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Research Article

Preservative Effects of *Ageratum conyzoides* Leaves Essential Oil on Poultry Feed

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ABSTRACT

Introduction: The reduced susceptibility of microorganisms to synthetic preservatives and consumer's demand for high-quality, and minimally processed green-label foods urged researchers to focus more on natural preservatives. This study aimed to evaluate the in vitro and in sacco antifungal activities of Ageratum conyzoides leaves essential oil (EO) against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, and *Penicillium* spp. in poultry feed.
Materials and methods: The poultry feed was stored for 30 days. The dilution plate method was then used to isolate the fungi present in the feed. The leaves of Ageratum conyzoides were collected and their EO was extracted using Steam distillations. The *in vitro* antifungal activity of EO (2.3; 3.4; 4.0; 4.5; 5.1 and 5.7; [µl/cm2]) was tested using the modified method, against the above fungi species during a 4-week of research. In sacco antifungal Screening of EO (5.7; 5.1; 4.5 and 4.0µl/Kg of feed) was done on poultry feed and kept for 10, 20, and 30 days.
Results: The most frequently isolated fungi in poultry feed was *Penicillium* spp. (84.07%). The smallest *in vitro* colony diameters were recorded by *Aspergillus niger* with 4µl of EO. The highest level of fungi growth reductions was recorded at 2.3µl of EO. Following 20 days of storage, 4.0µl of EO/kg of feed was shown to completely (100%) inhibit *Aspergillus niger* and *Aspergillus terreus* in the Sacco research. At 30 days of storage, there was no contamination in poultry feed especially in aspects of *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus*. *Penicillium* sp. contamination in poultry feed decreased as the EO concentration increased.
Conclusion: The results of the current study indicated that *Ageratum conyzoides* leaves essential oil at the level of 4.0µl of EO/kg of feed had the highest prevention effects on fungus growth.

1. Introduction

A number of factors linked to diet affect the active balance of the intestinal flora and consequently the health status and growth performance of the chickens[1,2]. Some of these are biological agents such as molds and mites which deteriorate the quality of the feed and cause the loss of large quantities of feedstuffs stored in farms. Globally, molds and their toxins cause feed losses estimated between 5 and 10%[3]. The infestation of poultry feed ingredients by different fungi strains causes adverse effect on health and productivity of poultry. Also, *Aspergillus sp.*, *Penicillium sp.*, *Fusarium sp.* and soil fungi produces mycotoxins which causes food spoilage and a huge economic and environmental loss especially during post-harvest processing and food conservation[3,4]. The growth of these molds in feed automatically leads to a drop in the feed quality[5]. Molds produce a number of secondary metabolites (mycotoxins) which are the most important potential risks to animal and human health[3,6,7]. They are recognized as inhibitors of nucleic acid and protein synthesis in animals[8]. However, the development of these fungi can be thwarted by factors related to the physico-chemical characteristics (temperature, humidity and electromagnetic radiation) of food[9]. To control mold growth and the accumulation of mycotoxins in foodstuffs,

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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[10,11]. The food industry has been pushed to concentrate more on natural preservation and stabilizing techniques due to the decrease in microorganism susceptibility to synthetic preservatives and consumer desire for high-quality, safe, minimally processed, preservative-free green label foods[12], plant products such as spices[13], plant extracts[14], and essential oils[15-17], are highly solicited because of their good antimicrobial ability, food conservation[18], their relatively low toxicity, and biodegradability[19]. Also, no detrimental effect deriving from their utilization has been documented. Essential oils have a wide variety of bioactivities and play an important role as an ideal natural source of antimicrobial, antioxidant, antitumor, anti-aflatoxigenic, antifungal, and chemopreventive agents[20]. They are a mixture of low molecular weight constituents that are responsible for their characteristic aroma[20]. They are volatile constituents obtained from aromatic plant materials, including leaves, rhizomes, flowers, roots, bark, seeds, peels, woods, whole plants, and fruits, a few are found in animal sources such as musk and whale sperm[20]. The variety of different properties ascribed to essential oils has led to their use in a range of applications including among others things as astringents, analgesics, antidepressants, antipyretics, antiviral, bactericides, bacteriostatics, deodorants, stimulants, fungicides, fungistatic, and insecticides[21]. Numerous essential oils produced by medicinal plants including *Ageratum conyzoides* plants have been tested for their ability in controlling aflatoxin contamination in culture medium conditions[22-25].

