Original Research

Preservative Effects of Neem Oil (*Azadirachta indica*) on Farm-Mixed Poultry Feed

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Abstract

In the present study, In vitro and in sacco antifungal activities of neem oil (NO) against Aspergillus flavus, Aspergillus niger, Penicillium sp and Fusarium sp were evaluated on farm-mixed poultry feed. In vitro concentrations of NO were 0.25, 0.5, 1, 1.5, 2, 2.5 and 3% (v/v) and In sacco concentrations were 10, 15, 20, 25 and 30 g/Kg of feed. The most frequently isolated fungi in poultry feed was Penicillium sp (83.87%). The lowest in vitro colony diameters of Aspergillus favus, Aspergillus niger and Fusarium sp were recorded with 3% of NO while, the highest fungi growth reductions were recorded with 2.5 and 3% of NO. Above the concentration of 0.5% NO showed fungistatic activity against A. flavus, A. niger and Penicillium sp. In sacco study of NO established the total inhibition (100%) of Aspergillus niger and Fusarium sp with 10, 15 and 30 g NO Kg^{-1} of feed after 20 days of storage. At 30 days of storage, there was no contamination of farm-mixed poultry feed especially by Aspergillus flavus and Aspergillus niger and the increasing NO concentration decreased the contamination of poultry feed by Penicillium sp. Given its accessibility, neem oil could be implemented as part of suitable integrated preservative agent for fungal contamination, as it has shown the inhibition of growth and number of colony.

Key words: Antifungal Activity, Fungi Count, Neem Oil, Poultry Feed, Preservative Effect

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Introduction

Poultry feed ingredients infestation by different strains of fungi have adverse effect on health and productivity. When mycotoxins-contaminant such as *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and soil fungi resulting from spoiled food are consumed, it causes a huge economic and environmental loss especially during post-harvest processing and food conservation (Bryden *et al*., 2012; Oguz *et al*., 2012). These fungi produce secondary metabolites mainly mycotoxins such as, aflatoxins, ochratoxin-A and

fumonisins which represent the most important potential risk to animal and human health (Bryden *et al*., 2012; Kana *et al*., 2013; Rodrigues *et al*., 2011). They are recognised as inhibitors of nucleic acid and protein synthesis in animals (Smith, 2011). However, the development of these fungi can be thwarted by factors related on physico-chemical characteristics of the food, such as temperature, humidity and electromagnetic radiation present around the food (Okoli *et al*., 2006).

To control the mold growth and accumulation of mycotoxins in foodstuffs, food preservatives mostly from synthetic origin are primarily employed alone or in combination with physical treatments to ensure the safety and stability of the products during storage (Javaid and Rauf, 2015). The reduce susceptibility of microorganism to synthetic preservatives and the consumers demand for high quality, preservative-free, safe and minimally processed green label foods urged the food industry to focus more on natural preservation and stabilizing approaches (Bhandari, 2014). Plant extracts and small molecules from plant origin have proven to be complementary control means as they displayed good antimicrobial ability and as a food conservation (Javaid and Iqbal, 2014; Javaid and Rauf, 2015). Their relatively low toxicity and biodegradability have led to a new door of safety (Ibrahim and Al-Ebady, 2014).

The neem plant (*Azadirachta indica*), a member of the family of Meliaceae is a fast-growing evergreen plant found in most parts of Africa including Kenya, Nigeria and Cameroon (Girish and Shankara, 2008). Neem is an effective source of environmentally-powerful natural food preservative and considered to be one of the most promising pesticides. It comprises several parts, such as fruit, seed, leaf and oil, with several active compounds (Govindachari *et al*., 1998; Martinez, 2002). The seeds contain many bioactive compounds and neem oil seed is most commercially relevant (Dual *et al*., 2009). A number of bioactive compounds have been isolated and characterized from neem oil, including azadirachtin, gedunin, quercetin, margolone, margolonone, nimbidin, nimbin, nimbolide and salannin (Valarmathy *et al*., 2010). These phytochemicals contribute to the various biological activities of neem oil, such as acaricidal, antibacterial, anticarcinogenic, antifungal, antihyperglycaemic, anti-inflammatory, antimalarial, antimutagenic, antiprotozoan, antioxidant, antiulcer, antiviral, immunomodulatory, insect-repellent and spermicidal properties (Ebong *et al*., 2008). Previous *In vitro* studies established, antifungal activities of neem oil against *Penicillium verrucosum* and *Penicillium brevicompactum* growth and ochratoxin A production (Mossini *et al*., 2009). Geraldo *et al.* (2010) established the antifungal activities of neem oil against *Fusarium oxysporum medicagenis, Fusarium subglutinans* and the production of fusaric acid toxin. *In vitro* activity of neem oil on *Aspergillus flavus* growth, sporulation and viability of spores, morphology and aflatoxins B1 production was also reported by Costa *et al.* (2010). Neem oil is reported to have considerably higher antibacterial activity than antibiotics such as tetracycline, ampicillin and ciprofloxacin (Jacela *et al*., 2010). Neem cake exhibits antimicrobial activity against bacteria spoiling the quality of retail fresh meat in

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a broth model meat system, thereby preserving the food system against microbial spoilage (Jacela *et al*., 2010).

