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Laboratory Evaluation of *Beauveria bassiana* Isolates on Red Flour Beetle *Tribolium castaneum* and Their Characterization by Random Amplified Polymorphic DNA

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ABSTRACT

***Beauveria bassiana* (Balsamo) Vuillemin is an important natural regulator of insect populations. Identification of a suitable molecular marker for detecting a virulent phenotype on a target pest would be useful in screening for effective isolates against the pest. Nine isolates of *B. bassiana* were tested for their virulence to adults of *Tribolium castaneum* (Herbst) in laboratory bioassay with 1×10^8 conidia mL^{-1}. DNA markers provide more detailed genomic information. DNA fingerprints were generated by RAPD markers. Fungal DNA was extracted by CTAB. Twelve random oligonucleotide primers were used for amplification. After bioassay, three arbitrary categories of isolates were chosen i.e. isolates that caused > 45%, 45-30% and < 30% mortality, and were classified as highly (H), moderately (M), and less (L) virulent isolates based on average mortality, respectively. Also, based on LT_{50} values, three arbitrary categories were chosen i.e. isolates with < 80 h, 80-100 h and > 100 h LT_{50} values, and were classified as highly (H), moderately (M), and less (L) virulent isolates, respectively. The results of bioassay showed that isolates IRAN 440C and DEBI 004 were the causative agents of mycoses with the highest and lowest lethal effect, respectively. The lowest LT_{50} value was related to DEBI 014. Cluster analysis of the RAPD data showed four clusters according to similarity, following cluster analysis using the Jaccard similarity coefficient and clustering was done using un-weighted pair group method with arithmetic (UPGMA). The results showed that there was genetic diversity between these isolates, but the groups based on virulence rating and LT_{50} values did not match with the RAPD clusters completely.**

Keywords: Entomopathogenic fungus, Virulence, Bioassay, *Tribolium castaneum*, RAPD analysis.

INTRODUCTION

Red flour beetle, *T. castaneum* (Herbst 1798) (Col.; Tenebrionidae) is a polyphagous, cosmopolitan pest in flour mills and wherever dried foods are processed or stored. Although its pest status is considered to be secondary, requiring prior infestation by an internal feeder, it can readily infest wheat or other grains damaged in the harvesting operation (Farrell, 2010).

Chemical control is the most commonly used method in insect pest management. Due to adverse effects on non-target organisms, toxicity to mammals and birds, and the risk of environmental pollution, chemical control measure should be replaced by the other environmentally friendly control methods to refrain from consumption and other ways it can be replaced (Fields, 1998). The pesticide

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revolution began in the early 1940s with the development of synthetic pesticides. These pesticides showed a remarkable ability to kill pests without any apparent side-effects. Biological, cultural, and mechanical controls were often underutilized or disregarded as viable pest management strategies. Although pesticides provided a short-term solution for many pest problems, the long-term negative effects of using pesticides did not begin to surface until the late 1950s (Rechcigl and Rechcigl, 2000).

Also, there are several reports showing that *T. castaneum* is resistant to many insecticides, such as malathion (Parkin, 1965). Therefore, with the current urgent and conflicting goals of reduced pesticide usage while maintaining adequate agricultural production, microbial control agents with selectivity and a low environmental impact could become ideal components of integrated pest management programs (IPM) in this century (Lacey and Goettel, 1995). *T. castaneum* was chosen for this study as a suitable indicator species for testing the effects of pesticides on insects.

Entomopathogenic fungi were among the first organisms to be used for biological control of pests. More than 700 fungal species from about 90 genera are pathogenic to insects (Hong, 2003). *Beauveria bassiana* (Balsamo) Vuillemin is the most important entomopathogenic fungus, which is an anamorphic form from phylum Ascomycota (Hypocreales: Cordycipitaceae). It may have host-specific strains that are virulent to pests but are not hazardous to non-target organisms. Its spores may be formulated and applied in a similar way as chemical pesticides and, therefore, could be adopted as a new technology. This includes oil-based formulations and ultra-low volume application, and they are cheap to produce and may provide low-cost control (Langle, 2006).

