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Inhibitory Effect of Essential Oils on Growth and Ochratoxin a Production by *Penicillium* Species

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ABSTRACT

The screening of essential oil of brassica (*Brassica oleracea*), castor (*Ricinus communis),* coconut (*Cocos nucifera*), eucalyptus (*Eucalyptus globulus*), groundnut (*Arachis hypogaea*), neem (*Azadirachta indica*), palmolive (*Elaeis guineensis*) and sunflower (*Helianthus annuus*) for their efficacy against growth and OTA production by *P. verrucosum* and *P. nordicum* was performed. Neem and eucalyptus oil were most effective inhibitors of biomass and OTA production by both the species of *Penicillium* under investigation. *P. nordicum* was totally inhibited at 15 μL mL^{-1} concentration of neem and eucalyptus oil, while *P. verrucosum* was inhibited only to the extent of 77.52-92.49%. Sunflower and palmolive oils were next in their toxicity against the growth and OTA production by both the species of *Penicillium* under investigation. Essential oil of brassica, castor, coconut and groundnut varied in their degree of inhibition against *P. verrucosum* and *P. nordicum*. A positive correlation coefficient (R) was observed between the inhibitory effect of essential oils on growth (0.8933) and OTA production (0.9242). In conclusion, neem and eucalyptus oils proved to be potential bio-control agents and help to prevent the infestation of stored foods and feeds by 2 species of *Penicillium* and elaboration of OTA.

Key words: *P. verrucosum*, *P. nordicum*, essential oils, growth, OTA, HPLC

INTRODUCTION

Mycotoxins are structurally diverse toxic secondary metabolites produced by a wide range of molds infesting different agricultural commodities (Reddy *et al*., 2010). Infestation of food grains by molds not only result in spoilage but also turn them to be toxic due to elaboration of mycotoxins (Fink-Gremmels, 1999). Mycotoxins are important bio-agents responsible for variety of health hazards of many animals including man (Hussein and Brasel, 2001). The OTA, which is a wide spread in nature is reported to be neurotoxic, teratogenic, genotoxic, immunosuppressive and possible to be human carcinogen (Pfohl-Leszkowicz and Manderville, 2007). *Penicillium verrucosum* and *P. nordicum* are the major producers of OTA in foods and feeds pose a potential health hazard to human beings (Sokolic-Mihalak *et al*., 2012).

Therefore, prevention of OTA formation is the best choice for protecting the consumer's health. However, prevention is not always feasible especially, OTA produced under field conditions. Several approaches have been suggested for prevention of OTA in foods and feeds (Diaz *et al*., 2004; Halasz *et al*., 2009). Though, chemical based method proved to be effective in reducing the OTA contamination, it is unsafe (Rao *et al*., 2015). Biological and enzymatic degradation of mycotoxins have been advocated as safe alternate (Pereira *et al*., 2010; Patharajan *et al*., 2011; Juodeikiene *et al*., 2012).

In recent times, considerable interest in the essential oils as in the prevention of mycotoxins in foods and feeds has been witnessed (Hua *et al*., 2014). Basil oil inhibited the growth of the *F. verticillioides*, *F. oxysporum*, *F. proliferatum* and *F. subglutinans* (Kocic-Tanackov *et al*., 2013). Essential oil of *Cymbopogon citratus, Ocimum gratissimum* and *Thymus vulgaris* could check the seed transmission of *Alternaria padwickii*, *Bipolaris oryzae* and *Fusarium moniliforme* (Nguefack *et al*., 2008). Aldred *et al*. (2008) have reported the efficacy of bay, clove and cinnamon oil in preventing the of growth and OTA production by *P. verrucosum* and *A. westerdijkiae* even under optimum water activity and temperature. Cvek *et al*. (2010), Dambolena *et al*. (2010), Tolouee *et al*. (2010), Tian *et al*. (2011), Passone *et al*. (2012), Avila-Sosa *et al*. (2012) and Yamamoto-Ribeiro *et al*. (2013) have also successfully controlled the mold growth and mycotoxin contamination of food commodities studied by them. Hence, the aim of the present study is to assess the efficacy of some of the common essential oils in prevention of growth and OTA production by two species of *Penicillium*.

