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Anticoccidial activities of essential oil of *Amomum subulatum* in broiler chicks

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Essential oils are potent medicinal substances that can control multiple diseases through their bioactivities. In the current research, the anticoccidial potential of the *Amomum subulatum* essential oil was estimated in broiler chicks. A day-old, 270 broiler chicks were divided equally into 6 groups, each having 3 replicates. All the groups, except the infected non-medicated control group, were given sporulated oocysts of mixed *Eimeria* spp. The first three groups received essential oil of *A. subulatum* @ 1%, 2%, and 3% (v/w) in the feed, and their activities were evaluated in comparison to the Symocox® (Toltrazuril) treated groups and infected non-medicated groups. Feed conversion ratio (FCR), feed intake, weight gain, rates of mortality, organ weight ratios, fecal scores, lesion scores, and oocyst per gram of feces were taken as the parameters of the study. Blood parameters e.g. red blood cell, white blood cells, packed cell volume, mean corpuscular hemoglobin, and serum metabolites like alanine aminotransferase, aspartate transferase, alkaline phosphatases, lactate dehydrogenase, urea, proteins, and albumins were also evaluated. *A. subulatum* essential oil @ 3% concentration had the best FCR, survival rates, and feed intakes. *A. subulatum* essential oils also significantly reduced lesions lowered oocyst counts and improved fecal scores. Hematological and serum profiles were improved by the essential oil of *A. subulatum* in the feed and the best results were achieved by the 3% concentration of the oil. This research concluded that the *A. subulatum* essential oil @ 3% concentration can be used for the treatment of avian coccidiosis however formulation of the dose requires further studies on broiler chicks.
Keywords: Essential oil; *A. subulatum*; coccidiosis; chicken; *Eimeria.*

INTRODUCTION

Amomum subulatum, known as Indian black cardamom or large cardamom, is a common plant found on the subcontinent (Joshi and Piya, 2019; Nair, 2020; Balkrishna *et al.*, 2023). It is a common kitchen item that is used in multiple local dishes and traditional medical practices (Joshi and Piya, 2019; Makhija *et al.*, 2022). Essential oil of *A. subulatum* (AEO) is a yellowish-colored volatile liquid that has a pleasant smell and a group of bioactive compounds (Thinh *et al.*, 2021). AEO has been used by multiple researchers for the control of multiple diseases of infectious origin (Parveen *et al.*, 2018; Van, 2021). AEO has been proven to be an effective medicinal plant because of its strong antioxidant activities (Mande and Sekar, 2021). Infectious diseases, especially parasitic diseases, have been a concern for life for a long time (Abdel-Naeem, 2021; Khallaf *et al.*, 2022). Coccidiosis is a disease of infectious origin caused by the genus *Eimeria*. *Eimeria* is notorious for high mortalities and economic losses in the poultry sector (Imran and Alsayeqh, 2022; Nahed *et al.*, 2022). Major pathological lesions of this disease are caused by oxidative stress by the anti-inflammatory response of the immune system (Nahed *et al.*, 2022; Rizwan *et al.*, 2022; Gul and Alsayeqh, 2023). Multiple researchers have suggested that antioxidant substances can control coccidiosis because of their ability to limit oxidative damage (Khater *et al.*, 2020; Moryani *et al.*, 2021; Imran and Alsayeqh, 2022). Many plant and essential oils have been searched for their anticoccidial potential. They have shown their potential to control coccidiosis because of the antioxidant-rich fractions present in them (Sorour *et al.*, 2018; Lee *et al.*, 2020b; Sidiropoulou *et al.*, 2020). Multiple essential oils have been proven to have anticoccidial properties e.g. essential oils of *Psidium guajava* (Langerudi *et al.*, 2022), *Citrus sinensis* (Salman and Imran 2022), *Melaleuca alternifola* (Khan *et al*., 2023) and essential oil based commercial products like Orgeo-stim ® are also being used to control coccidiosis (Yu-Wen-2008). A. *subulatum* essential oil has multiple compounds with proven antioxidant properties, so it can control coccidiosis (Abbas *et al.*, 2019b; Alam and Singh, 2021; Thinh *et al.*, 2021; Van, 2021). Its major components are cineole, terpineol, and terpinene which belong to the monoterpene and terpenoid groups (Sharma *et al.*, 2020; Alam and Singh, 2021). These compounds are great antioxidant substances and multiple

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essential oils showing anticoccidial activities possess these compounds (Batiha *et al.*, 2020; Abd El-Hack *et al.*, 2022; Raza *et al.*, 2022). Essential oil of *A. subulatum* has shown antibacterial, antiparasitic, anti-fungal, and antioxidant activities in multiple trials (Parveen *et al.*, 2018; Nethi and Mande, 2020; Mande and Sekar, 2021). This research was aimed at finding out the anticoccidial potential of AEO in broiler chicks.