No study so far has assessed the effects of neem oil as preservative of animal feed. The present investigation was carried out to study antifungal activity of neem oil against notorious animal feed pathogenic fungi as well as its potential in poultry feed preservation.

Materials and Methods

Study Area

This study was carried out in the Animal Nutrition and Production Research Unit of the University of Dschang, Cameroon. It is located at 05° 26 N latitude, 10° 26 E longitude and at an average altitude of 1420 m in the agro-ecological zone of the Western High Plateau of Cameroon. Climate is characterized by two seasons: a rainy season from mid-March to mid-November and a dry season for the rest of the year. The average rainfall is 2000 mm per year and the average temperature is around 21°C, the average annual insolation is 1873 hours and the average relative humidity is 76.8%.

Isolation and Identification of Fungi

The isolation of fungi was done following previously established dilution plate technique procedures (Kana *et al*., 2013). Five grams of feed was mixed with 45 mL of sterile distilled water on horizontal shaker at 220 rpm at 25°C for 20 minutes. Ten-fold appropriate serial dilutions were prepared and aliquots consisting of 1.0 mL of each dilution (in triplicate) were spread over potato dextrose agar (PDA) plates prepared with 1% chloramphenicol. The plates were then incubated at 27°C in the dark for one week (Kana *et al*., 2013). A pure culture of each colony type on each plate was obtained by sub-culturing each of the different colonies onto PDA plates, which were incubated at room temperature for 5 days. Pure fungal isolates were identified from their macroscopic and microscopic characteristics according to Samson *et al* (2010). Fungal isolates initially cultured on PDA were purified in the same medium. The relative density (RD) of each species was calculated according to Gonzalez *et al.* (1995).

Neem Oil Extraction

Fresh neem fruits were collected at Garoua in the Northern Region of Cameroon between September and October 2017. They were thoroughly washed with running tap water and separated from the sheet manually. The material was oven dried at 50ºC until constant moisture content was achieved. The dried seeds were ground in a mill for size reduction. The extraction was carried out by kneading the paste. During kneading, cold water was progressively added to the ground kernels in a pot. The amount of water added (50 ml/kg) was fundamental because the paste must remain thick. The paste was squeezed with a manual press; oil obtained was heated to evaporate water and conserved in a plastic bottle at room temperature, 1 Kg of neem

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fruits provided 0.5 kg of seeds yielding 150 ml of oil. The phytochemical analyses of neem oil were carried as described by Talukdar *et al.* (2010) for the presence of alkaloids, flavonoids, terpenoids, phenols, steroids, saponins and tannins.

In Vitro Antifungal Screening of Neem Oil

Neem oil solution was screened for antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp and *Penicillium* sp according to a method modified from the procedure reported by Kaur *et al*. (2015). About 20 ml of potato dextrose agar (PDA) medium with 1 % chloramphenicol (inhibit bacterial growth) was poured into petri plates and allowed to solidify. After solidification, 0.25, 0.5, 1, 1.5, 2, 2.5 and 3 % (v/v) of neem oil were spread on top using a sterile swab. Plate without neem oil was used as the negative control and plate with Nystatine® as positive control. The 5 mm discs of a 7-days old culture of respective test fungi were placed at the center of the above petri plates and incubated at 27°C. Plates were prepared in four replicates for growth, percentage inhibition and sporulation measurement. The diameter of colony was measured daily in two directions at 90° from each other to obtain the mean diameter for each colony (Bluma *et al*., 2008). The advantage of the method was that sequential records might be obtained from each colony, although only lateral growth was measured (Sutton *et al*., 1972).

Procedures for sporulation measurement followed Gusmán-de-Peña and Ruiz-Herrera (1997) with modifications. Agar discs were aseptically removed from the central, intermediate and peripheral zones of each replicate plate using a cork borer, transferred to flasks containing a sterile 0.1% Tween 80 solution (10 ml) and stirred for two minutes with a vortex to release the spores. After mycelium sedimentation, the supernatant containing the spores was recovered and estimated by a Neubauer counting chamber. The sporulation data were recorded in spores/cm of diameter colony. The toxicity of neem oil to fungi in term of percentage inhibition of mycelial growth was calculated according to Amandioha (2000).

$$\% \text{ Inhibition} = (dc-dt)/dc \times 100$$

Where,

dc= average increase in mycelial growth in control; dt = average increase in mycelial growth under treatment.