In tropical and subtropical regions, *Ageratum conyzoides* (Asteraceae family) is an invasive alien species that spreads quickly and has become a bothersome weed in various habitats[26,27]. It is an annual herb with a long history of traditional medicinal uses in many countries of the world, especially in the tropical and subtropical regions. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids, phenols, eugenol, ageratochromone, triterpenoids, steroids, conyzorium, methexnebilitin, and quercetin[28], have been isolated from this species. It is employed in folk medicine as purgative, febrifuge, antiasthmatic, antispasmodic, analgesic, antidiarrhoeic, antiinflammatory, against colic and for headache relief[29]. The essential oil of *Ageratum congziodes* (mentrasto) has inhibitory effects on mycelial growth and aflatoxin B1 production by *Aspergillus flavus*[24,30]. The essential oil of this plant has been reported to act as an insecticide[31,32], fungicide[33,24], anti-inflammatory Moura[34], and antitumor Momesso[35], agent. Based on the wide range of activities attributed to essential oil from *Ageratum conyzoide*, the present study was designed to contribute to the search for feed preservative antifungal substitutes in livestock feed. Specifically, it aimed to evaluate the *in vitro* and *in sacco* antifungal activities of *Ageratum coyzoides* leaves essential oil (EO) against *Aspergillus flavus, Aspergillus niger, Aspergillus terrus* and *Penicillium sp.* in poultry feed.

2. Materials and Methods

2.1. Ethical approval

The study was conducted according guidelines of the University of Dschang, Cameroon, and the ethical standards from the 1964 Helsinki Declaration with its later amendments.

2.2. 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. The climate is characterized by two seasons including 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. The average temperature is around 21°C, the average annual insolation is 1873 hours and the average relative humidity is 76.8%[36].

2.3. Isolation and identification of fungi

The feed was composted and kept in the building where chicken feed is stored at the University of Dschang for 30 days. The fungi in the feed were isolated using the dilution plate technique procedures[37]. In a bottle containing 45 ml of sterile distilled water 5g of feed was introduced[38]. This mixture was vigorously mixed from a vortex for 5 minutes to facilitate the release of spores. Ten-fold appropriate serial dilutions were prepared and a common measure of 1.0 mL of each dilution (in triplicate) was spread over potato dextrose agar (PDA) plates prepared with 1% chloramphenicol. The plates were then incubated at 28°C in the dark for 5 days[37]. The unalloyed culture of each colony type on each plate was obtained by sub-culturing each of the different colonies onto potato dextrose agar (PDA) plates, which were incubated at room temperature for 5 days. Unalloyed fungal isolates were identified from their macroscopic and microscopic characteristics according to Samson et al.[39]. 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.[40].

2.4. Extraction of essential oil

The leaves of *Ageratum conyzoides* were collected in the University of Dschang campus and its surroundings, then dried for three days at room temperature, and weight before transported to the "Monastère Saint Benoit de Babeté". At the monestry, the leaves were placed in the

plant chamber of a steel apparatus and then clean water was put in the water chamber. Steam distillation was then carried out for 4 hours. The generated steam passes through plant material thus softening the cells and letting the EO escape in vaporized form. The heating of the system was maintained to increment the EO vapor pressure, but it was not so high to destroy the plant or burn the plant hence the EO[41]. When tiny EO droplets are released and they mix with the steam, it is than converge into a cooling system. The mixture condenses and forms a bilayer liquid and the oil forms the top layer of the distillate since it is less dense them water. This was then separated easily using proper methods and instruments as described by Rao and Pandey[42]. The phytochemical analyses of *A conyzoides* leaves EO were carried as described by Talukdar et al.[43], for the presence of alkaloids, flavonoids, terpenoids, phenols, steroids, saponins, and tannins.