Distinctive markers that characterize individual isolates would be useful to determine their efficacy, host specificity, survival and spatial and spatial-temporal distribution in the field (Lacey and Goettel, 1995). Molecular diagnostics (typing) of *B. bassiana* isolates have been attempted by several methods, for example: isozyme (Castrillo and Brooks, 1998) and esterase profiles (Varela and Morales 1996), telomeric fingerprinting (Couteaudier and Viaud, 1997), polymorphisms in internal transcribed spacer regions of rDNA (Glare and Inwood, 1998) and random amplified polymorphic DNA (RAPD) analysis (Maurer *et al.,* 1997; Berretta *et al*., 1998; Castrillo and Brooks, 1998; Glare and Inwood, 1998; Luz *et al*., 1998).

DNA markers provide more detailed genomic information than isozymes do and they are not influenced by environmental or culture conditions (Tigano-Milani *et al*., 1995). RAPD-polymerase chain reaction (PCR) technology (Welsh and McClelland, 1990; Williams *et al*., 1990) has been utilized to identify species and strains of entomopathogenic fungi (Bidochka *et al*., 1994). This technique is highly sensitive to nucleotide differences between template DNA and the primer, allowing detection of single nucleotide changes (Williams *et al*., 1990). This feature makes RAPD markers ideal for detecting variation in closely related individuals and in less polymorphism species (Williams *et al*., 1991). Several reports have been studied using RAPD technique as a screening for genetic variation within several species of fungi like the genus *Alternaria* (Cooke *et al*., 1998); *Verticillium lecanii* (Zimmerman) and *B. bassiana* (Maurer *et al*., 1997); *Metarhizium anisopliae* (Metchnikoff) (Tigano-Milani *et al*., 1995), and *Fusarium mangiferae* (Britz) (Iqbal *et al*., 2006). Many researchers used this marker for detecting relation between genetic diversity and geographical origin, host or virulence against some pest (Berretta *et al*., 1998; Kaur and Padmaja, 2008).

In this study, we tested virulence and genetic diversity of different *B. bassiana* isolates and the relationship between virulence of these isolates and molecular clustering by using RAPD-PCR technique. Identification of a molecular marker linked to a virulent phenotype on a target pest would

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be useful in screening for effective isolates against the pest. The purpose of present research was to determine this relationship for selecting the most effective isolate without time consuming bioassay approach.

MATERIALS AND METHODS

Insect Rearing

Adult insects of *T. castaneum* were reared in plastic container (32×22×10 cm) and their doors were covered with mesh fabric. One third of the height of container was filled with wheat flour for adult feeding. Adults, 1 to 3 weeks old, were used for all bioassays. Beetles within this range of ages were obtained by harvesting pupae and holding them for 16 to 21 days at 26±1˚C, 70±5% RH, and a photoperiod of 16:8 (L: D) h before bioassay.

Fungal Isolates

The virulence of nine isolates of *B. bassiana* was investigated against *T. castaneum*. Also, we used newly obtained isolates that were not examined on this stored pest (Table 1).

Fungal Culture

All of the isolates were cultured on Sabouraud's dextrose agar with yeast extract (SDAY) at 25±2°C and a photoperiod 16:8 (L: D) h. Conidia from 21-day-old fungal cultures were used in the laboratory bioassays and DNA extraction.

Preparation of Conidial Suspension

Mycelium and conidia were moved into a tube with a scalpel, and 20 mL sterile water was added. Suspension was vortexed to ensure an even distribution of the conidia prior to use, then, it was filtered through mesh fabric in order to separate mycelium and transferred to a sterile tube. After that, 0.01% (v/v) Tween 80 (Sigma Chemical, St. Louis, Mo, USA) was added to all concentrations. The concentration of conidia was determined by counting with a haemocytometer (Paul Marienfeld GmbH and Co. KG, Germany). Most of isolates had LC_{50} values about 1×10^{8} conidia mL^{-1}, therefore, this concentration was used for comparison of their effects (Golshan *et al*., 2013)

Conidial Germination

To evaluate conidial germination, 1 mL of suspension (1×10^{7} conidia mL^{-1}) was dropped on SDAY medium in each Petri dish. These Petri dishes were incubated at 25±2°C, and a photoperiod of 16:8 (L:D) h for 18 h. Afterwards, 1 mL of formaldehyde 0.5% was poured into Petri dishes to stop germinated spores and, then, germination

Table 1. Isolates of fungus used in this study.