In sacco Antifungal Screening of Farm-Mixed Feed Poultry of Neem Oil

The farm-mixed poultry feed was formulated with following ingredients: maize (65%), cotton seed meal (5%), soybean meal (15%), fish meal (5%), wheat bran (5%), and oyster shell (1%) and premix (5%). Feed was mixed with respectively 10, 15, 20 and 30 g NO $Kg^{-1}$and stored at room temperature in sterile 45 × 20 cm bags. Each sample was replicated 3 times and conserved for 10, 20 and 30 days. After each period of storage, 3 samples were randomly collected from each treatment group and the fungal counts were

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determined. The serial dilution technique was used and treatments were carried out in a sterile beaker with gentle shaking (100 rpm) by a multi-shaker at room temperature. After serial dilution, 500 µl of each solution was poured on to a sterile plate (9 cm diameter) and 20 ml of potato dextrose agar was added and left to solidify at room temperature. The plates were then incubated at 27°C for 72 hours and colonies were counted as described previously (Kana *et al*., 2013).

Statistical Analysis

The Completely Randomized Design (CRD) was applied. All the data were analysed by two factors (Concentration of neem oil and fungi species) analysis of variance (ANOVA). The comparison among means was worked out using Duncan test at 5% level of significance (Steel and Torrie, 1984).

Results and Discussion

The present study of the qualitative phytochemical analysis revealed that neem oil possesses bioactive compounds such as alkaloids, steroids, flavonoids, phenols, triterpenoids and tannins (Table 1) that can affect the growth of fungi. Geraldo *et al*. (2010) and Mossini *et al.* (2009) also revealed the presence of flavonoids, alkaloids, tannins, phenols and steroids in neem oil and their *in vitro* antifungal activity against *Fusarium oxysporum medicagenis* and *Fusarium subglutinans.*

Table 1: Phytochemical composition of neem oil

Bio-active Constituents Classes	Observation
Triterpenoids	+
Alkaloids	+
Steroids	+
Saponins	-
Phenols	+
Flavonoids	+
Tannins	+

+: Present; - : Absent

Analysis of variance revealed a significant (*P*= 0.00) effect of the studied neem oil concentrations on colony diameter, sporulation and colony forming units per g. In all the fungal species, the diameter of colony decreased with increasing NO concentration from 0.25 to 1%. From 1 to 3% (v/v) NO showed fungistatic activity against *Aspergillus flavus, Aspergillus niger* and *Penicillium sp* (Fig. 1).

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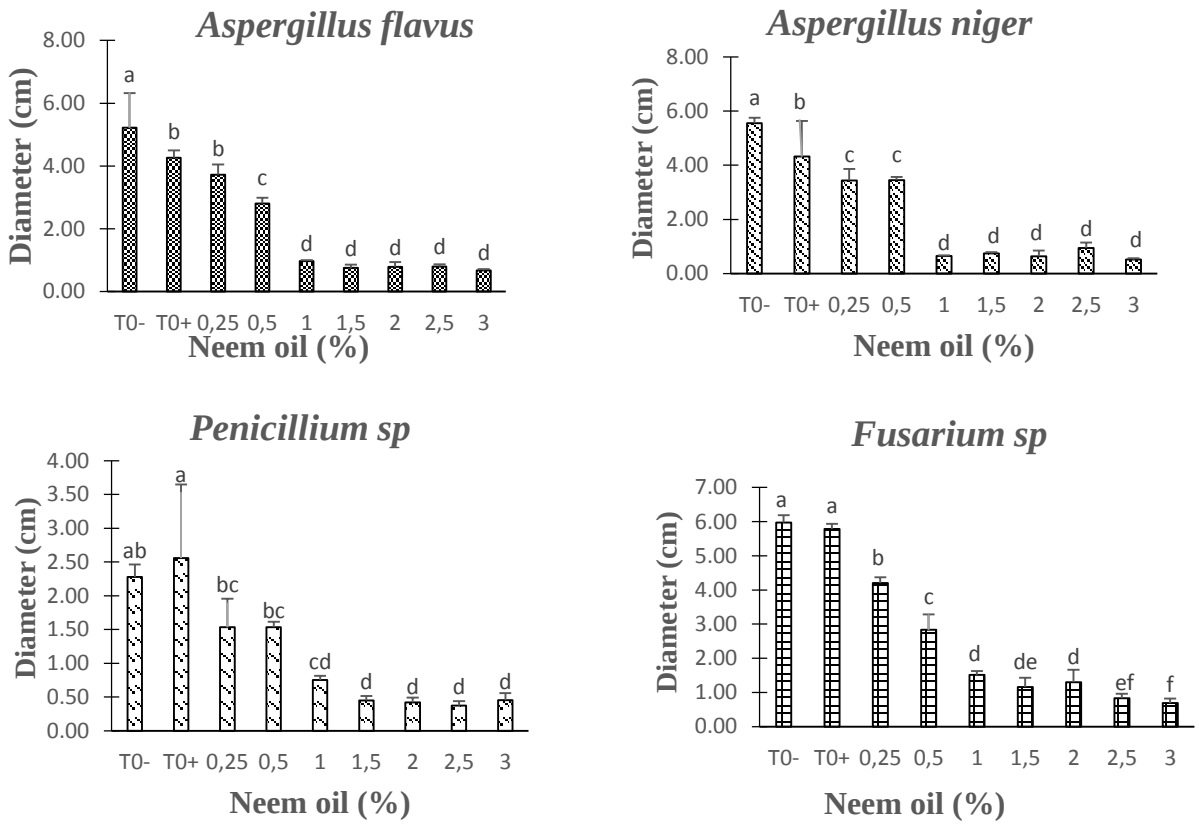

Fig. 1: Antifungal activity of neem oil against *Aspergillus flavus*, *Aspergillus niger*, *Pencicillium sp* and *Fusarium sp*.

