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Short note

Screening and virulence of Iranian isolates of *Beauveria bassiana* for potential management of Sunn Pest (*Eurygaster integriceps* Puton) in relation to cold activity

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Abstract
Environmental abiotic factors, such as low temperatures, restrict the application of entomopathogenic fungi well-known biological control agents against agricultural pests. Among 60 Iranian isolates collected in Central Iran that were exposed to cold stress, four isolates were identified as potential candidates for further investigation. One hundred percent of the conidia of these four isolates germinated and subsequently produced conidia. Their virulence against Sunn Pest (*Eurygaster integriceps* Puton) varied between isolates. After applying cold stress (4 °C), Vesh 1-8 isolate with sporulation of 6.5×10^7 conidia mL^{-1}, 50% mortality and LT_{50} in 3/4 days was the most cold-tolerant isolate. Cold-active germination and growth of the biocontrol fungal isolates are important to industrialize the products with a high potential against target pests in different environmental conditions.

Keywords
Beauveria bassiana, cold activity, Sunn Pest, *Eurygaster integriceps*, Iran

Introduction

Entomopathogenic fungi germinate and infect agricultural pest insects under environmental conditions. However, environmental abiotic factors can restrict the application of them for biological control. Considering that low temperatures can affect the virulence of entomopathogenic fungi against pests as well as their growth and sporulation, cold activity is defined as the ability to grow and germinate at low temperatures (as low as 5 °C) (Fernandes et al., 2008). The fungus *Beauveria bassiana* (Bals.) Vuill is an environmentally safe alternative to chemical pesticides to control agriculturally important pests. In this study, cold-active germination, growth and conidial production of Iranian *B. bassiana* isolates were studied after exposing to cold temperature along with their virulence against Sunn Pest *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae).

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Materials and methods

The isolates of *B. bassiana* were obtained from conidia collected in Sunn Pest cadavers and soil in the center of Iran including Esfahan, Kerman, Kermanshah, Markazi, Qum, Semnan and Tehran provinces in 2015-2017 (Parsi, 2019). The conidia were suspended in 10 mL of sterile distilled water with the addition of 0.02% Tween 80. The conidial suspensions were adjusted to 10^7 conidia mL^{-1}, and ten µL of conidial suspension was plated on quarter Sabouraud dextrose agar (¼SDA) medium. The plates were kept at 4 °C and microscopic observations of germination were recorded after 7 days (Shin et al., 2017). The radial growth (cm) of obtained isolates was measured after 30 days (Davari et al., 2018). Only four out of 60 isolates of *B. bassiana* which produced conidia at 4 °C were selected in this study. The controls were incubated at 25 °C (no cold stress). The virulence of the selected four isolates were tested against Sunn Pest *E. integriceps*. Ten adults in each plastic lab container were sprayed with 30 mL conidial suspension (10^7 conidia mL^{-1}) in sterile distilled water with the addition of 0.03% Tween 80 and the control sprayed only with water containing the same concentration of Tween 80. Each treatment was replicated three times and repeated on three separate days.

Comparison between the cold treatment and control in each isolate was performed with analysis of Fisher's least-significant-difference (LSD) test (Webster, 2007), using SAS software. Data were expressed as means ± standard error (SE) and statistical significance was set at the conventional $\alpha < 0.05$ level.

Total DNA of the superior isolate (Vesh 1-8) was extracted, and the internal transcribed spacer (ITS) region was amplified by PCR using the following universal primers; ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). PCR products of 600-700 bp were generated corresponding to the ribosomal DNA (ITS1, ITS2 and 5.8S rRNA). BLAST searches of the GeneBank nucleotide database were done, and Vesh 1-8 isolate sequence was deposited in the GeneBank (Table 1).

Results and discussion

Out of 60 isolates exposed to the cold stress (4 °C), only four isolates, including Vesh 1-8, N 4-2, V-8 and Virich 3-3 (with 100% germination), produced conidia after 30 days (Figs. 1a,b).

Although, the conidial production decreased in all four isolates in comparison with the controls (Fig. 1b); only the conidial production of the isolate Vesh V-8 (4.5×10^7 conidia mL^{-1}) was not significantly different from the control (Fig. 1b). On the other hand, the fungal growth rate declined in all four isolates exposed to the cold stress, and the results showed significant differences compared with the control (Fig. 1c). Based on the different reduction pattern (Figs 1b,c), it can be concluded that the responses to the abiotic factors are strain-dependent (Borisade and Magan, 2014). In virulence assay, the four isolates showed 36-50% accumulated mortality rates with median lethal time (LT_{50} day) 3.4-5.2 days, within 14 days (Table 1).

The isolates Vesh 1-8 and V-8 exhibited the highest virulence against Sunn Pest *E. integriceps* with 50% and 40% accumulated mortality rates, respectively (Table 1). Vesh 1-8 isolate with high mortality (Table 1), germination and conidial production (Figs 1a,b) showed the highest activity after the treatment at 4 °C.