Isolate	Host	Location area
DEBI 004	*Hyper postica* Gillenhal (Col.: Curculionidae)	Ghazvin (Alfalfa fields)
DEBI 005	Tenebrionidae (Insecta: Coleopteran)	Ghazvin (Alfalfa fields)
DEBI 014	*Lixus incanescens* Boh. (Col.: Curculionidae)	Ghazvin (Keshtosanate hezar jolfa)
EUT 116	*Galleria mellonela* Linnaeus (Lep.: Pyralidae)	Tehran
IRAN 187C	*Leptinotarsa decemlineata* Say (Col.: Chrysomelidae)	Ardebil
IRAN 440C	Soil	Atashgah (Karaj)
IRAN 441C	*Rynchophorus ferrugineus* Olivier (Col.: Curculionidae)	Saravan
IRAN 428C	*Chilo suppressalis* Walker (Lep.: Crambidae)	Rasht
IRAN 429C	*Chilo suppressalis* Walker (Lep.: Crambidae)	Hassan rood (Anzali)

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was checked under a microscope and results were recorded. Each experiment was replicated three times. All suspensions displayed >85% germination of conidia and were stored at 4˚C until used.

Bioassays

Virulence of *B. bassiana* isolates were tested on adults of *T. castaneum*. Fifteen adults were treated by fungal suspension for 20 seconds with submerging method. The impact of fungal isolates on *T. castaneum* was evaluated at 1×10^8 conidia mL^{-1}. Control insects were treated with 0.01% Tween 80, Sigma (Sigma, St. Louis, MO) solution in sterile distilled water. Treated insects were placed in incubator (25±2˚C and 85±5% RH). At the second day of incubation, wheat flour was added to each Petri dish. The dead adults after 24 hours were removed from experiment and were kept separately for appearance of the fungus in Petri dish with moistened filter paper and high RH in the desiccator. Mortality was recorded for 14 days. The bioassay was repeated three times. Experiments were performed according to the completely randomized design (CRD).

Molecular Techniques

DNA Extraction

Mixture of the mycelium and conidia of each isolate was powdered with liquid nitrogen by using mortar and pestle. The powder was extracted with 700 volumes of extraction buffer (PVP 1%, Tris-base 100M, EDTA-Na_2 20 Mm, NaCL 1.4M and CTAB 2%) and kept at 65°C for 2 hours. An equal volume of chloroform:isoamyl alcohol (24:1, by vol.) was added to the slurry. It was mixed gently and then centrifuged for 15 minutes at 13,000g. The aqueous phase was re-extracted with an equal volume of chloroform:isoamyl alcohol (24:1, by vol.). The separated aqueous phase was precipitated by 0.6 volume of isopropanol. This mixture was kept at -22°C for 30 minutes. DNA was washed with 70% (v/v) ethanol and dissolved in sterile double distilled water and RNase (50 µg mL^{-1}) and was incubated at 37°C for 20 minutes. In all cases, purity and quantity of DNA in the samples were estimated using a UV spectrophotometer and checked in ethidium bromide-stained 1% agarose TAE gels. DNA samples were diluted to 25 ng of DNA/µl.