The highest fungi growth reductions ranged from 72 to 81%, 84 to 88%, 59 to 75% and 70 to 86% respectively for *A. flavus*, *A. niger*, *Penicillium* sp. and *Fusarium* sp. with NO concentration ranging from 1 to 3 % except for *A. niger* which ranged from 0.5 to 3% (Fig. 2). The decrease in fungal count with neem oil might be due to the inhibition of environmental factors (relative humidity, little aeration and temperature) and tolerance activity of antifungal compounds present in neem oil such as triterpenoids. The above mentioned climatic and environmental conditions are highly favorable for the propagation of fungi, especially *Aspergillus* sp. and *Penicillium* sp. that produce and release spores (Mossini *et al*., 2009). There is a relationship between the chemical structures of the most abundant compounds in the neem oil and the antimicrobial activity (Farag *et al*., 1989).

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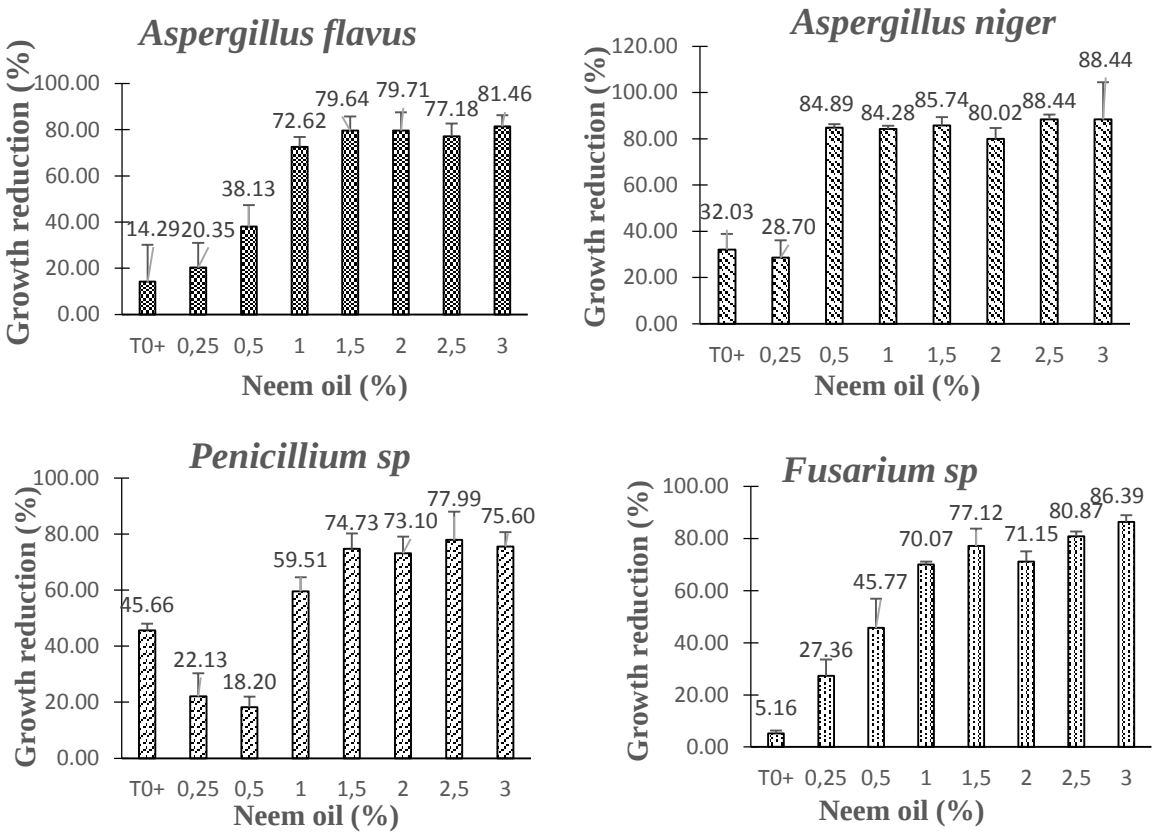

Fig. 2: Growth reduction of *Aspergillus flavus*, *Aspergillus niger*, *Pencicillium sp.* and *Fusarium sp.* as affected by the graded concentration of neem oil.

Bioactive compounds founds in plants exert antimicrobial activity, first, by interfering with the phospholipid bilayer of the cell membrane, which causes an increase in permeability and a loss of cellular constituents; second, by impairing a variety of enzyme systems, including those involved in the production of cellular energy and the synthesis of structural components (Conner and Beuchat, 1984); and third, by inactivating or destroying genetic material (Kim *et al*., 1995). However, the mode of action differs among the various compounds.