2.5. In vitro antifungal screening of essential oil from Ageratum congzoides leaves

The antifungal activity of the essential oil was tested against *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, and Penicillium spp.* according to the method modified from the procedure described by Kaur et al.[44]. About 20 ml of potato dextrose agar (PDA) with 1% chloramphenicol (antibiotic) was poured into petri dishes. After solidification, 0.1; 0.09; 0.08; 0.07; 0.06, and 0.04; (µl/cm^2) of the essential oil were spread over the medium using a sterile rod. Petri dishes without the essential oil were used as a negative control and another with 1% Nystatin® (antifungal) as a positive control. The 5 mm discs of a culture aged 7 days from each mold studied was placed in the center of each dish. The petri dishes were then incubated at 27 ° C for 7 days. The petri dishes were prepared in four replicates for growth, percentage inhibition, minimum inhibitory concentration and measurement of sporulation for each mold studied. The diameter of the colony was measured daily in two directions at 90° to each other to obtain the average diameter of each colony[45]. The advantage of the method was that sequential recordings could be obtained from each colony, although only lateral growth was measured. The procedures for measuring sporulation were those described by Gusmán-de-Peña and Ruiz-Herrera[46] with modifications. The Agar discs were aseptically removed from the central, intermediate, and peripheral areas of each replicated plate using a cork borer and then transferred to vials containing a sterile 0.1% Tween 80 solution (10 ml) and vortexed for two minutes to release the spores. After the sedimentation of the mycelium, the supernatant was collected and the spores were counted in the Neubauer counting chamber. The data was expressed in spores/cm of the colony in diameter. The toxicity of the essential oil vis-à-vis molds in terms of the percentage of inhibition of mycelial growth was calculated as described by Kana and Meimandipour[47], according to the 3 formula:

% Inhibition = | dc-dt | / dc × 100 where,

dc = average increase in mycelial growth in the control;

dt = average increase in mycelial growth under treatment.

2.6. In sacco Antifungal Screening of essential oil from Ageratum congzoides leaves on Poultry Feed

Into autoclave-sterilized jute bags, 1 kg of sterilized feed [(corn (65%), cottonseed meal (5%), groundnut meal (15%), fishmeal (5%), bran wheat (5%) and premix (5%)] was introduced respectively, containing different concentrations of the essential oil (5.7; 5.1; 4.5 and 4.0µl /Kg of feed). In another autoclave-sterilized jute bag, 1 kg of feed without the essential oil was used as a negative control and another with Nystatin® (antifungal) as a positive control. In this tail, the authors had all together 6 study groups. The doses of the essential oil used are those with fungicidal and fungistatic effects during the *in vitro* study previously carried out. Each concentration was repeated 3 times and kept for 10, 20, and 30 days. After each storage period, 3 samples were taken at random (base, medium, and high) in each treatment and the fungal counts were determined. The serial dilution technique was used and the treatment was carried out in a sterile beaker with gentle stirring (100 rpm) using a multiple stirrer at room temperature. After serial dilution, 500 µl of each solution was poured onto a sterile petri dish (diameter 9 cm), and 20 ml of dextrose potato agar was added and then allowed to solidify at room temperature. The petri dishes were then incubated at 27 ° C for 72 hours and the colonies were counted as described by Kana et al.[37].

