers OPB9 F/R677 and OPA15 F/R441 was specific to of *B. bassiana*

2. The highest infection rate by *B. bassiana*-G07 and mortality was observed in variants of both concentrations 2.1 x 10^8 conidia/ml, 2.1 x 10^9 conidia/ml; where mortality reached 86.3-100%.

**REFERENCES**