In this study, we examined twenty arbitrary primers, but, among them, we selected twelve primers that showed the best polymorphism (Table 2). PCR reactions were carried out with 10 ng genomic DNA in Thermal cycler (Eppendrof). The reaction mixture contained 3 µl $MgCl_2$, 1 µl of dNTP mix, 1 µl of primer (10 mM stock), 1 µl of template DNA (25 ng), 2.5 µl of Buffer PCR, 0.3 µl of *Taq* DNA polymerase and 12.5 µl of sterile double-distilled water in a final volume of 25 μl. PCR conditions were 1 cycle of 94°C for 5 minutes, denaturation at 94°C for 20 seconds, annealing at 30°C for 35 minutes, extension at 72°C for 1 minute, for 40 cycles, followed by final extension at 72°C for 7 minutes. The amplification products were resolved by electrophoresis in a 1% agarose gel at 85 volts for 2 hours with 1X TAE buffer (TAE 5X: Tris-base, 242 gr, EDTA 0.5M, 100 mL and Acetic acid, 57.1 mL, PH= 8). PCR products were visualized and photographed

Table 2. List of RAPD primers used in this study.

Primer	Sequence
OPA-09	5'-GGGTAACGCC-3'
OPA-10	5'-GTGATCGCAG-3'
OPA-11	5'-CAATCGCCGT-3'
OPB-07	5'-GGTGACGCAG-3'
OPD-01	5'-ACCGCGAAGG-3'
OPD-04	5'-TCTGGTGAGG-3'
OPR-11	5'-GTAGCCGTCT-3'
OPS-03	5'-CTACTGCGCT-3'
OPT-14	5'-AATGCCGCAG-3'
OPT-17	5'-CCAACGTCGT-3'
OPQ-14	5'-GGACGCTTCA-3'
OPQ-17	5'-GAAGCCCTTG-3'

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under UV light using a Polaroid camera (Intas).

Statistical Analysis

Bioassays

LT_{50} values and the mean percentage mortality of all replicates for each isolate was calculated and used to selection three arbitrary categories (Berretta *et al*., 1998; Kaur and Padmaja, 2008). Isolates that caused > 45%, 45-30%, and < 30% mortality were classified as highly (H), moderately (M) and less (L) aggressive isolates based on average mortality, respectively (Table 3). Also, isolates with <80h, 80-100h, and >100h LT_{50} values were classified as highly (H), moderately (M) and less (L) virulent, respectively (Table 4). Mortality percentage was normalized using arcsin transformation and subjected to analysis of variance (ANOVA) using SAS (2002). Means within groups of treatments were separated by the Fisher's protected LSD test (SPSS, 2004). Probit analysis was used to calculate values of LT_{50} for each isolate (SAS, 2002).

RAPD-PCR Analysis

A binary matrix for presence or absence (1/0) of all the polymorphs characterized by specific molecular weights in the isolates with each primer was compiled. The data were analyzed with the NTSYS.PC (numerical taxonomy and multivariate analysis system,) version 2.0 software (Rohlf, 1987). A similarity matrix was generated using the SIMQUAL program and Jaccard's similarity coefficient. A dendrogram displaying the similarities between the isolates was generated using the SAHN program by UPGMA (un-weighted pair group method with arithmetic) (Sneath and Sokal, 1973).

Table 3. Arbitrary rating of percentage mortality in concentration 1×10^8 conidia mL^{-1}.

Isolates	% Mortality±SE[a]	Arbitrary rating[b]
EUT 116	48.89 ± 4.44 ab	H
DEBI 004	15.55 ± 2.22 cd	L
DEBI 005	51.11 ± 4.44 ab	H
DEBI 014	42.22± 5.88 b	M
IRAN 187C	17.78 ± 5.88 cd	L
IRAN 440C	60.00± 7.70 a	H
IRAN 441C	24.45 ± 2.22 c	L
IRAN 428C	42.22 ± 2.22 b	M
IRAN 429C	42.22 ± 4.45 b	M

[a] Mean percentage mortality at concentration of 1×10^8 conidia mL^{-1}, [b] L: Less virulent; M: Moderately virulent, H: Highly virulent.