The production of new biomass depends on carbon assimilation, and the quick uptake and digestion of available carbon sources is necessary for fungal development in the host. These can comprise non-fermentable carbon sources such as amino acids and organic acids as well as fermentable sugars like glucose, fructose, and galactose[48,49]. Fungal infections have developed distinct carbon absorption profiles, perhaps as a result of their particular substrate. To determine the possible harm that feed may have to the health of chickens, the mold spore plate count is frequently employed. However, it was discovered that there was a lot of variation in this measurement across feed batches that were produced at various concentrations. Nonetheless, this test can provide results more quickly than mycotoxin contamination analysis at a lower cost in the number of mold spores that are viable per feed item Brothers and Wyatt[50]. One factor used to assess the sanitary quality of feed is the total number of fungus present. When livestock feed, mixes, and raw materials for animal feed contain more than 300.000 colonies forming unite per gram (cfu g-1) of forage body, to be used to feed oil animals or 50.000 cfu g-1 to be used to feed younger animals, they violate the regulations on harmful substances and components in animal feed Oliveria et al.[51].

2.7. Statistical analyzes

Data on *in vitro* growth, inhibition rate, and sporulation

were subjected to one-factor analysis of variance (ANOVA) according to a completely randomized design. Data on feed preservation was subjected to a 4×5 factorial (4 fungi × 5doses) design. Duncan's multiple range test was used to separate the mean at the 5% significance level. The statistical software SPSS 20.0 (Statistical Package for Social Science) was used for the analyses.

3. Results

The present study of the qualitative phytochemical analysis revealed that *A. conyzoides* leaves essential oil possesses bioactive compounds, such as steroids and triterpenoids (Table 1).

Analysis of variance revealed a significant effect of the studied *A. conyzoides* leaves essential oil concentrations on colony diameter, sporulation and colony forming units per gr ($p < 0.05$). In all the fungal species, the diameter of colony decreased with increasing *A. conyzoides* leaves essential oil concentration from 2.3 µl and 5.7 µl. *A. conyzoides* leaves essential oil showed fungistatic activity against *Aspergillus flavus* and *Aspergillus niger* (Figure 1).

The greatest reductions ($p < 0.05$) in fungal growth were observed in *A. flavus and A. terreus*, ranging from 39.78 to 52.13% and 42.92 to 48.5%, respectively. The *A. conyzoides* leaves essential oil varied between 2.3 and 5.7µl for *Aspergillus terreus* and between 4.5 and 5.1µl for *Aspergillus flavus* (Fig. 2). The fungal count drop with A. conyzoides leaves essential oil and the tolerance action of antifungal chemicals found in the oil, such as triterpenoids, may be caused by the inhibition of environmental conditions (relative humidity, minimal aeration, and temperature). The above-mentioned environmental and climatic factors are ideal for the reproduction of fungus, particularly *Aspergillus* and *Penicillium* species.

Bioactive compounds in plants exhibit antimicrobial activity in three different ways which are as follows: First, they disrupt the phospholipid bilayer of the cell membrane, thereby increasing permeability and resulting in the loss of cellular constituents; second, they hinder a range of enzyme systems, such as those that are involved in the synthesis of structural components and the production of cellular energy[52], and third, they inactivate or destroy genetic material[53]. However, each of the different chemicals has a different mode of action. The intensification of sporulation was linked to *A. conyzoides* leaves essential oil capacity to impede mycelial growth (Table 2). When the *A. conyzoides* leaves essential oil concentration was between 2.3 and 3.4 µl there was a highly significant increase in sporulation ($p < 0.05$). Above 3.4 µl, sporulation appears to decline as *A. conyzoides* leaves essential oil levels rise, regardless of the fungal species

Table1. Phytochemical composition of *Ageratum conyzoides* leaves essential oil

No	Extracts	Alcaloids	Phenols	Flavonoids	Sterols	Triterpenoids	Tannins	Saponins	Anthocyanins	Anthraquinons
1		-	-	-	+	+	-	-	-	-

