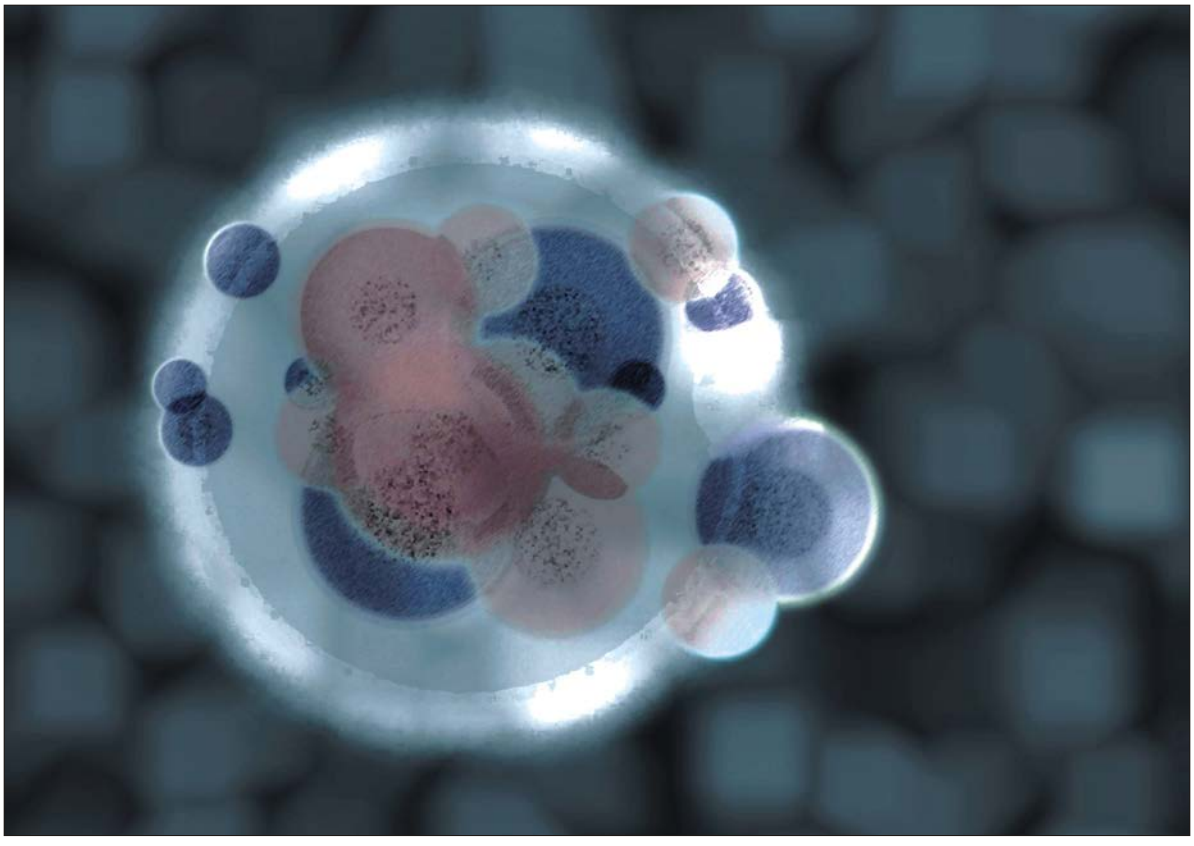

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 8 (4): 804-808, 2008
ISSN 1727-3048
© 2008 Asian Network for Scientific Information

Pathogenicity of Three Iranian Isolates of the Fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) Against Granary Weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae)

[1]Adel Khashaveh, [1]Mohammad Hassan Safaralizade and [2]Youbert Ghosta
[1]Department of Entomology, Faculty of Agriculture, Urmia University, Urmia, Iran
[2]Department of Plant Pathology, Faculty of Agriculture, Urmia University, Urmia, Iran

Abstract: Virulence of three indigenous Iranian isolates of the fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes), named DEMI001, IRAN 715C and IRAN 1018C, were evaluated as well as their virulence and their ability to suppress populations of the granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae). Five aqueous suspensions were prepared from each isolates, in a logarithmic series in Tween 80 (0.05% v/v). LT_{50} values ranged from 5.54 to 7.9 days following immersion in aqueous suspensions. Lowest LC_{50} on day 10 was $1/4\times10^5$ conidia mL^{-1} for DEMI001. Cumulative mortality 10 days after treatment varied from 9.4 to 88.88% for IRAN 1018C at low and high concentration, respectively.