The ability of NO to inhibit mycelial growth was associated with the enhacement of sporulation (Table 2). The sporulation was markedly (P = 0.00) higher with NO concentration ranging from 1 to 2%. Above 2%, the sporulation tends to decrease with increasing level of NO irrespective to the fungal species.

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Table 2: Effects of graded concentration of neem oil on *Aspergillus flavus*, *Aspergillus niger*, *Pencicillium sp.* and *Fusarium sp.* sporulation ($\times 10^5$/cm)

Fungi	Neem oil (%)									
	T_{0-}	T_{0+}	0.25	0.5	1	1.5	2	2.5	3	*p-value*
Aspergillus flavus	12.67±2.52d	6.84± 0.39^{d}	21.40±4.63b	59.58±8.54cd	249.33±6.93^{b}	372.67±39.11^{a}	322.67±102.79ab	118.4±48.64c	73.33±4.81cd	0
Aspergillus niger	17.67±1.68d	23.28±13.98^{d}	26.13±10.46cd	31.34±1.36cd	267.67±116.43^{a}	201.70±43.13^{ab}	95.81±48.80cd	78.90±23.06cd	123.40±72.36bc	0
Penicillium sp	51.25±29.55c	15.96±6.79^{c}	56.38±22.94c	79.42±26.38c	303.3±80.3b	440.22±42.71^{a}	267.78±89.89b	225.33±94.01^{b}	110.22±18.39a	0
Fusarium sp	14.27±1.81cd	6.87±1.15d	23.08±2.32cd	53.76±15.4bcd	174.7±130.7ab	138.81±23.86^{abc}	112.93±25.23abcd	190.89±66.72^{a}	75.41±41.21abcd	0.02

a, b, c, d: Means with the same superscript in the same row are statically non-significant (P>0.05) ; p- probability

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There was a significant increase in spore numbers with NO concentration from 1 to 3% (v/v) as compared to the negative and positive control treatment. The increasing neem oil concentrations were followed by the increasing number of spores. This result corroborates the findings of Costa *et al.* (2010) which showed that sporulation of *Aspergillus flavus* significantly increased with concentration of NO above 0.5 %. These results differ from some reports describing *in vitro* decrease in spores production of all fungi with 0.125; 0.250 and 0.500% NO (Mossini *et al*., 2009). The increasing spore number with increasing NO concentration in poultry feed may be either due to the solubility of the active oil compounds or inherent to fungal metabolism. Carbon assimilation is essential for the generation of new biomass and rapid fungal growth in the host relies on the efficient uptake and metabolism of available carbon sources. These can include fermentable sugars (such as glucose, fructose, and galactose) and non-fermentable carbon sources (such as amino acids and organic acids) (Ueno *et al*., 2011; Vieira *et al*., 2010). Fungal pathogens have evolved different carbon assimilation profiles that presumably reflect their different substrate. The mould spore plate count has often been used to estimate the potential risk that feed may pose to poultry health. This measurement, however, was found to be highly variable between batches of feed manufactured at different concentration. However, this test can be less expensive than analyses for mycotoxin contamination, while yielding a number corresponding to the number of viable mould spores per unit of feed-stuff (Brothers and Wyatt, 2000).

Total fungi count is one of the criteria used to evaluate the hygienic quality of feed. The regulation on harmful substances and components in livestock feed, mixtures and raw materials for animal feed are not in compliance with the standards of the hygiene quality if they contain above 300.000 cfu g^{-1} of forage mixture for old animal categories or 50.000 cfu g^{-1} for young animals (Oliveria *et al*., 2006). *In sacco* study of neem oil as a natural preservative to control farm-mixed poultry spoilage, revealed that after 10 days of storage, *Penicillium sp* was inhibited at 88.31, 85.75, 73 and 100%, respectively with 15, 20, 25 and 30g NO Kg^{-1}. At 20 days, the highest percentage inhibition (100%) of fungi was established with 10, 15 and 30g NO Kg^{-1} of feed for *Aspergillus niger*, *Aspergillus flavus* and *Fusarium sp* respectively. *Aspergillus flavus* (0± 0, 00 cfu g^{-1}) and *Aspergillus niger* (0±0, 00 cfu g^{-1}) were completely destroyed at 30 days of storage irrespective of the concentration of NO (Table 3).