MATERIALS AND METHODS

Chemicals: All the chemicals used in the present investigations were purchased from Merck (Mumbai, India) and acetic acid, acetonitrile and water were HPLC grade Sigma Aldrich (Mumbai, India).

Essential oils: Refined essential oils of Brassica, castor, coconut, eucalyptus, groundnut, neem, palmolive and sunflower were procured from standard companies located in Warangal, Telangana.

Fungal strains and culture conditions: The ochratoxigenic *Penicillium verrucosum* and *P. nordicum* isolated from poultry feeds and maintained on Malt Extract Agar (MEA) and preserved at 4°C (Rao *et al*., 2011) were employed.

Effect of essential oils on growth and OTA production: One hundred milliliters of basal medium taken in 250 mL Erlenmeyer conical flasks were supplemented with different essential oils so as to get 10 and 15 μL mL^{-1} concentration and sterilized in an autoclave at 15l bp for 30 min. The flasks thus prepared were inoculated with 1 mL of spore's suspension of 7 day old *P. verrucosum* and *P. nordicum*, after cooling to room temperature and incubated at 27±2°C on rotary shaker (Yiedher LM-450D) at 120 rpm for 12 days.

Biomass determination of *Penicillium* species: At the end of 12 day incubation period, cultures of *P. verrucosum* and *P. nordicum* were harvested on pre-weighed Whatman filter paper No. 1. The filter paper along with mycelial mat was dried in hot air oven at 65-75°C for 72 h to get a constant weight after cooling to room temperature in a desiccator. The amount of biomass yield was calculated per milliliter of medium.

Extraction and cleanup: At the end of 12 day incubation period, cultures of *Penicillium* species were acidified with 0.6 M o-phosphoric acid and equal volume of chloroform (1:1, v/v) was added and shaken thoroughly to extract ochratoxin A. The chloroform fraction of OTA was concentrated to 500 μL of acetonitrile on rotary evaporator. The OTA was estimated by RP-HPLC as suggested by Rao *et al*. (2013). The standard OTA (100 μg) was dissolved in 1 mL of acetonitrile and different concentrations were made for preparation of standard curve.

Detection of OTA by HPLC: Quantification of OTA was performed by HPLC (JASCO-975, Japan) using C18 isocratic reverse phase Luna column (250×4.6 mm internal diameter, 5 μM particle size) with mobile phase acetonitrile: water: acetic acid (99:99:2, v/v/v) at flow rate 1 mL min^{-1} by injecting 20 μL of extract under Ultraviolet (UV) detector at 333 nm. The amount of OTA was calculated with the help of standard curve of OTA.

Statistical analysis: All experiments were performed in triplicate and the obtained data was analyzed statistically to test the significance of difference ($p<0.005$) between different treatments by applying one way ANOVA and correlation coefficient using GraphPad InStat version 5.03 (GraphPad Software, Inc.) was performed to compare the growth and OTA production by species of *Penicillium*.

RESULTS AND DISCUSSIONS

Inhibitory effect of essential oils on biomass production by *Penicillium* species: Essential oils tried in the present investigation significantly, inhibited the mycelial growth and OTA production by *P. verrucosum* and *P. nordicum*, which varied with essential oil. Maximum inhibition of biomass of *P. verrucosum* (Fig. 1a) and *P. nordicum* (Fig. 1b) was recorded in the presence of neem, eucalyptus, sunflower, castor and palmolive oils at 15 μL mL^{-1} in a descending order. The essential oils of brassica, coconut and ground nut exhibited least toxicity. Rest of the essential oils were intermediate in their efficacy in the control of both the species of *Penicillium* under investigations. The effect of essential oil on growth and OTA production was statistically analyzed and their mean, minimum, maximum range presented in Table 1. The mean OTA production by *P. verrucosum* (8.64 mg mL^{-1}) and *P. nordicum* (7.21 mg mL^{-1}) was significant and ranged from 1.44-19.18 and 0.00-17.51 mg mL^{-1}, respectively. A positive correlation coefficient (0.9242) was observed on OTA production by *P. verrucosum* and *P. nordicum* and towards the inhibitory effect of essential oils assayed.