Key words: *Sitophilus granarius*, *Metarhizium anisopliae*, immersion bioassay, Iranian isolates, pathogenicity

INTRODUCTION

The granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) is a very serious primary pest of stored grain products, which is able to cause considerable economic losses (Hill, 1990). Application of insecticides is one means of preventing some losses during storage. However, the choice of insecticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food (Padin *et al*., 2002). The continuous use of chemical insecticides for control of storage grain pests has also resulted in serious problems such as resistance to the insecticides, pest resurgence, elimination of economically beneficial insects and toxicity to humans and wildlife (Adane *et al*., 1996; Padin *et al*., 2002).

Metarhizium anisopliae (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes) is a mitosporic haploid fungus with a global distribution. It represents a pathogen for many insect species including a wider range of important agricultural pests and therefore, it holds great potential for use as biological control agent (Butt *et al*., 2001).

Until present, limited number of published articles on biocontrol of stored grain insects using entomopathogenic fungi are available. *Beauveria bassiana*, for example, has proven highly effective against the major stored grain insects: *Sitophillus oryzae*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *Prostephanus truncatus* and *Tribolium castaneum* (Smith *et al*., 1999; Tanya and Doberski, 1984). In contrast, *M. anisopliae* has been less frequently reported for control of stored grain insects although it has been used effectively to control other insects especially termites, black field crickets, grasshoppers and locusts, tobacco whitefly and red spidermites (Batta and Abu Safieh, 2005).

Investigations since mid 1980s by Tanya and Doberski (1984) followed by Adane *et al*. (1996), Hidalgo *et al*. (1998), Bello *et al*. (2000), Ekesi *et al*. (2001) and Padin *et al*. (2002) suggested that isolates of *B. bassiana* and *M. anisopliae* are potential microbial control agents against some stored product pests.

In this research, the susceptibility of the granary weevil, *S. granarius* to three Iranian isolates of entomopathogenic fungus *M. anisopliae* was appraised. All experiments were carried out in room conditions to evaluate efficacy of different isolates. These isolates showed virulence on other stored product insect (Personal observations).

MATERIALS AND METHODS

***S. granarius* culture:** Adults of *S. granarius* were collected from a laboratory culture, was kept on whole wheat; at 27±1°C, 65±5% RH and continuous darkness that kept in laboratory cultures for > 3 years without exposing insecticides in the Department of Entomology in Urmia University, Iran. Adults used in the experiments were < 7 days old.

Corresponding Author: Adel Khashaveh, Department of Entomology, Faculty of Agriculture, Urmia University, Urmia, Iran
Tel: +98 441 277 9552 Fax: +98 441 277 99558

Source of *Metarhizium anisopliae* isolates: All fungal isolates; DEMI001, IRAN 715C and IRAN 1018C were obtained from the collection maintained by the Plant Pest and Diseases Research Institute, Tehran, Iran. The isolates were cultured and stored at 4°C on Sabouraud Dextrose Agar (SDA). The three isolates of *M. anisopliae* were used for the virulence test (Table 1).