RESULTS

Bioassays

The results showed that the virulence of nine isolates of *B. bassiana* against adults of *T. castaneum* in laboratory was differed among treatments (F= 13.47; df= 9, 29; P< 0/0001). At concentration of 1×10^8 conidia mL^{-1}, isolate IRAN 440C resulted in the highest mortality percentage (60%) and showed the higher virulence compared with other isolates (Table 3). The LT_{50} values for these isolates varied from 70 to 120 hours and the lowest LT_{50} value was related to DEBI 014 (Table 4). No mortality was observed in the control. According to mortality rates and LT_{50} values, these isolates were classified in three groups: highly (m>45%, LT_{50}<80h), moderately (m= 30-45%, LT_{50}= 80-100 h) and less (m< 30%, LT_{50}< 100 h) virulent isolates, respectively. The effective isolates were IRAN 440C, DEBI 005 and EUT 116 (Table 3).

RAPD-PCR Analysis

Twelve arbitrary primers (Table 2) showed amplification and polymorphism. An example of RAPD markers generated by primer OPT-14 with *B. bassiana* isolates is shown in Figure 1. A total of 227 characters were pooled from all 12 primers, with an

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Table 4. Arbitrary rating of LT_{50} in concentration 1×10^8 conidia mL^{-1}.

Isolates	LT_{50} (in hours)	χ^2	Slope±SE[a]	Fiducial limits	Arbitrary rating [b]
EUT 116	94.60	39.09	8.31 ± 1.33	137.04-262.08	L
DEBI 004	84.48	36.80	4.61 ± 0.76	74.88-146.4	M
DEBI 005	119.04	22.52	5.64 ± 1.19	79.44-199.52	L
DEBI 014	69.12	44.87	2.85 ± 0.43	48.48-88.56	H
IRAN 187C	98.88	32.51	4.62 ± 0.81	72.72-149.04	M
IRAN 440C	76.32	50.67	6.88 ± 0.97	119.76-210.48	H
IRAN 441C	77.28	46.74	3.21 ± 0.47	54.96-99.12	H
IRAN 428C	107.52	42.14	6.01 ± 0.93	100.8-187.92	L
IRAN 429C	75.84	14.27	2.96 ± 0.78	34.32-108.00	H

[a] LT_{50} at concentration of 1×10^8 conidia mL^{-1}, [b] L: Less virulent; M: Medium virulent, H: Highly virulent.

average of 18.92 characters scored per primer and the analysis produced a dendrogram (Figure 2). RAPD analysis indicated diversity between nine isolates used in this study. Molecular weights ranged from 294 to 5,249 bp. Cluster analysis based on similarities using the UPGMA method evidenced four groups with most basal bifurcation at 0.36 similarities (Figure 2).

The first and fourth groups had one isolate, EUT 116 and IRAN 187C, respectively. The second group branched out as two subgroups: the first subgroup included isolate IRAN 441C and the second subgroup consisted of isolates IRAN 428C and IRAN 429C. Isolates DEBI 004, DEBI 005 and DEBI 014 were clustered in the first subgroup of the third group and isolate IRAN 440C was separated out singly in another subgroup of this group (Figure 2).

RAPD data were analyzed by first generating the average distances between each pair of isolates (Table 5). The coefficient values were generated and the data in Table 5 represents similarity values between the isolates. The simple matching similarity coefficients for pairwise comparisons of the isolates varied from

