# Assessment of the implementation Degree in Handling Practices Which Contribute to Reducing Fungal Incidence and its Consequent Aflatoxins Production in Peanut Kernels

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## Abstract

*The peanut kernel intended for direct human consumption, while this has a high nutritional value, is very likely to be altered at different production stages. Thereby, fungal presence in grains decreases its quality as food and can produce mycotoxins, causing that grains be unfit for consumption. The purpose of this study was to evaluate the implementation degree in those handling practices which contribute to reducing fungal incidence and its consequent aflatoxins production in peanut kernels. Five representative samples of peanut kernels were evaluated. All these of direct consumption at three areas the central zone from Córdoba Province (Argentina). Variables evaluated were: fungal incidence, aflatoxins content, and implementation percentage in handling practices related to aflatoxins contamination. Determinations were carried out through a variance analysis model, with which areas as fixed effects about said model were compared to detect significant differences; between means, ANOVA was performed; and for comparisons, Tukey test ($p<0.05$); at last, correlations were calculated. Fungal incidence levels remained not directly related to aflatoxins concentration. The higher implementation percentage the GAP was associated with lower aflatoxins concentration, even if environmental conditions were predisposing.*

**Keywords:** Peanut - Aflatoxins- Fungal incidence - Good Agricultural Practices.

## *Introduction*

The peanut (*Arachis hypogaea* L.) was produced and marketed primarily as raw material the oil industry and for direct human consumption (Blengino, 2014). The Argentine peanut agribusiness sector was located mainly from Córdoba Province and constitutes a very important regional economy dedicated almost exclusively for export, given that 95% the produce was intended for international markets (Bolsa de Cereales de Córdoba, 2012).
The peanut is a high nutritional value food for direct human consumption, which needs a sustainable production system to ensure its safety. Therefore, peanut kernel is considered very likely to be altered at different production stages, due to abiotic and biotic factors. In particular, fungal presence in grains decreases its quality as food: causes discoloration, odors, nutritional and chemical disorders; and can produce mycotoxins, which bring about that peanuts or their derivatives be unfit for consumption (Christensen, 1982; March & Marinelli, 2005; Paster & Bullerman, 1988; Schneider & Sieber, 1999; Pitt *et al.*, 2012).

The fungal genera *Aspergillus, Penicillium, Rhizopus* and *Fusarium* are frequently detected in peanut kernels (Moraes & Mariotto, 1985; Cavallo *et al.*, 1994; and Cavallo *et al.*, 2005). Among genera mentioned, *Aspergillus* stands out as the most important aflatoxins producer (Chulze, 2005; Kumeda & Assao, 2001; March & Marinelli, 2005). Azaizeh *et al.* (1990), Van Egmont *et al*. (2004), and Cornejo & Villarroel (2007) have established that low levels of fungal colonization have also presented lower levels of aflatoxins. Accordingly, the *A. flavus* detection could be an indicator mycotoxins presence, becoming a simple method to prevent toxins contamination.
While the mycotoxins presence was traditionally a problem associated with storage, research has shown that contamination can occur before harvest (March & Marinelli, 2005).

Best way to prevent aflatoxins emergence in grains is adopt preventive measures for fungal controlling at all production stages (WHO, 2012). For this, it is necessary to identify, among crop handling practices, those which favor toxigenic fungal proliferation and its consequent mycotoxins generation. About, Chulze (2005) argues that handling strategies to minimize or eliminate aflatoxins contamination begins at countryside and ends at industrial process. Thus, Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) implementation decreases aflatoxins contamination risks in peanut kernels. (Bongiovanni *et al.*, 2012). So far, has not been established at Argentine production zone the implementation degree in those practices which contribute to reducing fungal incidence and its consequent aflatoxins production. The purpose of this study was to evaluate the implementation degree in those handling practices which contribute to reducing fungal incidence and its consequent aflatoxins production in peanut kernels, from central zone the Córdoba Province (Argentina).

