# First report of *Aspergillus allahabadii* Mehrotra and Agnihotri in vegetable fields in Northern Benin (West-Africa)

**A. Affokpon[5 & 7], D. L. Coyne[6], C. Tossou[5] and J. Coosemans[7]**

## Abstract

*Aspergillus allahabadii,* previously reported only from soils in India and El Salvador, was isolated from root-knot nematode egg masses (*Meloidogyne* spp.) in vegetable fields in Benin. Three populations were recovered from separate fields in the Guinea savannah region from tomato and/or cabbage. The populations were morphologically identified to genus level under the microscope and to species level from molecular sequences based on ITS 1 and ITS 2 regions using purified fungi. All populations were able to produce spores on potato dextrose agar following incubation at 25 °C as well as room temperature (24 ± 2 °C), producing as many as 7.9 x $10^8$ and 3.0 x $10^8$ spores per plate after 10 days, respectively. Pre-planting inoculation of the populations onto tomato (cv. Tounvi) seedlings in 1-L pots increased tomato shoot and root fresh weights by up to 13 and 24 %, respectively, compared to untreated controls after five weeks. The three populations all successfully established and remained in the rhizosphere of tomato plants until termination of the experiment at seven weeks after inoculation. Given that the populations were all isolated from nematode egg masses, the current study can be considered as the starting point for further research on their possible use for biological control.

**Key words:** Beneficial fungi, biological control, *Meloidogyne* spp., plant-parasitic nematodes, Benin.

# Première détection de *Aspergillus allahabadii* Mehrotra and Agnihotri sur les sites maraîchers au Nord-Bénin (Afrique de l'Ouest)

## Résumé

*Aspergillus allahabadii,* signalé jusque récemment que dans les sols en Inde et à El Salvador, vient d'être isolé des sacs d'œufs des nématodes à galles (*Meloidogyne* spp.) sur des sites maraîchers du Bénin. Trois isolats sont détectés sur différents sites sous la culture de la tomate et/ou du chou dans la zone de la savane guinéenne. Ces isolats sont identifiés d'abord à partir des caractères taxonomiques comme appartenant au genre *Aspergillus* à l'aide du microscope puis à partir de la séquence des régions ITS 1 et ITS 2 de l'ADN comme étant l'espèce *allahabadii*. Tous les isolats ont pu produire des spores aussi bien sur le milieu agar (Potato Dextrose Agar) à 25 °C après incubation qu'à la température ambiante (24 ± 2 °C) avec des densités de production pouvant atteindre respectivement 7,9.$10^8$ et 3,0.$10^8$ spores par boite de Pétri après 10 jours de culture. L'essai en pots a révélé une amélioration du poids frais des plants et des racines pouvant atteindre respectivement 13 et 24 %, suite à l'inoculation des sols avant le repiquage de la tomate. Les isolats ont pu également bien coloniser le sol et se maintenir dans la rhizosphère jusqu'à la fin de l'essai qui a duré 7 semaines après l'inoculation. Etant donné que ces isolats proviennent des sacs d'œufs des nématodes, la présente étude constitue le point de départ d'une série de recherches sur la possibilité de leur utilisation en lutte biologique.

**Key words:** Champignons bénéfiques, lutte biologique, *Meloidogyne* spp., nématodes parasites des cultures, Bénin.

## INTRODUCTION

*Aspergillus* spp. (Family of Trichocomaceae, order Eurotiales) are ubiquitous fungi that are adapted to various climates worldwide (Bennett, 2010). Species of *Aspergillus* are important medically and commercially, with some species able to infect humans and cause a range of diseases referred to as mycetomas (Thom and Rapper, 1945). In contrast, some species are a source of medicinal products

[5] MSc (PhD student), Antoine Affokpon, Centre de Recherches Agricoles Sud-Bénin, Institut National des Recherches Agricoles du Bénin, BP 03 Attogon, Tél. : (++229) 95 42 50 35/97 12 44 08, E-mail: affokpon_antoine@yahoo.fr, République du Bénin et Laboratory of Phytopathology and Plant Protection, Katholieke Universiteit Leuven, De Croylaan 42, 3001 Heverlee, Belgium.