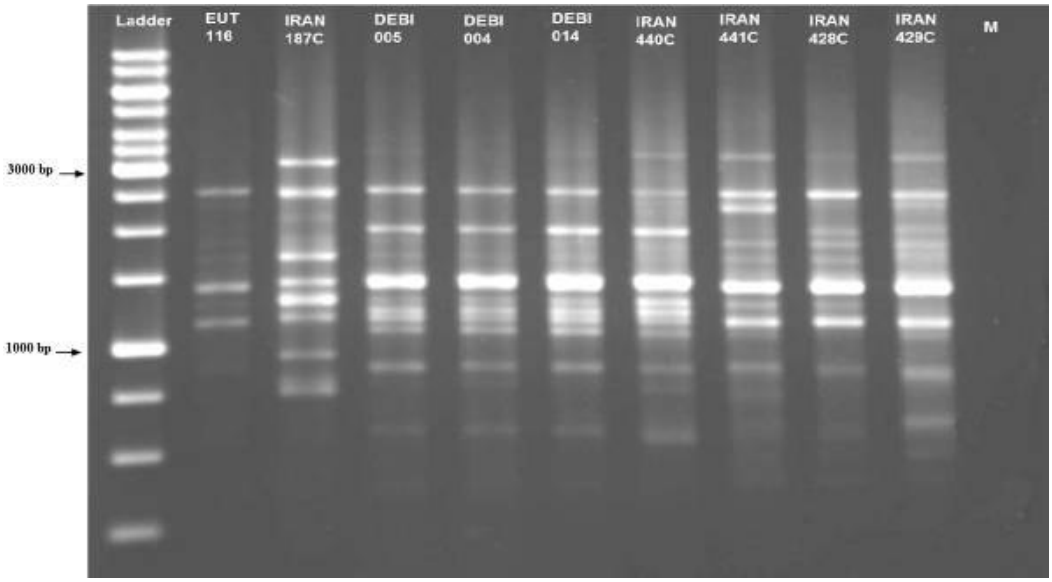

Figure 1. RAPD bands generated with primer OPT-14 and DNA from nine *B. bassiana* isolates. M: Negative control (without template DNA).

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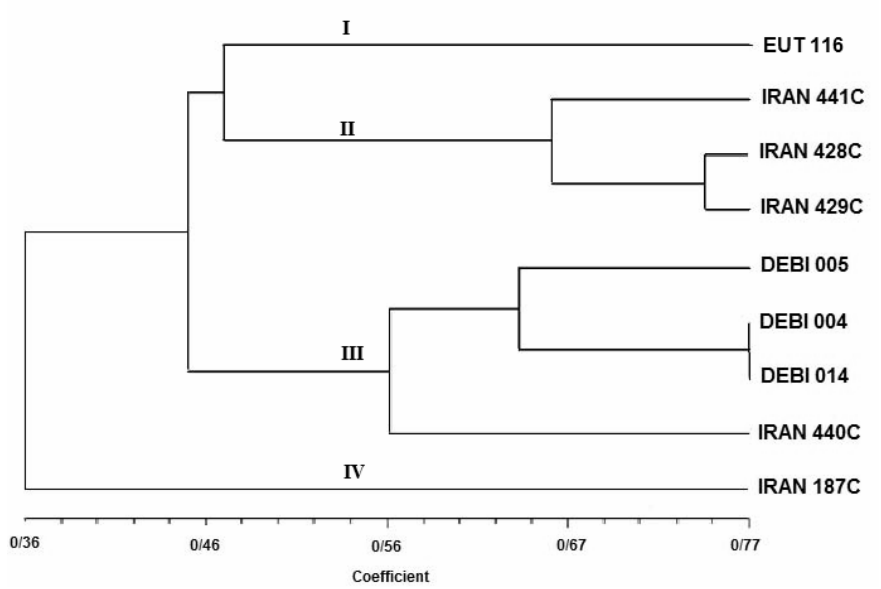

Figure 2. Dendrogram of nine isolate of *B.bassiana* using twelve primers by UPGMA based on simple matching coefficients.

29.24 to 77.14%. The maximum similarity value of (77.14%) was observed between isolates DEBI 004 and DEBI 014.

Correlation of RAPD data with virulence of fungal isolates showed that two isolates i.e. IRAN 428C and IRAN 429C, from the second molecular group and isolates IRAN 440C and DEBI 005 from the third molecular group (Figure 2) belonged to the highly virulent group. However, two isolates, namely, DEBI 004 and DEBI 014 with similar genetic characters were categorized in different virulent groups. The group with less virulence included three isolates i.e. DEBI 004, IRAN 187C, and IRAN 441C, which belonged to the third, fourth, and second molecular groups, respectively (Table 3). Correlation of RAPD data with LT_{50} values indicated that two isolates IRAN 441C and IRAN 429C from the second molecular group were in the highly virulent group and isolates IRAN 187C from the fourth group and EUT 116 from the first group were categorized in moderately virulent group (Table 4).