## *Materials & Method*

This research was carried out with peanut kernels intended for direct human consumption at three areas of production the peanut central zone from Córdoba Province (Argentina). The areas were identified as: Area 1 (Oncativo), Area 2 (Pasco), and Area 3 (La Palestina). Five samples per area at Campaign 2011-2012 were worked. Samples of 8.8 lb weight were taken representatively, according to Rule XIII (SAGPyA, 1994). The picking was performed with a *Geis-Cal*™ invert torn machine, consisting of a swath from four grooves. Completed the period at swath, its top was removed with a *Aipridec*™ machine of a single row. The pods were placed into polyethylene plastic burlap bags and were sent immediately to laboratory for analysis. Then, these were husked manually and evaluations were conducted with peanut kernels of confectionery category. Variables evaluated were:

***Fungal incidence:*** It was determined through modified blotter test method (Mathur & Kongsdal, 2003). Superficially, peanut kernels with healthy appearance were disinfected with sodium hypochlorite solution at 1.5% for two minutes and were rinsed twice with sterile distilled water. Later, these were placed on sterile filter paper moistened with sterile distilled water on plastic trays (6.7 x 9 x 1.6 in) formerly disinfected with alcohol at 95%. Finally, 3 replicates of 50 grains per each sample evaluated were placed to incubate into a chamber at 69.8 ± 35.6 ºF under UV light during cycles 12 hours light & 12 hours dark. Assessments in individual grains were performed seven days after sowing, through binocular lens and stereoscopic microscope (40x). Fungi were classified by techniques commonly used in mycology and with help of keys (Mathur & Kongsdal, 2003). Variables registered were: percentage the total fungal incidence and infected grains with *A. flavus, A. niger, Alternaria, Penicillium, Fusarium* and *Rhizopus* (Bringel *et al*., 2001).

***Total aflatoxins:*** It was preceded through direct competitive ELISA technique for total aflatoxins by *Romer Labs AgraQuant*™. For extraction, it was worked with 4.4 lb of peanut with husk for each sample. Randomly spaced, 1.76 lb of peanut was husked, which then were milled with a *Romer Series II*™ so that 75% passed through a No. 20 mesh. 0.04 lb of milled sample were weighed, and it was derived to alcoholic extraction with solution methanol/water at 70/30 (v/v). Concentrations were calculated by extrapolating optical density (OD) with respective calibration curve obtained. Results were expressed in parts per billion (ppb).

***Evaluation the implementation degree in handling practices, related to aflatoxins contamination:*** Data collection was performed using semi-structured personal surveys to technical managers for each area, under GAP for peanut crop (Bongiovanni *et al*., 2012; Pedelini, 2012). The checklist was grouped according to each stage the production process, from sowing to transport goods to plant. Assessment of each item was established as impact the executed task in relation to occurrence or possible fungal contamination. From addition of values obtained in each item, total score was obtained; and from this, implementation percentage was calculated. Determinations were carried out with a variance analysis model, with which areas considered as fixed effects about said model were compared. Mean values of each variable were subjected to statistical analysis to detect significant differences through ANOVA; comparisons were made with Tukey test ($p<0.05$) and correlations were calculated using *InfoStat*™ statistical program (Di Rienzo *et al.*, 2014).

## *Results & Discussion*

Average values the total fungal incidence obtained showed statistically significant differences at Area 2 relative to other areas evaluated (Figure 1).

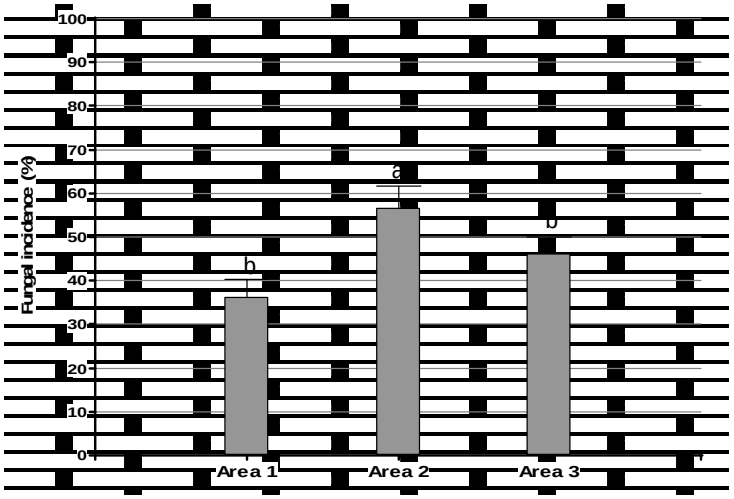


**Figure 1:** Total fungal incidence at three representative areas the peanut central zone from Córdoba Province. Equal letters indicate no significant differences (Tukey $p<0.05$).