DEAT (BSc student) Colette Tossou, Centre de Recherches Agricoles Sud-Bénin, Institut National des Recherches Agricoles du Bénin, BP 03 Attogon, Tél. : (++229) 98 13 49 29, E-mail: coltossou@yahoo.fr, République du Bénin

[6] Dr, Daniel L. Coyne, International Institute of Tropical Agriculture, IITA-Tanzania, Regional Hub, Plot 331, Kambarage Road, Mikocheni A, P.O. Box 34441, Dar-es-Salaam, Tanzania, Tél. : (++255) 222700092, E-mail: d.coyne@cgiar.org

[7] Prof. Jozef Coosemans, Laboratory of Phytopathology and Plant Protection, Katholieke Universiteit Leuven, De Croylaan 42, 3001 Heverlee, Belgium, Tél. : (++32) 16 56 06 79, E-mail: jef.coosemans@biw.kuleuven.be

used in the treatment of human diseases (Anonymous, 2000) or for industrial enzymes (e.g. *A. oryzae*, *A. niger*; Wortman *et al*., 2009). Others are plant pathogens and/or produce toxins which contaminate crop products (e.g. *A. flavus, A. fumigates, A. parasiticus;* Zareen *et al*., 2001, Ingram *et al.*, 2003). A number of species however, have been investigated for their potential as biological pest control agents (Ayoub *et al*., 2000; Siddiqui *et al*., 2001; Meyer *et al*., 2007), including against plant parasitic nematodes (Siddiqui *et al.,* 2001; Meyer *et al.*, 2007). The nematicidal effect of some species of *Aspergillus* (e.g. *A. niger*) was attributed to production of metabolites lethal to nematodes (Zuckerman *et al.,* 1994; Siddiqui *et al.,* 2004). The synergistic interaction of some species of *Aspergillus* also appeared to influence the biocontrol performance of bacteria (e.g. *Pseudomonas aeruginosa*, *Ps. fluorescens*; Siddiqui *et al*., 2001; Siddiqui *et al.,* 2004) and fungi (e.g. *Acremonium strictum*; Singha and Mathurb, 2010) against plant-parasitic nematodes.

*Aspergillus allahabadii* was first isolated from garden soil in Allahabad, India (Mehrotra and Agnihotri, 1962) and thereafter from soil in San Salvador, El Salvador only (http://nrrl.ncaur.usda.gov). However, there is no report to date on *A. allahabadii* effects on plants and/or nematodes. The current report describes the isolation of *A. allahabadii* from vegetable rhizosphere soils in northern Benin, West Africa during a survey of beneficial fungi for potential use as biocontrol agents against root-knot nematodes (*Meloidogyne* spp.). Preliminary results on *in vitro* culture, effect on tomato and rhizosphere competence are presented.

## MATERIEL AND METHODS

### *Origin, isolation and identification*

*Aspergillus allahabadii* were recovered from root-knot nematode egg masses and soil collected from vegetable fields during surveys undertaken across Benin between January and March 2007. A total of 88 samples of roots and rhizosphere soils were collected from 50 vegetable fields. Each sample consisting of a minimum of five root systems and 20 soil cores removed from 5 to 30 cm depth using an auger.

Prior to fungal isolation, soil and root samples were maintained on tomato (cv. Tounvi) in the plastic house for two months before processing. The fungus was isolated from rhizosphere soil and egg masses of root-knot nematodes using soil dilution plating techniques (Mauchline *et al.*, 2002; Van Damme *et al.,* 2005) on standard potato dextrose agar (PDA) and incubated at 25 °C. Concomitantly, fifteen individual and separate *Meloidogyne* spp. egg-masses (per root system) were removed from roots, rinsed three times with sterile distilled water (SDW) and then crushed in 10 ml SDW to release the eggs. Aliquots (0.2 ml) of the egg suspensions were spread onto PDA in triplicate Petri dishes and incubated.

The fungal colonies were first identified at genus level based on morphological features in open Petri dishes and under a compound microscope at 40x magnification. The fungal isolates were then purified by repeatedly inoculating 5 mm diameter plugs onto PDA and incubating at 25 °C for one week. Purified fungi were used for species identification by molecular techniques (DNA internal transcribed spacer ITS sequencing based on ITS 1 and ITS 2 regions using the primers ITS 1 together with ITS 4) by Scientia Terrae, Belgium.