Inhibitory effect of essential oils on OTA production by *Penicillium* species: Total inhibition of OTA production by *P. verrucosum* (Fig. 2a) and *P. nordicum* (Fig. 2b) was observed in the presence of neem and eucalyptus oil at 15 μL mL^{-1} concentration. Similarly significant inhibitory effect of sunflower and coconut oil on OTA production was recorded. Mossini *et al*. (2009) have also reported the efficacy of neem oil and neem leaf extracts in preventing growth and OTA

Table 1: Statistical analysis of inhibitory effect of essential oils on biomass and OTA production by *Penicillium* species

	Growth (mg mL^{-1})		OTA (μg mL^{-1})	
Statistical analysis	*P. verrucosum*	*P. nordicum*	*P. verrucosum*	*P. nordicum*
Minimum	1.4400	0.000	0.000	0.000
Median	8.2200	6.960	5.7900	6.590
Maximum	19.1800	17.510	22.8500	20.150
Mean	8.6440	7.211	6.4580	6.352
Std. Deviation	3.9730	4.377	5.5170	5.284
Std. Error	0.9637	1.062	1.3380	1.282
Paired t test	0.0028	-	0.8638	-
t, df	t = 3.5310 df = 16	-	t = 0.1743 df = 16	-
Mean of differences	1.4330	-	0.1059	-
95% confidence interval	0.5726-2.293	-	-1.1820-1.394	-
R square	0.4380	-	0.001894	-
Correlation coefficient (r)	0.9242	-	0.8933	-