In Figure 2, percentages the infected grains with each genus at areas evaluated are observed. Data indicated that fungal incidence with different genera wasn't homogeneous between areas evaluated. Genera detected were: *Aspergillus*, *Penicillium, Rhizopus* and *Fusarium;* standing, at Area 2, *Penicillium* presence which reached values of up to 35%. In all batches, there was fungal contamination; and all them, these was also coincidence with pollutants mostly found in peanut kernels (Cavallo *et al*., 1994; Cavallo *et al*., 2005; Moraes & Mariotto, 1985; Mazzani, 1989).

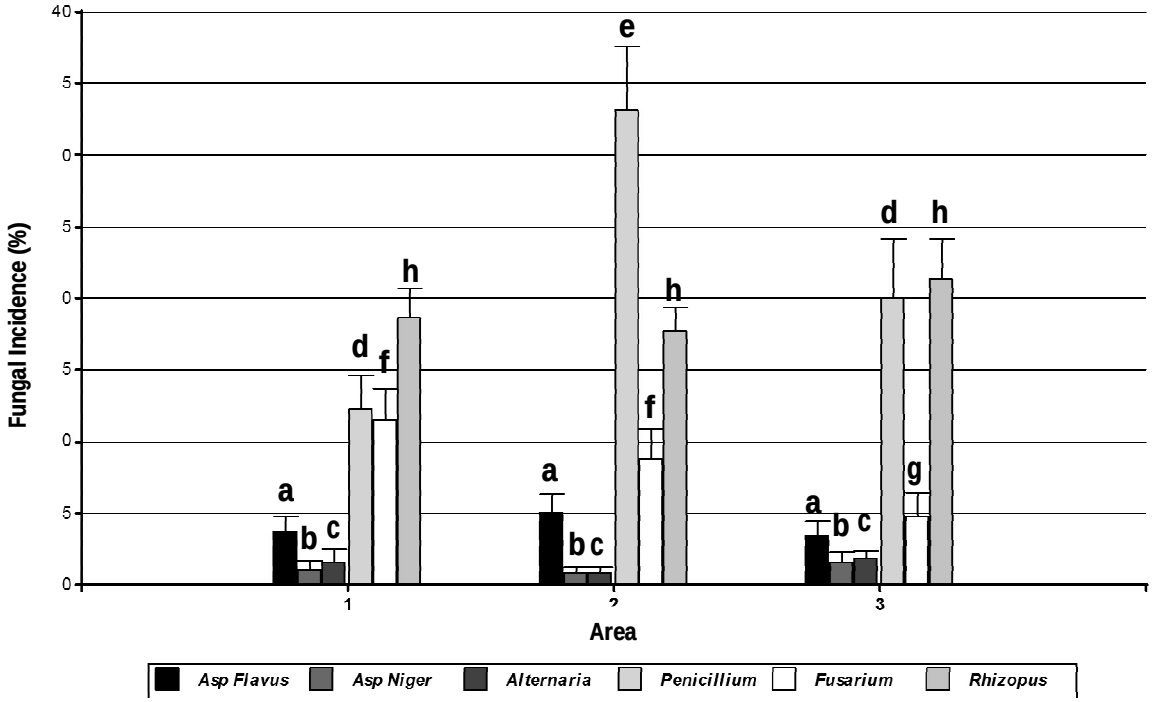


**Figure 2:** Fungal incidence per genus in peanut kernels at three representative areas the peanut central zone from Córdoba Province. Different letters indicate significant differences between evaluated areas (Tukey $p<0.05$).

*A. flavus* incidence between areas evaluated showed no significant differences ($p<0.05$) −Figure 2. This fungal specie, potential precursor the mycotoxins, was found equally at three areas. So that, this situation represents a potential risk which depends on environmental conditions and handling practices executed, according to proposals by Fonseca (1991), Melouk & Shokes (1995) and Schapovaloff *et al*. (2010).