### *Spore production ability of the isolates on agar*

The sporulation ability of the *A. allahabadii* populations was assessed on PDA at 25 °C in the incubator (55 % relative humidity) for 10 days in the dark; at room temperature (24 ± 2 °C, relative humidity between 70 and 85 %) for 10 days in the laboratory (wrapped in aluminum fold to create darkness); at 25 °C in the incubator for 5 days followed by a further 5 days at room temperature in the laboratory. Individual 3 mm-diam. plugs from sub-cultured plates of each isolate were singly transferred to the centre of three replicate Petri dishes. Following incubation, 3 ml SDW was applied to the agar surface and the fungal growth of each dish carefully scraped into a separate 100 ml beaker using a sterile glass-rod, adjusting the suspensions to 20 ml. After thoroughly mixing, 1 ml spore suspension from individual beaker was transferred separately to 9 ml SDW to make $10^{-1}$ dilution. Using a haemocytometer spore density was then determined.

### *Effect on tomato growth and rhizosphere competence in pots*

The phytopathogenicity status of the *A. allahabadii* populations was assessed on tomato in pots using sterilized soil (organic matter: 1.19 %, C/N: 10.5, pH: 5.7) after sub-culturing on PDA in Petri dishes for two weeks at 25 °C. Fungal suspensions were prepared as above, collected in 250 ml beakers, adjusted to 50 ml and then agitated using a hand mixer to separate and disperse the spores, before assessing spore density. Plastic pots (5 per treatment) containing 1,000 ml soil were inoculated with

fungal suspensions to achieve a rate approximating $10^6$ spores/ml soil (volume depending on spore concentration).

Pots were placed in the screenhouse (open temperature), mixing the soil every three days for two weeks to allow an even fungal establishment, before transplanting three week-old tomato seedlings (cv. Tounvi) singly per pot. Pots were arranged in a completely randomized design, with five replicates per treatment, which were watered manually as required. Five weeks after transplanting (= seven weeks after inoculation) plants were harvested and root and shoot fresh weights measured and fungal soil and root densities determined. Final fungal soil densities were estimated from a composite sample per pot consisting of four soil cores removed adjacent to plants using a 2 cm diam. borer, following the procedure described above. Root colonization was also assessed following a similar procedure using 1 g sub-sample of air-dried chopped roots crushed using a sterilized pestle and mortar in 9 ml of 0.05 % water agar . The numbers of clear colony-forming units (CFU) were counted and, based on the dilution with the most consistent results, the final densities per ml air-dried soil or g air-dried root were calculated as:

$$CFU = (N_c * D_f)/V_i \text{ , where:}$$

Nc = average number of colonies per plate; Df = dilution factor; Vi = volume of suspension plated).

All data were subjected to ANOVA using Statistica package (StatSoft Inc., 2001) and means separated using the LSD procedure ($p \leq 0.05$). Prior to analysis, fungal counts were transformed to log(x+1) in order that data conformed to normal distribution (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

Three populations of *A. allahabadii* were reported from the survey. All were isolated from both soil and *Meloidogyne* spp. egg masses from the north of Benin in the Guinean savannah (Table 1).

**Table 1. Origin of *Aspergillus allahabadii* populations from vegetable fields in the Guinea savannah zone of Benin**

| Population | Sites/Communes | Co-ordinates (GPS) | Plant host[a] | Substrates |
|---|---|---|---|---|
| *A. allahabadii* Asp-1 | Anandana/Copargo | 09°55.69N; 001°23.07E | Tomato | Soil + Egg masses |
| *A. allahabadii* Asp-2 | Ekpinda/Natitingou | 10°18.67N; 001°23.58E | Cabbage | Soil + Egg masses |
| *A. allahabadii* Asp-3 | Kika/Tchaourou | 09°18.05N; 002°44.17E | Cabbage, tomato | Soil + Egg masses |

**[a]More crops were cited where intercropping systems were present at the sampling time**

Due to their resemblance in morphological characters all the three isolates were initially recorded as *Paecilomyces* species. On PDA, the colonies were flat, powdery, and white, gradually turning brown with age as conidia began to form (Figure 1) and becoming unevenly shaped.


**Figure 1. *Aspergillus allahabadii* Asp-1 culture on PDA after two weeks incubation at 25 °C**

Later, all three populations were identified as *A. allahabadii* based on sequence analysis of ITS 1 and ITS 2 (Figure 2). The morphological description of *A. allahabadii* to date was based on culture on Czapek's solution agar by Mehrotra and Agnihotri (1962). These authors described colonies growing slowly and attaining a diameter of 2-3 cm in four weeks, at first white, later gradually turning greenish glaucus blue in the center of the colony; vegetative mycelium mostly submerged, yellow at some

places, surface growth mostly consisting of conidiophores and heads; reverse at first yellow turning brown with age. Phylogenetic analysis of sequence data based on the ITS regions and the 5.8S rRNA gene of *A. terreus* and related isolates showed that *A. allahabadii* belonged to the *A. niveus* clade (Varga *et al*., 2005) as reported by Mehrotra and Agnihotri (1962).