Although the selection of entomopathogenic fungi is done based on their virulence against target pests, survival outside the hosts and sensitivity to abiotic factors are crucial to their efficacy (Lee et al., 2015; Mossavi and Minassian, 2021). Therefore, field studies need to be conducted to evaluate their survival and ability to spread in the natural environment (Karimi et al., 2019). Cold activity of entomopathogenic fungi is as important as their thermotolerance (Parsi and Peighami Ashnaei, 2022),

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Table 1 - Virulence of the four isolates was evaluated against Sunn Pest (*E. integriceps*). The accumulated mortality rate and median lethal time (LT_{50} day) were calculated at 4 days and within 14 days, respectively.

Beauveria bassiana	**Mortality%±SE***	**LT_{50} (d)**	**Accession Number**
Vesh 1-8	50.0 ± 0.6^{ab}	3.4	OM760875
Virich 3-3	36.6 ± 0.7^{b}	5.2	-
V-8	40.0 ± 0.0^{ab}	5.0	-
N4-2	36.6 ± 0.7^{b}	4.9	-
GHA (standard isolate)**	56.6 ± 0.7^{a}	3.6	MZ951125
Tween 80 (0.03%)	0	0	

*Values followed by different letters are significantly different (LSD, 5%); **(BotaniGard wettable powder, USA from Parker et al., 2003)

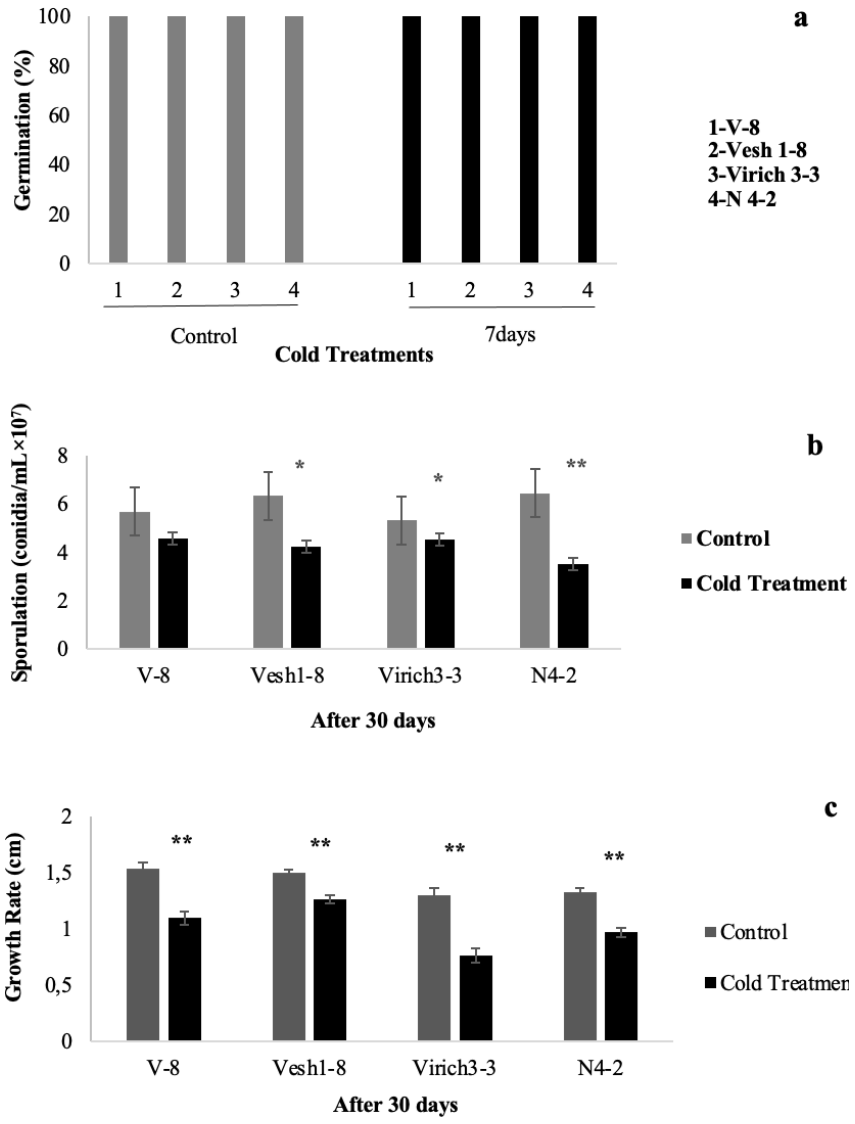

Fig 1. - Conidial germination (a) of four isolates of *Beauveria bassiana* (Vesh 1-8, N 4-2, V-8 and Virich 3-3) after exposure to 4 °C for 7 days; conidial production (b) and growth rate (c) of the four isolates after exposure to 4 °C for 30 days; control in 25 °C. Bars on the columns indicate standard error. Significant differences * = $P < 0.05$, ** = $P < 0.005$.

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to control both the overwintering adults and the summer generation in the crop (Edgington et al., 2007). Investigating and choosing the native entomopathogenic fungi to survive in a given location can be most promising to industrialize biological products (Lee et al., 2015). In future studies, the assessment of the expression of genes related to cold tolerance will highlight the genetic mechanisms involved in cold activity of the *B. bassiana* Iranian isolates studied here.

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