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Table 3: Effects of neem oil on farm-mixed poultry feed mycoflora ($\times 10^2$cfu g^{-1})

Doses (g/kg feed)	Fungi	10	Period (days) 20	30	*P-value*
0	*Aspergillus flavus*	1 ± 0.23^{Ba}	0.2 ± 0.23^{Bb}	0.3 ± 0.3^{Bb}	0.01
	Aspergillus niger	1.3 ± 0.76^{Ba}	0.2 ± 0.4^{Bb}	0 ± 0.00^{Bb}	0.01
	Penicillium sp	7.7 ± 3.17^{Aa}	0.4 ± 0.8^{Bb}	0.9 ± 0.6^{ABb}	0
	Fusarium sp	1 ± 0.23^{Ba}	2.7 ± 1.71^{Aa}	1.5 ± 1.05^{Aa}	0.16
	P	0	0.01	0.02	
10	*Aspergillus flavus*	0.5 ± 0.5^{Aa}	0.8 ± 0.34^{Aa}	0 ± 0.00^{Aa}	0.42
	Aspergillus niger	0.9 ± 0.8^{Aa}	0 ± 0.00^{Aa}	0 ± 0.00^{Aa}	0.4
	Penicillium sp	0.6 ± 0.5^{Aa}	1.5 ± 1.51^{Aa}	0.1 ± 0.2^{Aa}	0.21
	Fusarium sp	0.7 ± 0.82^{Aa}	2.1 ± 2.05^{Aa}	0.5 ± 0.6^{Aa}	0.52
	P	0.96	0.51	0.13	
15	*Aspergillus flavus*	0 ± 0.00^{Ba}	0 ± 0.00^{Ba}	0 ± 0.00^{Aa}	0.45
	Aspergillus niger	0.2 ± 0.1^{ABa}	0.7 ± 0.5^{Ba}	0 ± 0.00^{Aa}	0.28
	Penicillium sp	0.9 ± 0.6^{ABa}	0.9 ± 0.6^{Ba}	0.1 ± 0.1^{Aa}	0.08
	Fusarium sp	1.1 ± 1.00^{Aa}	3.9 ± 1.36^{Ab}	0.2 ± 0.4^{Ab}	0
	P	0.07	0	0.55	
20	*Aspergillus flavus*	1 ± 0.23^{Ba}	1.4 ± 0.29^{Aa}	0 ± 0.00^{Ba}	0.35
	Aspergillus niger	0 ± 0.00^{Ca}	0.8 ± 0.86^{Aa}	0 ± 0.00^{Ba}	0.08
	Penicillium sp	1.1 ± 0.38^{Ba}	1.4 ± 1.06^{Aa}	1 ± 1^{Aa}	0.81
	Fusarium sp	1.7 ± 0.6^{Aa}	1 ± 1.15^{Aab}	0 ± 0.00^{Bb}	0.03
	P	0	0.91	0.05	
25	*Aspergillus flavus*	1.8 ± 0.52^{Aa}	0.10 ± 0.2^{Bb}	0.10 ± 0.2^{Ab}	0
	Aspergillus niger	0.5 ± 0.38^{Ba}	0 ± 0.00^{Bb}	0 ± 0.00^{Ab}	0.02
	Penicillium sp	0.4 ± 0.00^{Ba}	0.3 ± 0.20^{Ba}	0.1 ± 0.2^{Aa}	0.52
	Fusarium sp	1.7 ± 1.05^{Aa}	1.9 ± 1.09^{Aa}	0 ± 0.00^{Aa}	0.13
	P	0.01	0.08	0.59	
30	*Aspergillus flavus*	0.4 ± 0.57^{Ba}	0.2 ± 0.4^{Aa}	0 ± 0.00^{Aa}	0.41
	Aspergillus niger	2.9 ± 1.24^{Aa}	0.1 ± 0.20^{Ab}	0 ± 0.00^{Ab}	0
	Penicillium sp	1.1 ± 1.4^{ABa}	0.1 ± 0.2^{Aa}	0 ± 0.00^{Aa}	0.35
	Fusarium sp	$1 \pm0.83A^{Ba}$	0 ± 0.00^{Aa}	0.6 ± 0.2^{Aa}	0.29
	P	0.07	0.72	0.43	

a, b : Means with the same superscript in the same row and A, B, C : Means with the same superscrit in the same column are statically non-significant (P>0.05) ; p- probability

This result contradicts the findings of Dalcero *et al.* (1998) who reported that *Aspergillus* (85%) and *Fusarium* (70%) genera were the most frequent in poultry feed samples with fungal counts ranging from 6.6×10^3 to 6.3×10^5 cfu g^{-1}. This difference can also be explained by the most important bioactive terpenoid azadirachtin present in neem oil (Godugu *et al.*, 2014) which contributed to the inhibition of ergosterol synthesis. Ergosterol is a major sterol component of fungal plasma membranes that aids in regulating fungal membrane fluidity and is a major sterol component required for the viability of all fungi. The findings of Oliveira *et al.* (2006) showed that fungal counts in poultry feed samples were $2.18 – 3.27 \times 10^3$cfu g^{-1} with *Penicillium spp.* (41.26%) and *Aspergillus spp.* (33.33%) having the highest isolation frequencies followed

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by *Fusarium spp.* (20.63%). Saleemi *et al.* (2010) reported the highest relative density of *Aspergillus spp.* (51.85%) followed by *Penicillium spp*. (25.93%) and *Fusarium sp.* (11.11%) isolated from poultry feed.