DISCUSSION

The pathogenicity of nine isolates of *B. bassiana* from different hosts and sources varied in their virulence towards adults of *T. castaneum*. Our results were in agreement with previous reports that showed low virulence of *B. bassiana* to *Tribolium* (Akbar *et al*., 2004; Wakefield, 2006; Khashaveh *et al*., 2011). Also, some authors investigated the virulence of different isolates of this fungus against other stored product pests, such as Mahdneshin *et al*. (2011) who evaluated the effect of isolate IRAN 441C of *B. bassiana* against *Callosobruchus maculatus* (Fabricius) (Col., Bruchidae). Their results showed that this isolate had high virulence against this stored product pests. This finding was inconsistent with our results. These can be due to differences in the studied isolates and also the stored product pests species. *Tribolium* species are known to

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Table 5. Simple matching coefficients of *B. bassiana* isolates as revealed by UPGMA tree analysis

EUT 116	...									
IRAN 187C	29.24	...								
DEBI 005	43.80	32.28	...							
DEBI 004	38.10	30.00	62.26	...						
DEBI 014	40.91	33.91	65.77	77.14	...					
IRAN 440C	40.25	41.36	53.96	52.74	63.19	...				
IRAN 441C	53.97	37.71	40.30	42.65	45.07	49.08	...			
IRAN 428C	44.76	42.37	44.20	44.68	48.97	53.33	68.15	...		
IRAN 429C	42.55	39.33	45.86	45.65	46.90	52.44	63.70	74.63	...	
	EUT 116	IRAN 187C	DEBI 005	DEBI 004	DEBI 014	IRAN 440C	IRAN 441C	IRAN 428C	IRAN 429C	

produce defensive quinones and it is possible that these chemicals may also inhibit germination. Inhibition of yeast and bacterial growth by the defensive secretions from *Tribolium* spp. has been already shown (Prendeville and Stevens, 2002).

The phylogenetic characterization of *B. bassiana* at the molecular level is of immense importance for understanding genomic organization, genetic variation, and gene stability (Kosir *et al*., 1991). Leal *et al*. (1994) found high similarity (60%) among 20 isolates of *M. anisopliae,* by RAPD-PCR method. Characterization of the isolates based on morphological or biochemical parameters alone would be inadequate for understanding the genetic basis for character differentiation (Kaur and Padmaja, 2008). Molecular markers have been used widely to consider host pathogen relationships among entomopathogenic fungi. According to the mentioned cases, in this study, RAPD marker was used to evaluate genetic diversity. Cluster analysis is a hierarchical method that will place isolates in groups with some level of implicit relatedness. The dendrogram from RAPD data clearly gave comparable results and showed that there were homogenous populations within the very diverse species *B. bassiana.*

Kaur and Padmaja (2008) have shown by RAPD analysis that there is no correlation between the virulence of the isolates against second instar larvae of *Spodoptera litura* (Fabricius) and the relatedness of the original insect host. But, our results showed less correlation between the pathogenicity of the isolates against *T. castaneum*. This difference may be related to an important sequence of primers because the sequences were selected arbitrary. Devi *et al*. (2001) reported no correlation between aggressiveness of the isolates and the relatedness of the original insect host, but a correlation was found between the RAPD grouping and the phenotypic classification of the isolates. Luz *et al*. (1998) found a similar situation in *B. bassiana* isolates tested against *Triatoma infestans.* The virulence of the isolates was not correlated

to phylogenetic groups in cluster analysis of RAPD markers. Rivera *et al*. (1997) and Valderrama *et al*. (2000) found no correlation between the clusters obtained by RAPD analysis of the insect host and the virulence of *Hypothenemus hampei* (Ferrari). Muro *et al*. (2003) revealed no significant correlation between the isolates and host and geographical origin, but AFLP technique revealed clonal populations of *B. bassiana* within Kenya.