+: Present; - : Absent

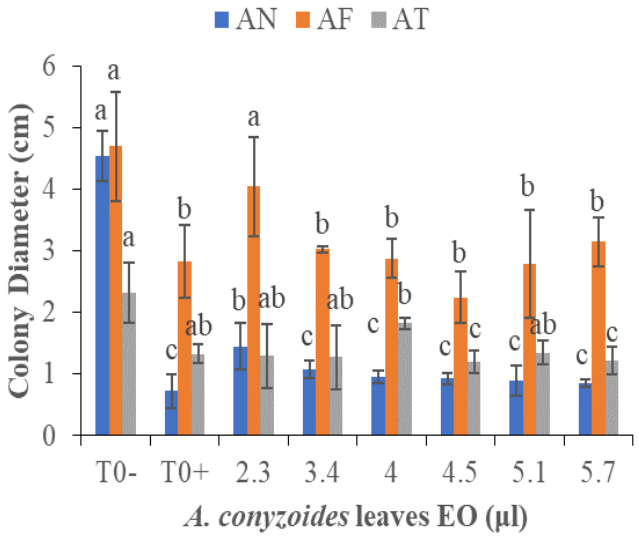

Figure 1. Effects of graded levels of *Ageratum conyzoides* leaves essential oil on the diameter (cm) of colonies of *Aspergillus niger(*AN), *Aspergillus flavus* (AF), *Aspergillus terreus* (AT*), and Penicillium sp* (PEN)

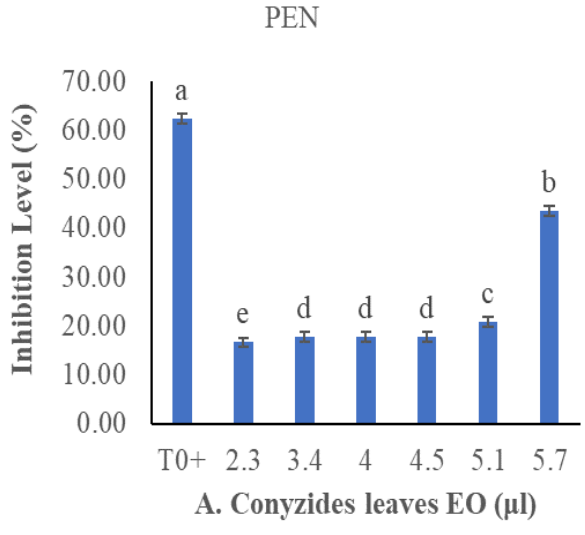

Figure 2. Effects of graded levels of *Ageratum conyzoides* leaves essential oil on the inhibition rate (%) of *Aspergillus niger (*AN), *Aspergillus flavus (*AF), *Aspergillus terreu*(AT), *and Penicillium sp* (PEN)

Table 2. Effects of graded levels of *A. conyzoides* leaves essential oil on the sporulation of fungal genera isolated from poultry feed

Fungal genus	Control		Essential oils (µl)						p
	T0-	T0+	2.3	3.4	4	4.5	5.1	5.7	
Aspergillus terrus	893.25 ± 9.11^{a}	33.75 ± 8.38^{e}	574.75 ± 5.12^{b}	496.75 ±3.59^{c}	124.25 ± 5.06^{d}	104.75 ± 3.78^{de}	74.00 ± 6.98^{de}	42.50 ±10.12^{e}	0.00

Aspergillus niger	2905.3 ± 9.27^{a}	475.00 ± 4.27^{b}	384.75 ±1.26^{c}	347.25 ± 4.27^{d}	162.00 ±5.89^{e}	94.00 ± 1.41^{f}	65.50 ±2.08^{fg}	45.00 ± 7.53^{g}	0.00
Aspergillus flavus	853.50± 9.79^{a}	168.00 ± 7.55^{c}	400.00 ± 7.07^{b}	257.00 ± 4.83^{b}	160.25 ± 9.54^{c}	329.75 ± 6.65^{b}	57.00 ± 4.08^{d}	43.50 ± 3.00^{d}	0.00
Penicilliium sp	1672 ± 6.34^{a}	122.25 ± 6.34^{c}	859.25 ± 7.04^{b}	118.25 ± 6.24^{c}	80.50 ± 3.69^{de}	85.00 ± 7.75^{de}	57.00 ± 4.08^{de}	42.00 ± 2.94^{e}	0.00

a, b, c, d, e, f, g : Means with the same letter on the same line are not significantly different (p> 0.05). T0: 0µl of essential oils, T0^{+}:/kg of feed; T0+: 50 mg nystatin, p: Probability