**(A)**

TGGAAAAAAAGTTGTTATGCGTCGGCGGGCGCCGGCCGGGCCTGCAGAGCGGAAGACAAAGCC
CCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGAGCC
GGGGGACGAGGGCCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCAT
GCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCA
ATTCACATTAGTTATCGCATTTCGCTGCGTT

(B)

GTCACCTGGAAAAAAAGTTGTTATGCGTCGGCGGGCGCCGGCCGGGCCTGCAGAGCGGAAGAC
AAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGG
GAGCCGGGGGACGAGGGCCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACA
GGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATT
CTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGACAAT

(C)

AACCTGGAAAAAAAGTTGTTATGCGTCGGCGGGCGCCGGCCGGGCCTGCAGAGCGGAAGACAA
AGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGG
AGCCGGGGGACGAGGGCCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAG
GCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTC
TGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGAT

**Figure 2. DNA sequences of the ITS 1 and ITS 2 regions of Aspergillus allahabadii Asp-1 (A), Asp-2 (B) and Asp-3 (c) from Benin, using the primers ITS 1 and ITS 4**

The fungal isolates showed variability on their potential to produce spores *in vitro* and also responded differently to incubation conditions (Table 2). The maximum spore production was recorded from incubation at 25 °C in darkness. For a given incuba tion condition, the Asp-2 population produced the most spores per plate. In the laboratory, temperatures were more or less similar to that in the incubator; however, due to the use of the air-conditioner during daytime the humidity was higher (> 70 %) than in the incubator. Humidity may have therefore influenced sporulation, which could be a factor explaining the absence of the fungus in other agro-ecological regions of the country.

**Table 2. Spore production of three *A. allahabadii* populations on standard potato dextrose agar under various incubation conditions in Benin**

| Population | Number of spores / plate (x $10^8$) | | |
|---|---|---|---|
| | 25 °C for 10 days darkness | Room temperature for 10 days | 25 °C for 5 days darkness and 5 days at room temperature |
| *A. allahabadii* Asp-1 | 3.4 c[a] (a)[b] | 2.1 b (b) | 3.2 c (a) |
| *A. allahabadii* Asp-2 | 7.9 a (a) | 3.0 a (c) | 6.2 a (b) |
| *A. allahabadii* Asp-3 | 6.6 b (a) | 2.1 b (c) | 5.2 b (b) |

**Data are means of three replicates. Statistical analysis and mean separations were undertaken on log(x+1) transformed data; backtransformed data presented.**

**[a]Values in columns followed by different letters are significantly different ($p \leq 0.05$) based on Fisher LSD test.**

**[b]For a given isolate, values followed by different letters in parenthesis within a row are significantly different ($p \leq 0.05$) based on Fisher LSD test.**

All inoculated tomato plants appeared in good health, with no death, before the termination of the experiment, indicating their non-pathogenic nature on tomato (Table 3). Pre-planting application of the fungi furthermore improved plant growth and root development by up to 13 % and 23 %, respectively. All three populations also established well in the plant rhizosphere and on tomato roots (Table 3). Assessing the effect of filtrates of seven *Aspergillus* species on tomato for protection against root-knot nematodes, Zareen *et al*. (2001) reported a significant positive effect on tomato growth following application either as soil drench or bare root dip of seedlings. This effect was also confirmed by Siddiqui *et al.* (2004) who reported increased ($p<0.05$) plant height and shoot weight following treatment with *A. niger*. In recent study, soil application of Talc based formulation of *A. terreus* showed

successful establishment and persistence of the fungus in tomato rhizosphere (Singha and Mathurb, 2010).