In the study of Berretta *et al*. (1998), isolates of *B. bassiana* were highly virulent (four out of six) to *Diatraea saccharalis* (Fabricius) formed one phonetic group with 85% similarity. Castrillo and Brooks (1998) used isozymes and RAPD and detected variation among 24 *B. bassiana* isolates. Further, they reported better resolution of the differences between the strains with respect to RAPD markers. *B. bassiana* isolates in several instances collected from the same insect species and from the same region were genetically dissimilar (Berretta *et al*., 1998) or similar genetic types were described from widely separated geographic locations (Bidochka *et al*., 1994). Clear relationship between the population structure of *B. bassiana* and some defined host species was shown by RFLP and RAPD analysis (Maurer *et al*., 1997).

Understanding the processes that drive genetic changes is important in the development of control strategies (McDonald *et al*., 1989), as well as in the selection and evaluation of biocontrol agents. This study showed no complete correlation between virulence and RAPD analysis. It can be due to sequence of primers used to amplify. Therefore, it seems that some important part of DNA was not amplified. According to the results of this study and other researches, RAPD-PCR is a good marker for revealing genetic diversity, but, other methods like RFLP, AFLP (Muro *et al*., 2003), minisatellite locus markers, telomere fingerprinting and SCAR markers (Castrillo *et al*., 2003) should be used to provide more accurate results for differentiating the isolates and characterizing their phylogenetic relationships.

Finding a suitable marker for screening an effective isolate can be very useful and may be performed by bioassay or genetic method. Fungal bioassay is a time-consuming method. Because of the dependence of all features of organisms on their genetic characteristics, the genetic methods can be used since they are very fast and accurate.

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بررسی آزمایشگاهی جدایه‌های قارچ *Beauveria bassiana* بر روی سوسک قرمز آرد *Tribolium castaneum* و طبقه‌بندی آنها بوسیله تکثیر تصادفی قطعات DNA

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چکیده

قارچ *Beauveria bassiana* (Balsamo) Vuilemin یکی از مهمترین تنظیم‌کننده‌های طبیعی جمعیت حشرات است. شناسایی یک نشانگر مولکولی مناسب برای شناسایی فنوتیپ بیماریزا جهت انتخاب موثرترین جدایه بر روی آفت هدف، مفید می‌باشد. جهت بررسی شدت بیماریزایی قارچ *B. bassiana* در غلظت 1×10^{8} کنیدی در میلی‌لیتر بر روی حشرات کامل *Tribolium castaneum* (Herbst) در شرایط آزمایشگاهی، نه جدایه از این قارچ انتخاب شد. نشانگرهای DNA، اطلاعات ژنومی فراوانی فراهم می‌کنند .انگشت‌نگاری DNA توسط نشانگرهای RAPD و استخراج DNA قارچ‌ها به روش CTAB انجام شد. جهت انگشت‌نگاری از ۱۲آغازگر الیگونئوکلوتیدی تصادفی استفاده شد. بعد از انجام آزمایشات زیست‌سنجی، بر اساس میانگین مرگ و میر سه طبقه‌بندی اختیاری - جدایه های با >۴۵٪، ۳۰-۴۵٪ و <۳۰٪ مرگ و میر به ترتیب به عنوان جدایه های با بیماریزایی زیاد، متوسط و کم انتخاب شدند. همچنین بر اساس سرعت کشندگی– جدایه-های با >۱۰۰ساعت، ۸۰-۱۰۰ ساعت و <۸۰ ساعت LT_{50} به ترتیب به عنوان جدایه‌های با بیماریزایی زیاد، متوسط و کم طبقه‌بندی شدند. نتایج زیست‌سنجی نشان داد که جدایه‌های IRAN 440 و DEBI 004 به عنوان عوامل ایجاد کننده بیماری‌های قارچی، بیشترین و کمترین اثر کشندگی را داشتند. جدایه‌ی DEBI 014 کمترین مقدار LT_{50} را نشان داد. بر اساس دندروگرام ترسیم شده با روش UPGMA با استفاده از ضریب تشابه جاکارد، چهار گروه ژنتیکی ایجاد شد. نتایج نشان دادکه بین جدایه‌های مورد آزمایش، تنوع ژنتیکی وجود دارد اما بین شدت بیماریزایی این قارچ و داده‌های مولکولی حاصل از RAPD، رابطه‌ی زیادی وجود ندارد.

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