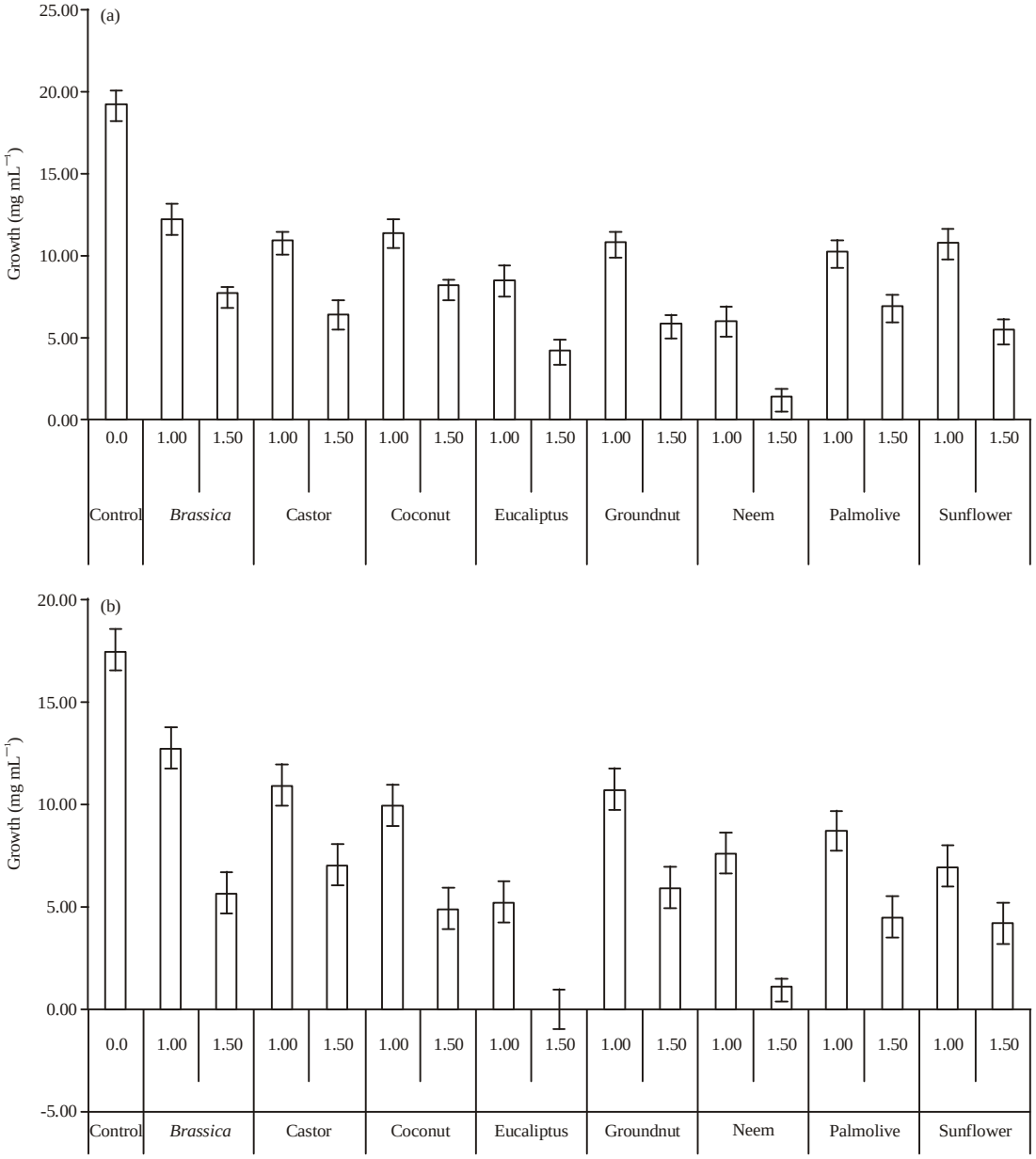

Fig. 1(a-b): Inhibitory effect of oils on growth of, (a) *P. verrucosum* and (b) *P. nordicum*

production by *P. verrucosum* and *P. brevicompactum*. Fungi toxicity of neem may be attributed to presence of biologically active compounds like triterpenoidal, tetranortriterpenoid, azadirachtin, 6-deacetyl-nimbin, azadiradione, nimbin, salannim and epoxyazadiradione (Martinez, 2002). The fungitoxicity of oil extracted from caraway (Carum carvi) against *A. flavus* and *A. parasiticus* was also reported by Soher (1999). Hua *et al.* (2014) have reported the inhibition of *A. ochraceus, A. flavus, A. parasiticus* and *F. moniliforme* by the essential oil of thyme, cinnamon, anise spearmint and basil oil at 3000 ppm. Cinnamon oil was also reported to be fungistatic against mycotoxigenic fungi studied by Mukherjee and Nandi (1994) and found the useful in protecting