Determination the total aflatoxins showed significant differences between areas (Figure 3). The Area 2 was it lowers aflatoxins contamination.

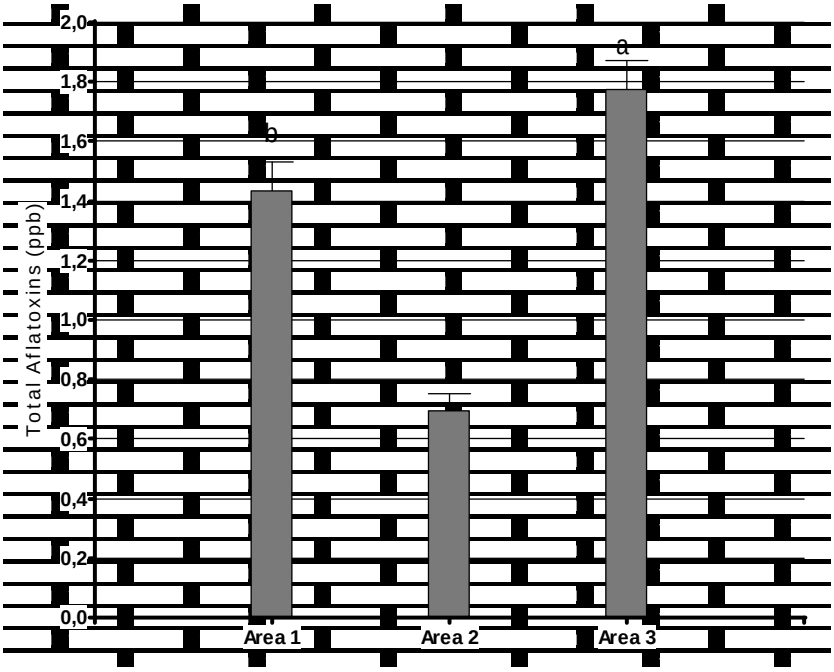


**Figure 3:** Aflatoxins concentration in peanut samples at three representative areas the peanut central zone from Córdoba Province.

Different letters indicate significant differences (Tukey $p<0.05$). Results presented by PAHO (1983); March & Marinelli, (2005); Fernández *et al.* (2006); and Cornejo & Villarroel (2007) indicate that *A. flavus* presence is closely associated with aflatoxins formation. Results found in this research didn't show a direct correlation between *A. flavus* percentage and aflatoxins values detected ($R^2= 0.03$). By comparing studied areas, different aflatoxins concentration was established for same level *A. flavus* contamination.

Regarding handling practices that reduce fungal incidence and subsequent aflatoxin contamination, the higher implementation percentage, according to surveyed data, was obtained for Area 2 (Table 1).

**Table 1:** Implementation percentage the practices at three areas of production from Córdoba Province.

| Etapa | Cumplimiento (%) | | |
|---|---|---|---|
| | **Área 1** | **Área 2** | **Área 3** |
| **Previo a la siembra** | 25.0 | 78.3 | 53.3 |
| **Siembra** | 85.7 | 85.7 | 85.7 |
| **Crecimiento del cultivo** | 100.0 | 100.0 | 100.0 |
| **Cosecha** | 20.0 | 33.3 | 20.0 |
| **Post cosecha** | 0.0 | 0.0 | 0.0 |
| **Total** | **32.5** | **52.1** | **40.5** |

The comparative analysis between evaluated areas demonstrates that, while *A. flavus* incidence levels showed no significant differences (Figure 2), the higher implementation percentage the handling practices at Area 2 determined lower aflatoxins level ($p<0.05$). These results are contrasted to proposals by Cornejo & Villarroel (2007) and Nyirahakizimana *et al.* (2013), who indicate a simple and direct relation between percentage the fungal incidence with *A. flavus* and aflatoxins contamination level in peanut kernels. However, these are consistent with proposals by Chulze (2005) and Pitt *et al.* (2012), who hold that mycotoxin formation depends not only of fungal incidence, but interacting environmental factors such as humidity, temperature and crop handling. The predisposing environmental conditions for aflatoxins formation weren't those that occurred at pre-harvest stages at areas evaluated (temperatures no higher than 77 ºF, absence moisture deficit − Figure 4). Nevertheless, at post-harvest stages, during the time of permanence at swath, rainfalls accumulated during the months of April and May were higher at Area 2 (52 mm), regarding Area 1 (22 mm) and Area 3 (42 mm).