When the concentration of *A. conyzoides* leaves essential oil was increased from 2.3 to 5.7 µl the number of spores decreased significantly in comparison to the negative control treatments but it was comparable to the positive control treatments.

A. conyzoides leaves essential oil was used as a natural preservative in an *in-Sacco* study to prevent poultry feed from spoiling, and the results showed that after 10 days of storing feed with 4.0, 4.5, 5.1, and 5.7µl of EO/Kg of feed, *Penicillium sp*. was inhibited at 73.64, 68.36, 100, 91.21% respectively. *Aspergillus niger, Aspergillus flavus*, and *Aspergillus terreus* showed the maximum percentage suppression (100%) of fungi at 10 days and 20 days when fed 4.0, 4.5, and 5.7µl EO/Kg-1 of feed. Regardless of the *A. conyzoides* leaves essential oil concentration and the number of days stored, *Aspergillus niger* (0±0, 00 cfu g-1) and *Aspergillus terreus* (0±0, 00 cfu g-1) were totally eliminated after 30 days of storage (Table 3).

Table 3. Effects of graded levels of *A. conyzoides* leaves essential oil on the microflora (10CFU/g) of feed stored 10, 20, and 30 days

Essential oil (µl/kg)	Species	Period of days			P-value
		10	20	30	
0	*Aspergillus niger*	4.03 ± 0.05^{Aa}	2.93 ± 0.59^{Ab}	3.16 ± 0.28^{Bb}	0.03
	Aspergillus terrus	3.36 ± 0.10^{Ca}	3.26 ± 0.24^{Aba}	3.26 ± 0.24^{Ba}	0.79
	Aspergillus flavus	3.63 ± 0.35^{Aba}	3.86 ± 0.23^{BCa}	3.73 ± 0.23^{Ba}	0.59
	Pencillium sp	3.79 ± 0.18^{Ab}	4.53 ± 0.40^{Ca}	4.53 ± 0..40^{Aa}	0.06
	P-value	0.02	0.01	0.00	
4.0	*Aspergillus niger*	0.00 ± 0.00^{A}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}	/
	Aspergillus terrus	0.00 ± 0.00^{A}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}	/
	Aspergillus flavus	0.00 ± 0.00^{Ab}	0.33 ± 0.14^{Ba}	0.00 ± 0.00^{Ba}	0.00
	Pencillium sp	1.00 ± 0.00^{Ab}	2.67 ± 0.29^{Aa}	3.00 ± 0.50^{Aa}	0.00
	P-value	/	0.00	0.00	
4.5	*Aspergillus niger*	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	/
	Aspergillus terrus	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	/
	Aspergillus flavus	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	/
	Pencillium sp	2.90 ± 0.42^{Aa}	0.33 ± 0.14^{Ab}	2.77 ± 0.40^{Aa}	0.00
	P-value	0.00	0.00	0.00	
5.1	*Aspergillus niger*	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	/
	Aspergillus terrus	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	/
	Aspergillus flavus	2.20 ± 1.90^{Aa}	0.87 ± 0.12^{Ba}	0.90 ± 0.15^{Ba}	0.12
	Pencillium sp	0.00 ± 0.00^{Ba}	3.53 ± 0.25^{Ab}	3.96 ± 0.31^{Ab}	0.00
	P-value	0.05	0.00	0.00	
5.7	*Aspergillus niger*	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{C}	/
	Aspergillus terrus	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ±0.00^{C}	/
	Aspergillus flavus	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Ab}	0.62 ± 0.08^{Ba}	0.00
	Pencillium sp	0.33 ± 0.14^{Bb}	0.00 ± 0.00^{Ab}	2.48 ± 0.35^{Aa}	0.00
	P-value	0.00	/	0.00	