Conclusion

The present study established the preservative ability of neem oil on farm-mixed poultry feed against fungi and *In vitro* fungistatic activity against the most important fungi found in feed. Given its accessibility and the numerous bioactives compounds present, neem oil is a promising natural preservative to reduce losses of poultry feed and to preserve the health of animal and the consumers of animal products.

References

1. Amandioha, A.C. (2000). Controlling rice blast in vitro and in vivo with extracts of *Azadirachta indica*. *Crop Protection*, 9: 287-290.
2. Bandhari, G. (2014). An overview of agrochemicals and their effects on environment in Nepal. *Applied Ecology and Environmental Sciences*, 2(2): 66-73.
3. Bischoff, T.W. & Garraway, M.O. (1985). Ammonium production by races and mating types of *Bipolaris maydis* and its relationship to mycelium dry weight, sporulation and pH of the culture medium. *Ohio Journal of Science*, 85: 155-158.
4. Bluma Romina, V. & Etcheverry Miriam, G. (2008). Application of essential oils in maize grain: Impact on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. *Food Microbiology*, 25(2): 324-34.
5. Brothers, A.M. & Wyatt, R.D. (2000). Factors influencing the mold spores plates count of poultry feed. *Journal Applied of Poultry Resources*, 9: 433-441.
6. Bryden, W.L. (2012). Mycotoxin contamination of the feed supply chain: Implication for animal productivity and feed security. *Animal Feed Sciences Technology*, *173*, 134-158.
7. Conner, D. E. & Beuchat, L.R. (1984). Sensitivity of heat-stressed yeasts to essential oils of plants. *Applied Environment of Microbiology*, 47(2): 229-337.
8. Costa da, C.L., Geraldo, M.R.F., Arrotéria, C.C. & Kemmelmeier, C. (2010). In vitro activity of neem oil (Awadirachta indica A. Juss (Meliaceae)) on *Aspergillus flavus*, sporulation, viability of spores, morphology and Aflatoxins B_1 and B_2 production. *Advances in Biosciences and Biotechnology*, 1: 292-299.
9. Dalcero, A., Magnoli, C., Luna, M., Reynoso, M., Chiacchiera, S., Miazzo R. & Palacios, G. (1998). Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. *Mycopathologia*, 141: 37-43.
10. Dua, V.K, Pandey A.P., Kamaraju,R., Gupta, A., Sharma, T. & Aditya, P.D. (2009). Larvicidal activity of neem oil (Azadirachta indica) formulation against mosquitoes. *Malaria Journal*, 8:124.
11. Ebong, P.E.; Atangwho, I.J.; Eyong, E.U. & Egbung, G.E. (2008). The antidiabetic efficy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf)," *The American Journal of Biochemistry and Biotechnology*, 4 (3): 239–244.
12. Farag, R.S.; Daw, Z.Y.; Hewed, F.M. & El-Baroty, G.S.A. (1989). Antimicrobial activity of some Egyptian spice oils. *Journal of Food Protection*, 52: 665-667.
13. Geraldo, M.R.F., Arrotéia, C.C. & Kemmelmeier, C. (2010). The effects of neem (*Awadirachtina indica A. Juss* (Meliaceae)) oil on Fusarium oxysporum medicagenis and Fusarium subglutinans and the production of fusaric and toxin. *Advances of Bioscience and Biotechnology*, 1:1-6.
14. Girish, K. & Shankara, B.S. (2008). Neem a green treasure. *Electronic Journal of Biology*, 4(3):102-111.