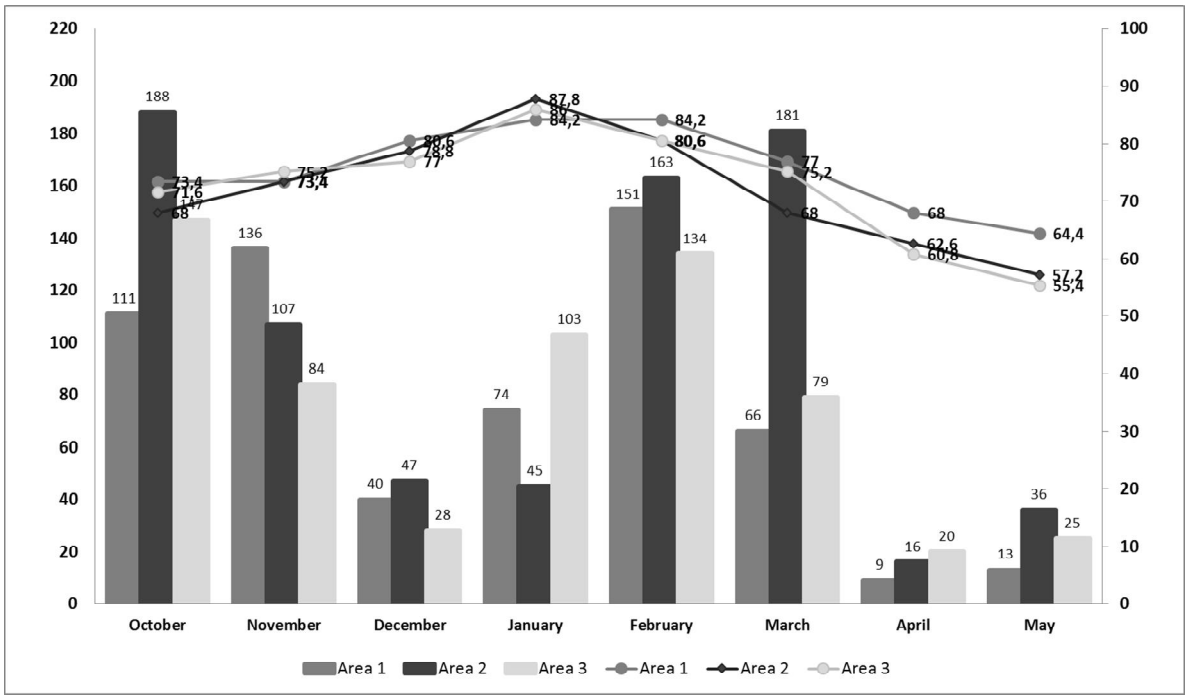


**Figure 4:** Rainfalls (mm) and maximum temperatures (ºF) monthly average for Agricultural Campaign 2011-2012 at Areas 1, 2 & 3 (Córdoba Grain Exchange).

The higher implementation percentage the GAP (Table 1) was associated with lower aflatoxins generation, even if environmental conditions were predisposing (Area 2). The stages in which this area had the higher GAP implementation were: at prior-sowing stage (crop rotation, fallow execution and mechanical tillage), at sowing stage (quality assurance seed, determination sowing time), and at harvest stage (decrease permanence at swath). Stages with the lower implementation percentage the GAP were observed at harvest stages until the time of entry to processing plant.

## *Conclusion*

The fungal incidence levels at studied areas remained not directly related to aflatoxins concentration, which cannot be estimated mycotoxin risk just to health test the peanut kernels. The higher implementation percentage the GAP, related mycotoxins formation, was associated with lower aflatoxins concentration −even if environmental conditions were predisposing. It's noteworthy that while at different areas evaluated, the higher contamination risk was verified at post-torn stages, it's necessary to implement GAP throughout peanut crop cycle, order to encourage grains production of better quality intended for direct human consumption.