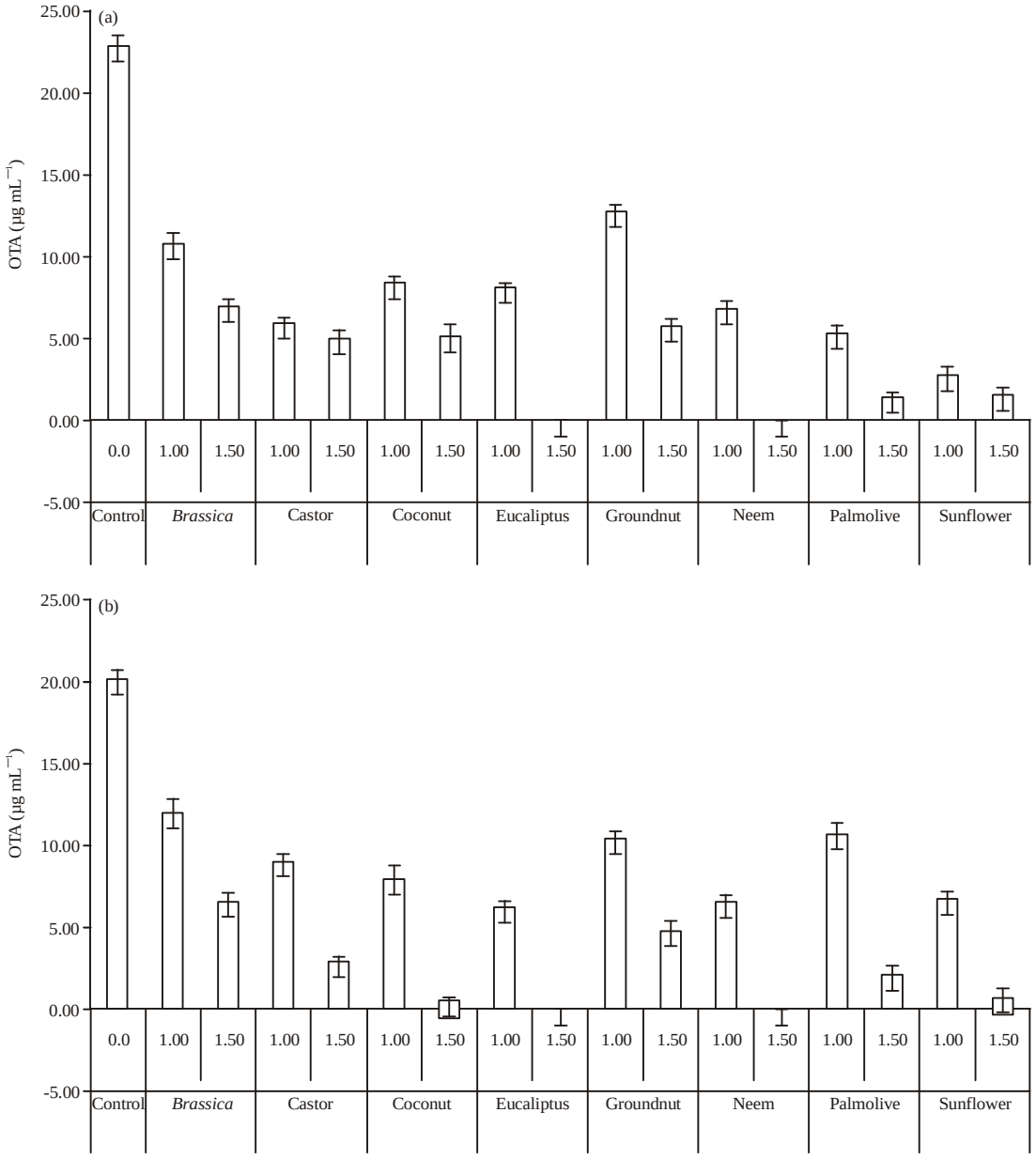

Fig. 2(a-b): Inhibitory effect of oils on OTA production by, (a) *P. verrucosum* and (b) *P. nordicum*

foods and feeds. Sunflower and coconut oils caused the inhibition of OTA production at 15 µL mL^{-1} concentration to the extent of 97% OTA and 75% of the biomass production by *P. nordicum*. The inhibitory effect was found to be dose dependent.

Brassica, castor, ground nut and palmolive oils were least toxic and failed to inhibit OTA production by both the species of *Penicillium* under investigation. Rest of the essential oils were intermediate in their toxicity on the OTA production by both the species of *Penicillium*. The effect of essential oils on OTA production was statistically analyzed and their mean, minimum, maximum ranges are presented in Table 1. The mean OTA production by *P. verrucosum* (6.45 µg mL^{-1}) and *P. nordicum* (6.35 µg mL^{-1}) was significantly recorded range (0.00-22.85 µg mL^{-1}) and

(0.00-20.15 μg mL^{-1}) with *P. verrucosum* and *P. nordicum*, respectively. A positive correlation (0.8933) was observed between growth of *P. verrucosum* and *P. nordicum* and the inhibitory effect of essential oils tried.

Infestation of mycotoxigenic fungi and mycotoxins contamination may not be eliminated completely during seasons of poor weather and crop conditions. The present investigations reveal that neem oil was effective inhibitors of OTA production. No positive correlation could be observed between the inhibitory effect on biomass and OTA production. Hope *et al*. (2005) failed to observe correlation between mycotoxin elaboration and growth of *Fusarium culmorum*, *Fusarium graminearum*. Similarly, Magan *et al*. (2002), who also observed sub-optimal levels of fungicides stimulated Deoxynivalenol (DON) production by *F. culmorum* in wheat grain. Therefore, in depth analysis conductions leading to elaboration of mycotoxins reveals critical role of environmental conditions, type of foods and feeds infesting moulds.