**Table 3. Shoot fresh weight (SFW) and root fresh weight (RFW) per plant of tomato cv. Tounvi and fungal densities at five weeks after transplanting in 1000 ml pots. Pots were inoculated two weeks before transplanting with $10^6$ *A. allahabadii* spores/ml soil**

| Population | SFW (g) | RFW (g) | CFU/ml soil (x$10^4$)[a] | CFU/g root (x$10^4$)[a] |
|---|---|---|---|---|
| *A. allahabadii* Asp-1 | 30.3 bc | 9.4 b | 9.7 b | 1.3 b |
| *A. allahabadii* Asp-2 | 33.1 a | 10.1 a | 14.7 a | 3.3 a |
| *A. allahabadii* Asp-3 | 31.4 b | 10.8 a | 16.2 a | 2.7 a |
| Control (no fungi) | 29.2 c | 8.12 c | 0.0 c | 0.0 c |

**Data are means of five replicates. Values in columns followed by different letters are significantly different ($p \leq 0.05$) based on Fisher LSD test.**

**[a]Statistical analysis and mean separations were undertaken on log(x+1) transformed data; backtransformed data presented. CFU= colony forming units**

Microorganisms that are able to colonize the rhizosphere are ideal for potential use as biological control agents since this is the front line area for defense against attack by soilborne pathogens (Weller, 1988). The biological control effect of *A. allahabadii* against nematodes or other soilborne constraints are yet to be determined. However, in relation to *Meloidogyne* spp., fungi isolated directly from egg sacs have proved to be among the more adapted and effective at suppressing and/or parasitizing nematodes (Affokpon *et al.*, 2008). To date there is no information on the biology and pest management potential of *A. allahabadii*, although other species of *Aspergillus* have demonstrated biocontrol activity against plant-parasitic nematodes. For example, *A. flavus* and *A. fumigates* were found to parasitize cyst (*Heterodera* spp.) and root-knot nematodes (Khan and Saxena, 1995); *A. nidulans, A. tamari* and *A. terreus* on females, eggs and egg masses on root-knot nematodes (Zareen *et al*., 2000). The biocontrol activity of *Aspergillus* spp. on plant-parasitic nematodes have been demonstrated using a variety of applications, such as a conidial suspension application to the soil (Siddiqui *et al*., 2001), fungal culture broth (Meyer *et al.,* 2007), talc based formulation (Singha and Mathurb, 2010), and filtrates (Zareen *et al*., 2001). The compatibility of some *Aspergillus* species with other beneficial microorganisms is also an interesting aspect for potential exploitation. *Aspergillus niger*, for example, enhanced the antagonistic activity of *Ps. fluorescens* strain CHA0 towards *Meloidogyne javanica* on tomato (Siddiqui *et al*., 2004), and suppression of *M. incognita* was most effective when *Acremonium strictum* was combined with *Aspergillus terreus* (Singha and Mathurb, 2010).

## CONCLUSION

The current study provides the first report of *A. allahabadii* in Benin and also in Africa. Moreover, it is the first time that this species is found associated with root-knot nematodes. Given that species of *Aspergillus* produce toxins that affect various target organisms, further research is needed to assess the pathogenicity of these populations towards crop pests and pathogens, which will contribute for minimizing the use of chemical pesticides in the intensive vegetable production systems in Benin. The screen house study demonstrated their avirulent nature against tomato, although further biosafety analysis would be necessary to confirm this before recommending their use for plant protection purposes.

## ACKNOWLEDGMENTS

We acknowledge Vlaamse Interuniversitaire Raad (VLIR) in Belgium for supporting in the PhD scholarship framework allocated to the lead author. Thanks also to Scientia Terrae (Belgium) for the molecular identification of the fungi and to Gande Claude (IITA-Cotonou) for his help during the *in vitro* culture of the fungi.

## REFERENCES

Affokpon, A., G.K. Mutua, J. Coosemans, D.L. Coyne, 2008: Evaluation of *Pochonia* and *Trichoderma* from Benin as biocontrol agents against root-knot nematodes. *Journal of Plant Pathology*, abstract, 9, 339-340.

Anonymous, 2000: Halimide, a cytotoxic marine natural product, and derivatives thereof. US Patent 6069146. http://www.freepatentsonline.com/6069146.html, retrieved on 02/10/2010 at 12:31.

Ayoub, R., A. Zareen, M.J. Zaki, S.M. Saifullah, 2000: Female parasitism by *Aspergillus* species and their effect on root penetration by root-knot nematodes *Meloidogyne javanica*. *Pakistan Journal of Phytopathology*, 12, 115-117.

Bennett, J.W., 2010: An overview of the genus *Aspergillus*: 1-17. *In*: Machida, M., Gomi, K., (eds), *Aspergillus*: Molecular Biology and Genomics. Caister Academic Press, Norfolk, UK.