$^{a, b, c}$ Means with the same letter on the same line are not significantly different (p > 0.05), /: No fungi are present. A, B, C: Means with the same letter on the same column for the same species are not significantly different (p> 0.05) p: Probability

4. Discussion

The present study revealed the presence of triterpenoids and steroid compounds in *A. conyzoides* leaves essential oil. These results concur with that of Patil et al.[54], who also revealed the presence of triterpenoids and steroids in *A. conyzoides* essential oil and their antifungal. The fungi isolated from farm-made food in the current study belong to the post-harvest flora group (*Apspergillus flavus, Aspergillus niger*, *Apspergillus terreus,* and *Penicillium spp).* The above result agree with that of[55,37], who isolated the same groups of fungi in the broiler feed. The aforementioned group of fungi was also isolated by Kana et al.[40], from samples of poultry feed collected in the different agroecological zones of Cameroon. In contemporary academic work, the most dominant mold genera are *Aspergillus flavus, Penicillium spp, Aspergillus niger,* and *Aspergillus terreus*. The presence of these molds could be explained by the poor harvesting conditions of cereal seeds and most especially poor storage conditions which have a great influence on the development of molds[56]. These molds were identified based on morphological criteria. The results in this present study concur with the work carried out by[36,57], who reported that the isolated and identified molds (*Apspergillus flavus, Aspergillus niger*, *Apspergillus terreus,* and *Penicillium spp)*, were them used to carry out antifungal tests.

The increasing rate of *Ageratum conyzoides* leaves essential oil resulted in a significant drop in colony diameter irrespective of the mold type. This result agrees

with that of Mafouo et al.[36], who reported that neem oil causes a drop in colony diameter of *Aspergillus flavus, Aspergillus niger, Penicillium sp* and *Fusarium sp*. In addition, Renata et al.[30], reported that *Ageratum conyzoides* leaves essential oil inhibits the growth of *Aspergillus flavus*. Likewise, Nogueira et al.[31], noted that *Ageratum conyzoides* essential oil inhibits the growth of *Aspergillus flavus* and also completely inhibits aflatoxin production at concentrations above 0.10 µg/mL. Furthermore Patil et al.[54] , review that *Ageratum conyzoides* essential oil inhibits the growth of *A. parasiticus* and the production of aflatoxin. This *Ageratum conyzoides* essential oil (0.75 mg mL−1) totally inhibited the growth of *A. parasiticus* and inhibited more than 84% aflatoxin production of test fungi, at a concentration of 0.5 mg mL−1. The Essential oil (1500 ppm) also showed complete inhibition of the *Aspergillus* group of fungi in the poisonous medium technique. The antifungal effect of *Ageratum conyzoides* leaves essential oil in the present study could be explained by the presence of compounds such as sterols and triterpenoids which have been proven to have antifungal power.

The amount of mold spore plaques discovered in feed has frequently been used to determine the possible damage that the feed may provide to the health of poultry. The present result revealed that the number of spores produced depends on the concentration of *Ageratum conyzoides* leaves essential oil. Regardless of the mold, the number of spores decreases with the increasing rate of oil. This result concur the conclusions of Renata et al.[30], who showed that *A. conyzoides* leaves essential oil decreases or inhibits sporulation of *Aspergillus flavus*. This result concurs with that of Sun et al.[58], who reported that the diameter of the spore size linearly decreased with the increasing of concentration of essential oils. Jaya et al.[59], also agree with these results by reporting that the corresponding decrease in fungal mycelia growth was as a result of increasing concentration of *A. conyzoides, H. suaveolens,* and *C. aromaticus* EO. In addition, the results of Mossini et al.[60], also agrees with the above results by reporting that spore production with 0.125; 0.250 and 0.500% neem oil decrease. This data contradicts the findings of Mafouo et al.[36]. who demonstrated that *Aspergillus flavus, Aspergillus niger, Fusarium sp. and Penicillium sp.* sporulation increased dramatically with neem oil concentrations from 1 to 3% (v/v) and Costa et al.[61], who demonstrated that *Aspergillus flavus* sporulation increased dramatically with concentrations of neem oil above 0.5%. The solubility of the active oil compounds or property of fungal metabolism may be the cause of the decreasing spore number in poultry feed as the concentration of *A. conyzoides* leaves essential oil rises.