CONCLUSION

In conclusion, essential oils could be safely used as preservatives of some kinds of foods. Neem and eucalyptus oils, which were responsible for total inhibition OTA production by *P. verrucosum* and *P. nordicum* at low concentration can be helpful in protecting foods and feeds at post harvest stage. However, an in depth study is needed to fully understand the mechanism of action essential oils and mode of application in order to exploit these ecofriendly oils in protection of foods and feeds.

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REFERENCES

Aldred, D., V. Cairns-Fuller and N. Magan, 2008. Environmental factors affect efficacy of some essential oils and resveratrol to control growth and ochratoxin a production by *Penicillium verrucosum* and *Aspergillus westerdijkiae* on wheat grain. J. Stored Prod. Res., 44: 341-346.

Avila-Sosa, R., E. Palou, M.T.J. Munguia, G.V. Nevarez-Moorillon, A.R.N. Cruz and A. Lopez-Malo, 2012. Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films. Int. J. Food Microbiol., 153: 66-72.

Cvek, D., K. Markov, J. Frece, T. Dragicevic, M. Majica and F. Delas, 2010. Growth inhibition of *Aspergillus ochraceus* ZMPBF 318 and *Penicillium expansum* ZMPBF 565 by four essential oils. Arch. Ind. Hygiene Toxicol., 61: 191-196.

Dambolena, J.S., M.P. Zunino, A.G. Lopez, H.R. Rubinstein and J.A. Zygadlo *et al*., 2010. Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by Fusarium verticillioides. Innovat. Food Sci. Emerg. Technol., 11: 410-414.

Diaz, D.E., W.M. Hagler Jr, J.T. Blackwelder, J.A. Eve and B.A. Hopkins *et al*., 2004. Aflatoxin binders II: Reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. Mycopathologia, 157: 233-241.

Fink-Gremmels, J., 1999. Mycotoxins: Their implications for human and animal health. Vet. Q., 21: 115-120.

Halasz, A., R. Lasztity, T. Abonyi and A. Bata, 2009. Decontamination of mycotoxin-containing food and feed by biodegradation. Food Rev. Int., 25: 284-298.

Hope, R., V. Cairns-Fuller, D. Aldred and N. Magan, 2005. Use of antioxidants and essential oils for controlling mycotoxins in grain. BCPC Crop Sci. Technol., 5B: 429-436.

Hua, H., F. Xing, J.N. Selvaraj, Y. Wang and Y. Zhao *et al*., 2014. Inhibitory effect of essential oils on *Aspergillus ochraceus* growth and ochratoxin a production. PloS One, Vol. 9. 10.1371/journal.pone.0108285

Hussein, H.S. and J.M. Brasel, 2001. Toxicity, metabolism and impact of mycotoxins on humans and animals. Toxicology, 167: 101-134.

Juodeikiene, G., L. Basinskiene, D. Vidmantiene, T. Makaravicius and E. Bartkiene, 2012. Benefits of β-xylanase for wheat biomass conversion to bioethanol. J. Sci. Food Agric., 92: 84-91.

Kocic-Tanackov, S., G. Dimic, J. Levic, I. Tanackov and D. Tuco, 2013. Antifungal activities of basil (*Ocimum basilicum* L.) extract on Fusarium species. Afr. J. Biotechnol., 10: 10188-10195.

Magan, N., R. Hope, A. Colleate and E.S. Baxter, 2002. Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. Eur. J. Plant Pathol., 108: 685-690.

Martinez, S.S., 2002. O Nim Azadirachta Indica: Natureza, Usos Multiplos, Producao. Instituto Agronomico do Parana, Londrina, pp: 142.

Mossini, S.A.G., C. Carla and C. Kemmelmeier, 2009. Effect of neem leaf extract and Neem oil on *Penicillium* growth, sporulation, morphology and ochratoxin A production. Toxins, 1: 3-13.