MATERIALS AND METHODS

Essential oils: Seeds of the A. subulatum were purchased from the local market of Faisalabad and verified by the Department of Botany, University of Agriculture, Faisalabad. These seeds were powdered and soaked in the water and put in the distillation apparatus using the hydro distillation method. After that, essential oils were extracted from the hydro distillation apparatus within 5 hours of exposure (Gavahian et al., 2012).

Parasite Material: Freshly passaged oocysts of mixed species of Eimeria (*E. tenella, E. mivati, E. necatrix, E. maxima*) were obtained from the guts and ceca of the infected birds. The sporocysts were sporulated and preserved in the 2.5% Potassium dichromate solution following the guidelines of (Ryley *et al.*, 1976).

***Housing and Management of Birds*:** Birds were housed in the Poultry Shed of the Department of Parasitology, University of Agriculture, Faisalabad. Fumigation and disinfection were performed before the arrival of the chicks. Birds were reared on the floor. A standard vaccination schedule was followed and feeding was done using feeding pens. Feed formulation is given in Table 1. All the birds were reared in a single shed and similar conditions.

Table 1. Composition of broiler ration

Feed Ingredients	Starter (%)	Finisher (%)
Maize	45.00	51.00
Rice grains	11.00	11.00
Canola meal	13.00	13.00
Soybean meal	13.00	13.00
Rice polishing	4.00	3.00
Molasses	4.00	3.00
Corn gluten meal 60%	3.00	2.00
Fish meal	4.00	2.00
Dicalcium Phosphate	2.00	1.00
Vitamin mineral Premix	0.63	0.65
DL-methionine	0.13	0.10
L–lysine	0.24	0.25

Experimental Design: 270 broiler chicks (one day old) were purchased from the hatchery and reared as per standard management practices. On the 7th day of age, the chicks were divided into six groups A, B, C, D, E, and F. Each group had three replicates, each replicates having 15 chicks. On the 10th day of age, all these groups were infected with the sporulated oocysts of Eimeria @ 100,000 oocysts/ bird except group F which was kept as a non-infected, non-medicated group. Same-day Groups A, B, and C were given A. subulatum essential oil at 1%, 2%, and 3% (v/w) in the feed, while group D was given Symocox® (Toltrazuril) at the rate of 1mL/L in the water. These medicines continued for 7 days. Group E served as the infected non-medicated control group, while Group F was the non-infected non-medicated control group. Essential oils were mixed manually in the feed by calculating the percentage of the feed weight and adjusted with leftovers.

Performance Parameters: Feed conversion ratio (FCR), weight gain, mortality rates, and organ weight ratios were used as the parameters for the comparison of birds' performance. Mortality rates were calculated by following the equation (Abbas *et al.*, 2019a):

Percent mortality = (Number of chicks died due to coccidiosis/ total chicks in the group) × 100

FCR was calculated by the following formula:

FCR = Average feed consumed/ average weight.

***Anticoccidial Parameters*:** Anticoccidial parameters used in the study were fecal score, lesion score (Johnson and Reid, 1970), oocyst score, and oocyst per gram (OPG) of feces (Ryley et al., 1976; Youn et al., 1993).

Fecal scores were obtained by the visual scoring of feces on a scale from 0 to 4 where 0 showed no change in the fecal texture and 4 meant the feces with a high amount of blood. Lesion scores were calculated by the slaughtering of birds on day 7 post-infection and measuring the extent of lesions (Johnson and Reid, 1970). Fecal samples were brought to the chemotherapy lab where oocyst scoring was done. The oocyst score was given on the visual quantity of the oocysts while oocyst per gram was done through the McMaster technique (Ryley *et al.*, 1976; Youn *et al.*, 1993).

***Serum and Hematological Parameters*:** Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and packed cell volume (PCV) were taken by auto hemocytometer. Total red blood cells (RBC), total white blood cells (WBC), heterophils, and Lymphocytes were the blood parameters taken in the study which were calculated using the Natt-Herricks-Tic® hemocytometer. (Abbas et al., 2019a). Alanine aminotransferases (ALT), Aspartate transferase (AST), Alkaline phosphatase (ALK.P), total proteins, total albumins, globulins, urea, and lactate dehydrogenase (LDH) were serum parameters included in the study. Serum parameters were estimated using the spectrophotometric technique by the kits (Merck, Germany) except the globulins which were calculated by subtraction of albumins from the serum proteins.

Statistical analysis: Results were adjusted on an Excel sheet and mortality rates, feed intake, and feed conversion ratio were calculated using Microsoft Excel. Anticoccidial parameters and hematological parameters were subjected to statistical analysis. Analysis of variance was performed and the Tuckey test was performed for the comparisons of the

means. Minitab® 20.3 statistical software was used for the statistical analysis. A 5% significance level was observed for the statistical comparison of the means (P<0.05).

RESULTS

Anticoccidial Parameters: Anticoccidial parameters were estimated, i.e., lesion scores, oocyst scores, fecal scores, and OPG. The lesion scores were done to observe the coccidial lesion and comparison of the efficacy of various concentrations of the oil. Oocyst score informed about the coccidial oocysts in the feces which indicate the amount of Eimeria being excreted while OPG is a rather more precise way to access the output of oocysts. Fecal score informed the status of blood and water being depleted through the feces. Group C (chicks receiving 3% AEO (v/w) in the feed) had the comparable (P>0.05) results to Group D (Symocox ® treated chicks) and had significantly different (P<0.05) results from Group E (infected non-medicated chicks) (Figure 1, Table 2).