The above results were *A. conyzoides* leaves essential oil was used as a natural preservative for poultry feed, in accordance with the findings of Bing Han et al.[62], who reported that *cinnmaldehyde and citral* essential oil were used to preserve poultry feed, and after 7 days *Aspergillus flavus* using was inhibited to almost 100% in all treatments. Also, the above results concur with the findings of Jaya et al.[59], which sate that EO of *A. conyzoides, H. suaveolens,* and *C. aromaticus* inhibit the growth of *Aspergillus niger, A. terreus, A. fumigatus, Fusarium roseum, Alternaria alternata, Cladosporium cladosporiodes, Penicillium italicum, Curvularia lunata,* and *Trichoderma viride* in food commodities (stored wheat).

The above result *A. conyzoides* leaves essential oil was used as a natural preservative in an *in Sacco* study to prevent poultry feed from spoiling, is in accord with the findings of Mafouo et al.[36], who reported that the most frequently isolated fungi in poultry feed were *Penicillium* sp. (83.87%) and that this could be due to the climatic condition and the presences of terpenoid in neem oil. Meanwhile, the results were in contrast to the findings of Dalcero et al.[63], who found that the most common genera in chicken mix feed samples were *Aspergillus* (85%) with fungal counts ranging from 6.6 × 103 to 6.3 x 105 cfu/ g. This discrepancy can also be explained by the bioactive terpenoid found in *A. conyzoides* leaves essential oil[54,64] which helped to inhibit the synthesis of ergosterol. Ergosterol is a significant sterol component of fungal plasma membranes that is essential to the survival of all fungi and helps to regulate the fluidity of the fungal membrane. Oliveira et al.[51], also disagree with the above results by reporting that fungal counts in poultry feed samples were highest in *Aspergillus spp.* (33.33%) followed by *Penicillium spp.* (20.63%) and they exhibited the greatest isolation frequencies, respectively, ranging from 3.27 to 2.1 × 103 cfu g–1. Furthermore Abu and Shoushan[64], also contradict the above results by stating that *Aspergillus spp*. had the highest relative density (51.85%) followed by Penicillium spp. (25.93%) in poultry feed.

5. Conclusion

The current study demonstrated the *in vitro* fungistatic efficacy of *A. conyzoides* leaves essential oil against the most significant fungi present in feed including *Apspergillus flavus, Aspergillus niger*, *Apspergillus terreus,* and *Penicillium spp,* as well as the oil's potential to preserve poultry feed against fungi. *A. conyzoides* leaves essential oil is a promising natural preservative to reduce feed losses in poultry and to protect the health of the animals and consumers of animal products because of its accessibility and the abundance of bioactive chemicals it contains.

It would be wise in future studies to extract and isolate the bioactive compounds (steroids and triterpenoids) present in *Ageratum conyzoides* leaves essential oil and evaluate their effect on fungal growth.

Declarations

Competing interest

The authors declare that they have no competing interests.

Authors' contribution

Ngwa Evelyn Bih and Teguia Alexis conceived, designed the experiment, and wrote the manuscript. Katte Brigitte

and Edie Nounamo Langston Wilfried conducted data collection and analysis. Ebile Dayan Agwah revised the manuscript. Its final version was read and approved by all authors.

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Availability of data and materials

The data from this trial could be available with the agreement of the corresponding author.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

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