Mukherjee, P.S. and B. Nandi, 1994. Poultry feed preservation from fungal infection by cinnamon oil. J. Mycopathol. Res., 32: 1-5.

Nguefack J., V. Leth, J.B.L. Dongmo, J. Torp, P.H.A. Zollo and S. Nyasse, 2008. Use of three essential oils as seed treatments against seed-borne fungi of rice (*Oryza sativa* L.). Am. Eurasian J. Agric. Environ. Sci., 4: 554-560.

Passone, M.A., N.S. Girardi and M. Etcheverry, 2012. Evaluation of the control ability of five essential oils against Aspergillus section Nigri growth and ochratoxin A accumulation in peanut meal extract agar conditioned at different water activities levels. Int. J. Food Microbiol., 159: 198-206.

Patharajan, S., K.R.N. Reddy, V. Karthikeyan, D. Spadaro, A. Lore, M.L. Gullino and A. Garibaldi, 2011. Potential of yeast antagonists on *in vitro* biodegradation of ochratoxin A. Food Control., 22: 290-296.

Pereira, P., A. Nesci, C. Castillo and M. Etcheverry, 2010. Impact of bacterial biological control agents on fumonisin B1 content and *Fusarium verticillioides* infection of field-grown maize. Biol. Control, 53: 258-266.

Pfohl-Leszkowicz, A. and R.A. Manderville, 2007. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Mol. Nutr. Food Res., 51: 61-99.

Rao, V.K., P. Shilpa, S. Girisham and S.M. Reddy, 2011. Incidence of mycotoxigenic penicillia in feeds of Andhra Pradesh, India. Int. J. Biotechnol. Mol. Biol. Res., 2: 46-50.

Rao, V.K., M.V. Ramana, S. Girisham and S.M. Reddy, 2013. Culture media and factors influencing ochratoxin a production by two species of *Penicillium* isolated from poultry feeds. Nat. Acad. Sci. Lett., 36: 101-110.

Rao, V.K., K.N. Rao, S. Girisham and S.M. Reddy, 2015. Efficacy of food preservatives and volatile compounds in the control of Ochratoxin A production by *Penicillium* species. Proc. Natl. Acad. Sci. India Section B: Biol. Sci., (In Press). 10.1007/s40011-015-0513-1

Reddy, K.R.N., B. Salleh, B. Saad, H.K. Abbas, C.A. Abel and W.T. Shier, 2010. An overview of mycotoxin contamination in foods and its implications for human health. Toxin Rev., 29: 3-26.

Soher, E.A., 1999. Prevention of the growth and a atoxin production of *Aspergillus flavus* by some spice essential oils. Minu ya J. Agric. Res., 24: 563-576.

Sokolic-Mihalak, D., J. Frece, A. Slavica, F. Delas, H. Pavlovic and K. Markov, 2012. The effects of wild thyme (*Thymus Serpyllum* L.) essential oil components against Ochratoxin-Producing Aspergilli/Majcina Dusica (*Thymus Serpyllum* L.) I Njezine Komponente Protiv Okratoksikotvornih Vrsta Aspergillusa. Arch. Ind. Hygiene Toxicol., 63: 457-462.

Tian, J., X. Ban, H. Zeng, J. He, B. Huang and Y. Wang, 2011. Chemical composition and antifungal activity of essential oil from *Cicuta virosa* L. var. latisecta Celak. Int. J. Food Microbiol., 145: 464-470.

Tolouee, M., S. Alinezhad, R. Saberi, A. Eslamifar and S.J. Zad *et al*., 2010. Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of *Aspergillus niger* van Tieghem. Int. J. Food Microbiol., 139: 127-133.

Yamamoto-Ribeiro, M.M.G., R. Grespan, C.Y. Kohiyama, F.D. Ferreira and S.A.G. Mossini *et al*., 2013. Effect of *Zingiber officinale* essential oil on *Fusarium verticillioides* and fumonisin production. Food Chem., 141: 3147-3152.