 DOI 10.5455/ijlr.20181027101318

15. Godugu Chandraiah, Apurva Patel, R.; Ravi Doddapaneni; Jaganmohan Somagoni; & Mandip Singh (2014). Approaches to Improve the Oral Bioavailability and Effects of Novel Anticancer Drugs Berberine and Betulinic Acid. *PLOS ONE*, 9(3):89-91.
16. Gonzalez, H.H.L.; Resnik, S.L.; Boca R.T. & Marasas, W.F.O. (1995). Mycoflora of Argentinian corn harvested in the main production area in 1990. *Mycopathologia*, 130: 330-338.
17. Govindachari, T.R.; Suresh, G.; Gopalakrishnan, G.; Banumathy B. & Masilamani S. (1998). Identification of antifungal compounds from the seed oil of *Azadirachta indica*. *Phytoparasitica*, 26:109-116.
18. Gusmán-de-Peña, D. & Ruiz-Herrera, J. (1997). Relationship betzeen aflatoxin biosynthesis and sporulation in *Aspergillus parasiticus*. *Fungal Genetical Biology*, 21: 198-205.
19. Ibrahim, F.A.; & Al-Ebady A.N. (2014). Evaluation of antifungal activity of some plant extracts and their applicability in extending the shelf life of stored tomato fruits. *Journal of Food Processing and technology*, 5(6): 34.
20. Jacela, J.Y., Joel, M.D., Mike, D.T., Robert D.G., Jim L.N., David G.R. & Steve S.D. (2010). Feed additives for swine: fact sheets-prebiotics and probiotics, and phytogenics. *Journal of Swine Health Production*, 18 (3): 132-136.
21. Javaid A. & Iqbal D. (2014). Management of collar rot of bell pepper (*Capsicum annum* L.) by extracts and dry biomass of Coronopus didymus shoot. *International Journal for Sustainable Production Systems*, 30 (3): 164-172.
22. Javaid A. & Rauf S. (2015). Management of basal rot desease of onion with dry leaf biomass of Chenopodium album as soil amendment. *International Journal of Agriculture and Biology*, 17 (1): 142-148.
23. Kana J. R., Gnonlonfin B. J. J., Harvey J., Wainaina J., Wanjuki I., Skilton R. A. & Teguia A. (2013). Assessment of aflatoxin contamination of maize, peanut meal and poultry feed mixtures from different agroecological zones in Cameroon. *Toxins (Basel)*, 5 (5): 884–894.
24. Kaur, P., Thakhur R., Barnela, M., Chopra M., Manuja A. & Chaudhury A. (2015). Synthesis, characterization and in vitro evaluation of cytotoxicity and antimicrobial activity of chitosan-metal nanocomposites. *Journal Chemistry of Technology and Biotechnology*, 90 (5): 867-873.
25. Kim, J., Marshall, M.R; & Wei C.I. (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry*, 43:2839-2845.
26. Martinez, S.S. (2002). O Nim – *Azadirachta indica* Natureza, Usos Múltiplos, Produção. *Editions IAPAR*: Londrina, PR, Brazil.
27. Mossini, S.A. G.; Carla Arrotéia, C. & Carlos Kemmelmeier. (2009). Effect of neem leaf extract and neem oil on *Penicillium* growth, sporulation, morphology and Ochratoxin A Production. *Toxins* 1(1): 3-13.
28. Oguz, T., Akoglu, E. & Salihoglu B. (2012). Current state of overfishing and its regional differences in the Black sea. *Ocean and coastal management*, 58: 47-56.
29. Okoli, I.C., Nweke, C.U., Okoli, C.G. & Opara M.N. (2006). Assessment of the mycoflora of commercial poultry feeds sold in the humid tropical environnment of Imo states, Nigeria. *International Journal Environnment of Sciences Tehnology*, 3:9-14.
30. Oliveira, G.R., Ribeiro, J.M., Fraga, M.E., Cavaglieri, L.R., Direito, G.M., Keller, K.M., Dalcero, A.M. & Rosa, C.A.R. (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia*, 162: 355-362.
31. Rodrigues, I., Handl, H. & Binder, E.M. (2011). Mycotoxin occurence in commodities, feeds and feed ingredients sourced in the Middle East and Africa. *Food Additives and Contaminants*, 4(3): 168-179.
32. Saleemi, M.K., Khan, M.Z., Khan, A. & Javed, I. (2010). Mycoflora of poultry feeds and mycotoxins producong potential of Aspergillus Species. *Pakistan Journal of Botany*, 42 (1): 427-434.
33. Samson, R.A., Hoekstra E.S. & Frisvad J.C. (2004). Introduction to Food and Airborne Fungi. 7th Edition. *Centraalbureau voor Schimmelcultures, Utrecht.*

DOI 10.5455/ijlr.20181027101318

34. Smith, M.E., Terry.W., Henkel, M., Catherine,A.K. & Vilgalys F.R. (2011). Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. *New phytologist*, 192 (3): 699-712.
35. Steel, R.G.D. & Torrie, J.H. (1984). Principles and procedures of statistics. Inten. Student ed. Tokyo: McGraw Hill.
36. Sutton, S., Harding, P. & Burn D. (1972). Key to British Woodlice. Ginn and Company London, UK, p. 106.
37. Talukdar, A.D., Choudhary, M.D., Chakraborty, M., & Dutta BK. (2010). Phytochemical screening and TLC profiing of plant extracts *Cyathea gigantea* (Wall. Ex. Hook.) Haltt and Cyathea brunoniana Wall. Ex. Hook. (Cl. & Bak.). *Journal of Science and Technology: Biological and Environmental Sciences* 5: 70-74.
38. Ueno, K., Matsumoto, Y., Uno, J., Sasamoto, K., Sekimizu, K., Kinjo, Y. & Chibaba, H. (2011). Intestinal resident yeast Candida glabrata requires Cyb2p-mediated lactate assimilation to adapt in mouse intestine. *PLoS ONE*, 6: 247-259.
39. Varmathy, K., Gokularikrishman, M., Kausar, M.S. & Paul, K. (2010). A study of antimicrobial activity of ethanolic extract of various pla,t leaves against selected microbial species. *International Journal of Pharma Sciences and Research*, 1 (8): 293-295.
40. Vieira, N., Casal, M., Johansson, B., MacCallum, D.M., Brown, A. J. & Paiva, S. (2010). Functional specialization and differential regulation of short-chain carboxylic acid transporters in the pathogen *Candida albicans*. *Molecules of Microbiology* 75: 1337–1354.

DOI 10.5455/ijlr.20181027101318