Gomez, K.A., Gomez, A.A. 1984: Statistical procedures for agricultural research. Wiley, J. & Sons Inc. New York, USA, 680 p.

http://nrrl.ncaur.usda.gov, retrieved on 24/10/2010 at 22:58

Ingram, K.T., B.C. Ahohuendo, B. Diarra, G. Hoogenboom, 2003: *Aspergillus flavus* infection and aflatoxin contamination of peanut. New tools for research on *Aspergillus flavus* infection and aflatoxin contamination of peanut. *Annales des Sciences Agronomiques du Bénin*, 4, xx pp.

Khan, T.A., Saxena, S.K., 1995: Studies on the degree of parasitism and chitinolytic activity of fungi associated with root-knot and cyst nematodes in Uttar Pradesh, India. *Annals of Plant Protection Science*, 3, 137-140.

Mauchline, T.H., B.R. Kerry, P.R. Hirsch, 2002: Quantification in soil and the rhizosphere of the nematophagous fungus *Verticillium chlamydosporium* by Competitive PCR and comparison with selective plating. *Applied and Environmental Microbiology*, 68, 1846-1853.

Mehrotra, B.S., Agnihotri, V.P., 1962: Two new species of *Aspergillus* from India. *Mycologia*, 54, 400-406.

Meyer, S.L., D.K. Lakshman, I.A. Zasada, B.T. Vinyard, D.J. Chitwood, O.N. Shemshura, N.E. Bekmakhanova, M.N. Mazunina, B.K. Yeskalieva, E.P. Masler, 2007: Clove oil and fungus compounds: Can nematode suppression be achieved without phytotoxicity? *Journal of Nematology* abstract, 39, 92.

Siddiqui, I.A., N.I. Ali, M.J. Zaki, S.S. Shaukat, 2001: Evaluation of *Aspergillus* species for the biocontrol of *Meloidogyne javanica* in mungbean. *Nematologia Mediterranea*, 29, 115-121.

Siddiqui, I.A., S.S. Shaukat, A. Khan, 2004: Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHA0. *Letters in Applied Microbiology*, 39, 74-83.

Singha, S., N. Mathurb, 2010: Biological control of root-knot nematode, *Meloidogyne incognita* infesting tomato. *Biocontrol Science and Technology*, 20, 865-874.

StatSoft Inc., 2001: Statistica release 6. Statsoft. Tulsa, USA.

Thom, C., Rapper, K.B., 1945: A manual of the *Aspergilli*. Baltimore, MD: The Williams & Co., 373 p.

Van Damme, V., A. Hoedekie, N. Viaene, 2005: Long-term efficacy of *Pochonia chlamydosporia* for management of *Meloidogyne javanica* in glasshouse crops. *Nematology*, 7, 727-736.

Varga, J., B. Toth, S. Kocsubé, B. Farkas, G. Szakàcs, J. Téren, Z. Kozakiewicz, 2005: Evolutionary relationships among *Aspergillus terreus* isolates and their relatives. *Antonie van Leeuwenhoek*, 88, 141-150.

Weller, D., 1988. The role of antibiosis in the biological control of plant diseases. *Annual Review of Phytopathology*, 26, 75-91.

Wortman, J.R., J.M. Gilsenan, V. Joardar, J. Deegan, J. Clutterbuck, M.R. Andersen, D. Archer, M. Bencina, G. Braus, P. Coutinho, H. von Döhren, J. Doonan, A.J.M. Driessen, P. Durek, E. Espeso, E. Fekete, M. Flipphi, C.G. Estrada, S. Geysens, G. Goldman, *et al.*, 2009: The 2008 update of the *Aspergillus nidulans* genome annotation: a community effort. *Fungal Genetics and Biology*, 46, S2-13.

Zareen, A., I.A. Siddiqui, M.J. Zaki, 2000: Fungal parasites of root-knot nematodes. *Pakistan Journal of Biological Sciences*, 3, 478-480.

Zareen, A., M.J. Zaki, N.J. Khan, 2001: Effect of fungal filtrates of *Asperillus* species on development of root-knot nematodes and growth of tomato (*Lycopersicon esculentum* Mill.). *Pakistan Journal of Biological Sciences*, 4, 995-999.

Zuckerman, B.M., M. Matheny, N. Acosta, 1994: Control of plant-parasitic nematodes by a nematicidal strain of *Aspergillus niger*. *Journal of Chemical Ecology*, 20, 33-43.