Production of conidial suspension: All fungal isolates were cultured on Potato Dextrose Agar (PDA, Merck and Co., Inc., Germany) in 9 cm diameter Petri dishes and then placed in dark at 24±2°C and 45±5% RH (in room conditions) for 15 days for complete sporulation. After this period, a mixture of conidia and hyphae was harvested by flooding the Petri dishes with sterile distilled water containing 0.05% (v/v) Tween 80 (Sigma Chemical, St. Louis, MO, USA) and agitating with glass rod. All sample vortexed for 3 min to break up the conidial chains or clumps. Conidia were separated from hyphae and substrate materials by filtration of the suspension through a five layers of cheese-cloth. The conidia concentration was counted with Haemocytometer (Improved Neubauer, 0.1 mm depth). Germination was assessed by counting 300 conidia after fixing with lacto phenol cotton blue (Kassa *et al*., 2002).

Dose-response bioassay by immersion method: Five aqueous suspension were prepared from 1×10^{8} down to 1×10^{4} conidia mL^{-1} in Tween 80 (0.05%v/v) for primary experiments. On the basis of preliminary tests, for each isolate and insect five concentrations of conidia were prepared for main experiments (Robertson and Preisler, 1992). Each concentration was replicated four times. For each replicate, thirty adults (>7 days old) were treated by immersion for 5 sec in 5 mL suspension. The control insect were immersed in sterile distilled water with Tween 80 (0.05%v/v). The treated insects and the suspension (1 mL) were subsequently poured into a plate containing filter paper (9 cm diameter) and sealed with parafilm to prevent insects from escaping. The filter paper helped to absorb the excess moisture and increased conidial load in each insect by allowing secondary spore pick up. (Adane *et al*., 1996). The treated insects were kept without food for 24 h at 24±2°C and 45±5% r.h. After 24 h, the treated insects in each replicate were transferred into glass pots (7 cm diameter and 8.5 cm height) with perforated lid containing 30 g wheat grains (variety Zarrin) and then kept at 24±2°C and 45±5% RH (at room conditions) for 10 days. The experiment was arranged in a completely randomized design and mortality was recorded at 48 h interval. Dead insects from each treatment were washed in 70% ethanol, rinsed in sterile distilled water three times and kept separately in Petri dishes. These plates were then incubated in a plastic box with high RH (approximately 100%) to observe the outgrowth of fungus. The same procedure were carried out for the control insects.

Data analysis: Control mortality was corrected by using Abbott's (1925) formula. For dose-mortality bioassay, cumulative mortality percentage was normalized using arcsine transformation and subjected to analysis of variance (ANOVA) using SAS (1999). Means were separated by using the Tukey-Kramer honestly significant difference test at $p\leq0.05$. Probit analysis was used to estimate LC_{50}, LC_{95} and LT_{50} of the isolates with 95% Confidence Limits (CL) after 10 days (SPSS, 2002).

RESULTS AND DISCUSSION

Results of this study sowed that the mean viability of conidia of all *M. anisopliae* isolates ranged between 89 to 94% (Table 1). The mortality within the control group was very low (2.49±1.59%) and no fungal growth was observed on the control insects. LT_{50} values for *M. anisopliae* isolates varied from 5.54 to 7.9 days, with an average of 6.63 days (Table 2). Among the *M. anisopliae* isolates, the isolate DEMI001 demonstrated the shortest LT_{50}. The parameters of the probit analysis and LC_{50} and LC_{95} are given in Table 3. At all isolates, *M. anisopliae* were pathogenic. The lowest and highest LC_{50} and LC_{95} values were observed in the isolates DEMI001 (1.4×10^{5} and 1.5×10^{7}) and IRAN 715C (1×10^{7} and 5.1×10^{11}), respectively. Mortality percentages for adults increased with increasing conidial concentration in all isolates (DEMI001: $F = 38.973$, $p\leq0.0001$), (IRAN 1018C:

Table 1: The host, location and germination percentage of the isolates of *Metarhizium anisopliae* used against *Sitophilus granarius*

Isolate	Host (Order: Family)	Location	Germination (%)
IRAN 715C	Lacust (Orthoptera: Acrididae)	Ahvaz-Khozestan	89±4.2
IRAN 1018C	*Parandra caspica* (Coloptera.: Cerambycidae)	Nour-Mazandaran	91±1.8
DEMI001	*Rhynchophorus ferrugineus* (Col.: Curculionidae)	Saravan-Balochestan	94±2.2