## *References*


Azaizeh, H. A., Pettit, R.E., Sarr, B. A. & Phillips, T.D. (1990). Effect of peanut tannin extracts on growth of Aspergillus parasiticus and aflatoxin production. Mycopathologia, 110, 125-132.

Blengino, C. 2014. Maní: Informe Sectorial N°1. (On line). Área de Estudios Sectoriales. Dirección de Agroalimentos. Available in http://www.alimentosargentinos.gov.ar/contenido/sectores/otros/mani/informes/2014_05May.pdf.

Bolsa de Cereales de Córdoba. 2012. Producción. (On line). Available in http://www.bccba.com.ar/produccion-7168.html.

Bongiovanni, R. Troilo y L. Pedelini R. 2012. Buenas prácticas agrícolas para la producción de maní. Manfredi, Córdoba. Argentina. Ediciones INTA. Estación Experimental Agropecuaria Manfredi. 73 p. ISBN 978-987-679-119-9.

Booth, C. (1971). The Genus Fusarium. Commonwealth Micological Institute, 237p. England.

Bringel, J. M. M.; Moraes M. H. D.; Menten J.O.M.; y Bedendo I. P. 2001. Qualidade sanitária e fisiológica de sementes de soja produzidas na Região de Balsa. Summa Phytopathologica, Jaboticabal. P. 438-441.

Casini C. y Bragachini M. 2010. Buenas prácticas de manejo para disminuir el riesgo de aflatoxinas en el cultivo del maní.Informe técnico. INTA E.E.A. Manfredi. (On line). Available in http://www.cosechaypostcosecha.org/data/articulos/calidad/aflatoxinasmani.asp.

Cavallo, A.R. 2005. Sanidad de semillas. En Enfermedades del maní en Argentina. March G. J. y Marinelli A.D (ed.). p. 97-102.

Cavallo, A.R., R.J. Novo y C.W. Robledo. 1994. Flora fúngica transportada por semilla de maní (Arachis hypogaea L.) en la Provincia de Córdoba, Argentina. Facultad de Ciencias Agropecuarias. U.N.C. Córdoba. Agriscientia. Vol 11, p 43-48.

Cavallo, A.R.; Novo, R.J.; Perez, M.A. 2005. Eficiencia de fungicidas en el control de la flora fúngica transportada por semillas de maní *(Arachis hypogaea*L.) En la Argentina. Argentina. Agriscientia. V.1, p. 9-16.

Christensen, C.M. 1982. Storage of Cereal Grains and Their Products. American Association of Cereal Chemists, St Paul. P.145-217.

Chulze, S. 2005. Aflatoxinas en maní. Enfermedades del maní en Argentina. March G. J. y Marinelli A.D (ed.). p. 103-113.

Cornejo J. C. y Villarroel O. G. 2007. Antecedentes generales sobre las aflatoxinas y otras micotoxinas y elementos a tener en cuenta para el diseño de prácticas correctas de cultivo y elaboración de nueces. Ministerio de Salud, departamento de Alimentos y Nutrición. (On line). Available in http://web.minsal.cl/portal/url/item/72fd6274dad8792ee04001011f0109e4.pdf.

Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzales, L.; Tablada, M.; Robledo, C.W. InfoStat versión 2014. Grupo InfoStat. FCA. Universidad Nacional de Córdoba. Argentina. (On line). Available in http://www.infostat.com.ar

Fernandez, E. M.; Giayetto, O. y Cholaky, L. 2006. Crecimiento y desarrollo. El cultivo de maní en Córdoba. Rio Cuarto, Córdoba. P. 73-89. ISBN-13:978-950-665-407-8.

Fonseca, H. 1991. Sistema de amostragem para análise de aflatoxinas en grãos. Rev. Microbiol. Vol. 21, p. 66-70.

Kumeda, Y. y Assao, T. 2001. Heteroduplex panel analysis, a novel method for genetic identification of Aspergillus Section Flavus strains.Applied and Environmental Microbiology. Washington. Estados Unidos. Vol. 57, N. 9, p. 4084-4090.