Table 2: LT_{50} values (day) with 95% confidence limits following immersion of *S. granarius* adults in aqueous suspensions of *M. anisopliae* isolates in high concentration

		95% confidence limits	
Isolate	LT_{50}	Lower	Upper
IRAN 1018C	6.46	4.66	8.96
IRAN 715C	7.90	5.18	19.71
DEMI001	5.54	3.87	7.82
Control	46.68	NC*	NC

*Non calculated

Table 3: LC_{50} and LC_{95} values (with 95% confidence limit) and probit analysis parameters for adults of *S. granarius* 10th day after immersion in aqueous conidial suspensions of *M. anisopliae* (three isolates)

Isolate	LC_{50} (conidia mL^{-1})	LC_{95} (conidia mL^{-1})	χ^2	p	Slop (b)	Intercept (a)
DEMI001	1.4×10^5	1.5×10^7	5.30	0.15	0.804	0.854
	($1\text{-}1.9\times10^5$)	(7.5×10^6-4.3×10^7)				
IRAN 715C	1×10^7	5.1×10^{11}	0.94	0.81	0.351	2.525
	(5×10^6-2.18×10^7)	(5.3×10^{10}-2.4×10^{13})				
IRAN 1018C	8.8×10^5	3.8×10^7	5.077	0.16	1.010	-1.003
	(6.8×10^5-1.1×10^6)	(2.1-8/3×10^7)				

Table 4: Cumulative mortality percentage (corrected) ±SE of *S. granarius* adults 10th day after immersion in aqueous conidial suspensions of *M. anisopliae* (three isolates)*

Isolates	Concentration (conidia mL^{-1})					F	p
DEMI001	7×10^3	3.1×10^4	1.3×10^5	6.1×10^5	2.7×10^6	38.973	<0.0001
	13.67±1.63d	30.77±1.64cd	52.98±2.56bc	61.53±4.41b	88.03±4.29a		
IRAN 715C	3.8×10^5	2.5×10^6	1.6×10^7	1.1×10^8	7.3×10^8	61.085	<0.0001
	32.47±1.63d	41.07±081cd	49.56±2.15c	61.53±2.56b	76.96±2.91a		
IRAN 1018C	5×10^4	2×10^5	7×10^5	2.6×10^6	1×10^7	80.408	<0.0001
	9.4±2.21d	31.62±2.62c	41.02±4.27c	64.1±2.96b	88.88±2.92a		

*Mean within a row followed by the same letter do not differ significantly by Tukey-Kramer test at $p \leq 0.05$

F = 80.408, $p<0.0001$), (IRAN 715C: F = 61.085, $p \leq 0.0001$). Maximum and minimum mortality rates observed in IRAN 1018C (88.88 and 9.4%, respectively). In general isolate DEMI001 had better effect on *S. granarius* because the range of concentration was very low and the high concentration in this isolate was lower rather than the two other isolates and had same mortality rate with IRAN 1018C (88.03%) (Table 4).

Insect cuticle, the first barrier against fungal pathogens, consists of a thin outer epicuticle, containing lipid and proteins and a thick procuticle, consisting of chitin and proteins. Entomopathogenic fungi produce proteases, chitinases and lipases which can degrade insect cuticle (Weiguo *et al*., 2005). Laboratory assessment of entomopathogenic fungi is an essential step in identifying virulent strain prior to field or large scale use. Entomopathogenic fungi are being developed worldwide for the control of insect pests and some products are already available commercially (Ekesi *et al*., 2001). Results of the current study indicated that all fungal isolates were virulent to granary weevil. The isolate DEMI001 can provide better control of *S. granarius* because it had lower LT_{50}, LC_{50} and LC_{95} . Within these taxa, individual isolates can exhibit substantially restricted host range and isolates recovered from a target host and closely related species are generally more virulent than isolates from non-related species (Inglis *et al*., 2001). Because the isolate DEMI001 was originally from a curculionid pest, its potential for the control of *S. granarius* was great. This observation highlights the need for screening for more virulent isolates against storage pests for use in the management of these pests. Other investigators have reported that treatment of stored grain pests with entomopathogenic fungi, especially *M. anisopliae* and *B. bassiana* can be effective (Hidalgo *et al*., 1998; Kassa *et al*., 2002; Batta, 2005). Obtained results are in accordance with their results. Wakefield *et al*. (2005) demonstrated that some *B. bassiana* isolates can provide 100% mortality in *Oryzaephilus surinamensis* (saw-toothed grain beetle-organophosphate resistant strain), *Ephestia kuehniella* (Mediterranean flour moth), *Epinotus patruelis* (black domestic psocid) and *Acarus siro* (flour mite) 10 days after treatment in 1×10^8 conidia mL^{-1}. Adane *et al*. (1996) demonstrated that several isolates of *B. bassiana* tested against *Sitophilus zeamais* (Motsch.) adults showed pathogenicity to insects, but there were highly significant differences among the isolates with respect to virulence. These isolates caused 37 to 100% mortality in *S. zeamaiz*. They also had been recorded median lethal time for different isolates. The lowest time (2.74 days) was recorded for isolate 189-481. Rodrigues and Pratissoli (1990) reported 6 months protection of maize grains and bean grains from damage by *S. Zeamais* and *Acanthoscelides obtectus* (Say) following treatment with *Beauveria brongniartii* (Sacc.) Petch. and *M. anisopliae* at a dose of 1×10^8 conidia mL^{-1}. Unformulated conidia of *M. anisopliae* isolate MaPs and *B. bassiana* Isolates BbPs and BbGc at the rate of 0.15 g a.i. to 50 g a.i. rice grain could cause 77.5, 88.75 and 90% of *S. oryzae* adults mortality, respectively (Hendrawan and Ibrahim, 2006). Bourassa *et al*. (2001) found that *B. bassiana* IMI330194 led to 100% mortality of *P. truncatus* larvae. Cherry *et al*. (2005), also have demonstrated that different isolates from *M. anisopliae* and *B. bassiana* could provide good control of *C. maculatus* by immersion bioassay. They represented that the LT_{50} values for *B. bassiana* and *M. anisopliae* isolates was varied from 3.11 to 6.13 days (with an average of 4.61 days) and 3.27 to 5.62 days (with an average of 4.60 days).

In conclusion, study research showed a high susceptibility of adult *S. granarius* to *M. anisopliae*. Based on this finding, we may suggest *M. anisopliae* as a useful candidate for the management of the storage pests, *S. granarius*. However, further investigations are strongly recommended to be carried out on the possibility of field application as well as finding other isolates of entomopathogenic fungi that have potential as biopesticides against such storage pests.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Adane, K., D. Moore and S.A. Archer, 1996. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. J. Stored Prod. Res., 32: 105-113.

Batta, Y.A., 2005. Control of the lesser grain borer (*Rhyzopertha dominica* Fab., Coleoptera: Bostrychidae) by treatments with residual formulations of *Metarhizium anisopliae* (Metsch.) Sorokin. J. Stored Prod. Res., 41: 221-229.

Batta, Y.A. and D.I. Abu Safieh, 2005. A study of treatment effect with *Metarhizium anisopliae* and four types of dusts on wheat grain infestation with Red Flour Beetle (*Tribolium castaneum* Herbs, Coleoptera: Tenebrionidae). J. Islamic Univ. Gaza (Series of Natural Studies and Engineering), 13: 11-22.

Bello, G.D., S. Padina, C.L. Lastrab and M. Fabrizio, 2000. Laboratory evaluation of chemical biological control of rice weevil, *Sitophilus oryzae* L. in store grain. J. Stored Prod. Res., 37: 77-84.

Bourassa, C., C. Vincent, C.J. Lomer, C. Borgemeister and Y. Mauffette, 2001. Effects of entomopathogenic Hyphomycetes against the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and its predator, *Teretriosoma nigrescens* Lewis (Coleoptera: Histeridae). J. Invert. Path., 77: 75-77.