March, G. J. Y Marinelli, A. D. (Ed.). 2005. Enfermedades del maní en Argentina. Cap 6, p. 103-114. ISBN 987-43-8755-6.

Mathur, S. B. y Kongsdal, O. 2003. Common laboratory seed health testing methods for detecting fungi. International Seed Testing Association. Zürich. P. 111-280. ISBN 3-906549-35-6.

Mazzani, C. B. 1989. Evaluación de la resistencia de genotipos de maní (*Arachis hypogaea L.*) a la colonización de sus semillas por hongos del género Aspergillus. Tesis MSc. Maracay, VE. Facultad de Agronomía, Universidad Central de Venezuela. P. 105.

Melouk, H.A. Y Shokes, F.M. 1995. Management of soilborne fungal pathogens. Peanut health management. Ed. The American Phytopathological Society. Minnesota USA. P. 75-82.

Moraes, S.A. y Mariotto, P.R. 1985. Diagnóstico da patologia de sementes de amendoim no Brasil. Revista Brasileira de Sementes. Brasília, BRA. Vol. 7, n°1, p. 41-43.

Nyirahakizimana, H.; Mwamburi, L.; Wakhisi, J.; Mutegi, C. K.; Christie, M. E. y Wagacha, J. M. 2013. Occurrence of Aspergillus Species and Aflatoxin Contamination in Raw and Roasted Peanuts from Formal and Informal Markets in Eldoret and Kericho Towns, Kenya. Advances in Microbiology. Scienctific Research. (On line). Available in http://dx.doi.org/10.4236/aim.2013.34047.

OMS, Organización Mundial de la salud. Organización De Las Naciones Unidas Para La Alimentación y La Agricultura. 2012. Codex Alimentarius: Prevención y Reducción de la Contaminación de los Alimentos y Piensos. Primera edición.(On line). Available in ftp://ftp.fao.org/codex/publications/Booklets/Contaminants/CCCF_2012_ES.pdf. ISSN 1020-2579.

OPS, Organización Panamericana de la Salud. 1983. Criterio de Salud 11. Micotoxinas. Publicación Científica N° 453, p. 73.

Paster, N. y Bullerman, L.B.1988. Mould spoilage and mycotoxins formation in grains as controlled by physical means. Int. J. Food Microbiol. Vol. 7, n° 3, p. 257-265.

Pedelini, R. 2012. Maní: Guía práctica para su cultivo. Instituto Nacional de Tecnología Agropecuaria. INTA General Cabrera. Estación Experimental Agropecuaria Manfredi. Boletín de divulgación técnica. Segunda edición. 20 p. ISSN 1851-1084. (On line). Available in http://www.ciacabrera.com.ar/Documentos/cia%20revista3.pdf

Pitt, J.I.; Taniwaki, M.H. y Cole, M.B. 2012. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives. SciVerse Science Direct. Food Control 32, p. 205-215.

SAGPyA, Secretaría de Agricultura, Ganadería, Pesca y Alimentos. 1994. Normas de calidad para la comercialización de granos y subproductos. Res. ex SAGPyA N° 1075/94. Capítulo II: Muestreo, Norma XXII Muestreo en granos.

Schapovaloff, M. E.; Señuk, I.A.; Vedoya, M. C. y Medvedeff, M.G. 2010. Ensayos preliminares in vitro de la capacidad aflatoxigénica de Aspergillus flavus aislados de maní. Cátedra de Micología. Facultad de Ciencias Exactas, Químicas y Naturales. Universidad Nacional de Misiones. Rev. Cienc. Tecnol. Año 12 Nº 12. (On line). Available in http://exactas-unam.dyndns.org/recyt/images/stories/Descargas/revista12a_4.pdf.

Schneider, K. y Sieber, H. 1999. Micotoxinas. Peligros Ocultos en los Alimentos. (On line). Available in www.postcosecha.org.ni/documentos/micotoxinas.doc

Van Egmont, H.P.; Jonker, M.A.; Magan, N. y Olsen, M. 2004. Mycotoxins in food. Detection and control. Editorial wood-head publishing limited. Cap. 3, p. 49-68.