Butt, T.M., C.W. Jackson and N. Magan, 2001. Introduction-Fungal Biological Control Agents: Progress, Problems and Potential. In: Fungi as Biocontrol Agents: Progress, Problems and Potential, Butt, T.M., C.W. Jackson and N. Magan (Eds.). CABI Publishing, Wallingford, UK., ISBN-10: 0851993567.

Cherry, A.J., P. Abalo and K. Hell, 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. J. Stored Prod. Res., 41: 295-309.

Ekesi, S., E.A. Egwurube, A.D. Akpa and I. Onu, 2001. Laboratory evaluation of the entomopathogenic fungus, *Metarhizium anisopliae* for the control of the groundnut bruchid, *Caryedon serratus* on groundnut. J. Stored Prod. Res., 37: 313-321.

Hendrawan, S. and Y. Ibrahim, 2006. Effect of dust formulations of three entomopathogenic fungal isolates against *Sitiphilus oryzae* (Coleoptera: Curculionidae) in rice grain. J. Biosains, 17: 1-7.

Hidalgo, E., D. Moore and G. Le Patourel, 1998. The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais* in stored maize. J. Stored Prod. Res., 34: 171-179.

Hill, D.S., 1990. Pests of Stored Products and Their Control. 1st Edn. Belhaven Press, London, ISBN 1-85293-052-7.

Inglis, G.D., M.S. Goettel, T.M. Butt and H. Strasser, 2001. Use of Hyphomycete Fungi for Managing Insect Pests. In: Fungi as Biocontrol Agents Progress, Problems and Potential. Butt, T.M., C.W. Jackson and N. Magan (Eds.). CABI Publishing, Wallingford, ISBN-10: 0851993567, pp: 23-69.

Kassa, A., G. Zimmermann, D. Stephan and S. Vidal, 2002. Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to entomopathogenic fungi from Ethiopia. Biocontrol Sci. Technol., 12: 727-736.

Padin, S., G.D. Bello and M. Fabrizio, 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. J. Stored Prod. Res., 38: 69-74.

Robertson, J.L. and H.K. Preisler, 1992. Pesticide Bioassays with Arthropods. 1st Edn. CRC Press, Boca Raton, Florida, ISBN-10: 0849364639.

Rodrigues, C. and D. Pratissoli, 1990. Pathogenicity of *Beauveria brongniartii* (Sacc.) Petch. and *Metarhizium anisopliae* (Mots.) Sorok. and its effect on the corn weevil and the bean beetle. Anais Soc. Entomol. Brasil, 19: 301-306.

SAS, 1999. SAS/STATTM guide for personal computers. Version 8, Cary, North Carolina: SAS Institute Inc.

Smith, S.M., D. Moore, L. Karanja and E.A. Chandi, 1999. Formulation of vegetable fat pellets with pheromone and *Beauveria bassiana* to control the larger grain borer, *Prostephanus truncatus* (Horn.). Pest. Sci., 55: 711-718.

SPSS, 2002. Statistical product and service solution. Systat Statistical Software, System User's Guide, Ver. 11.

Tanya, S. and J. Doberski, 1984. An investigation of the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. as a potential biological control agent for *Oryzaephilus surinamensis* (L.). J. Stored Prod. Res., 20: 17-23.

Wakefield, M.E., P.D. Cox, D. Moore, M. Aquino De Muro and B.A. Bell, 2005. Mycopest: Results and perspectives. Proceedings of the 6th meeting of COST Action 842 Working Group IV Biocontrol of Arthropod Pests in Stored Products, 10-11th June, Locorotondo, Italy, pp: 17-26.

Weiguo, F., B. Leng, Y. Xiao, K. Jin, J. Ma, Y. Fan, J. Feng, X. Yang, Y. Zhang and Y. Pei, 2005. Cloning of *Beauveria bassiana* Chitinase gene *Bbchit1* and its application to improve fungal strain virulence. Applied Environ. Microbiol., 71: 363-370.