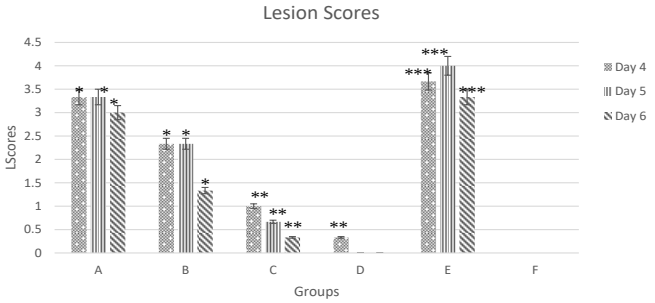

Figure 1. Effect of *A. subulatum* essential oil on lesion scores on days 4, 5, and 6 post-infection. A: 1% *A. subulatum* Essential Oil; B: 2% *A. subulatum* Essential Oil; C: 3% *A. subulatum* Essential Oil; D Infected Medicated Control; E: Infected Non-medicated Control; F: Non-infected Non-medicated control. Columns represent the average values taken from replicates, and the bars represent the standard errors. Columns having similar superscripts are non-significant statistically (P>0.05).

Table 2. Effects of *A. subulatum* essential oil on oocyst per gram, lesion score, and oocyst scores in broiler chicks

Groups	OPG (x10^4)	Lesion scoring	Oocyst scoring
A	5.48±0.42^{b}	3.66±0.47^{a}	3.33±0.47^{a}
B	3.25±0.54^{c}	2.66±0.47^{ab}	2.33±0.47^{ab}
C	1.20±0.15^{d}	1.33±0.47^{bc}	1.33±0.47^{bc}
D	0.78±0.16^{de}	0.33±0.47^{c}	0.66±0.47^{bc}
E	10.20±0.39^{a}	3.66±0.47^{a}	4.00±0.81^{a}
F	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{c}

A: 1% *A. subulatum* Essential Oil; B: 2% *A. subulatum* Essential Oil; C: 3% *A. subulatum* Essential Oil; D Infected Medicated Control; E: Infected Non-medicated Control; F: Non-infected Non-medicated control. The means having similar superscripts in the columns are non-significant (P>0.05).

***Performance Parameters*:** Broiler farming mainly depends upon the birds' growth performances, which are measured by Feed intake, weight gain, mortality rates, and FCR. Coccidiosis disturbs these parameters so, estimation of these parameters is crucial because if these are not being improved then medicine is not useful for broiler farming. The chicks receiving AEO @ 3% (v/w) in the feed had the best FCR, weight gain, and feed intake values. They had a 100% survival rate with no mortalities. In contrast to them, infected non-medicated chicks (Group E) had the poorest values with the maximum mortalities (Figure 2, Table 2). Organ weight ratios of group C (3% AEO treated group) were comparable to the value of Symocox ® treated chicks (Table 3).

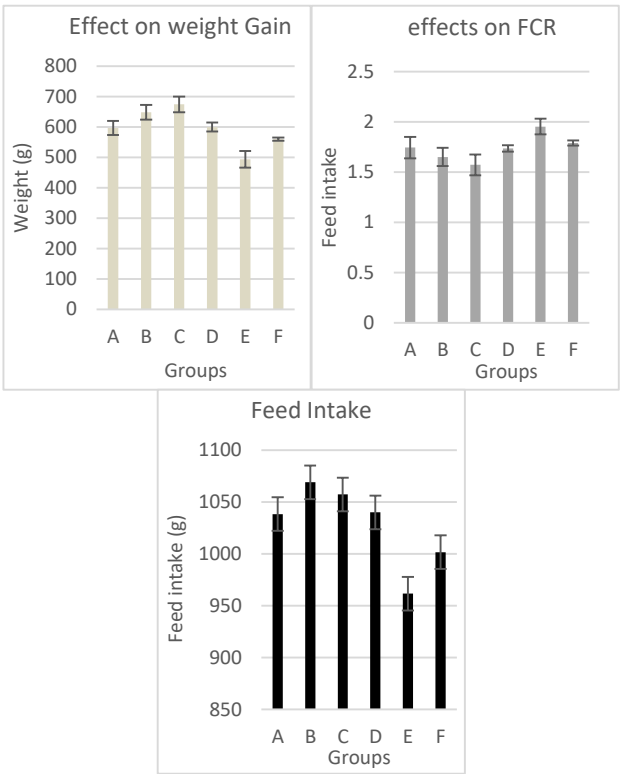

Figure 2. Effect of *A. subulatum* essential oil on feed conversion ratio (FCR), feed intake, and weight gain of broiler chicks. A: 1% *A. subulatum* Essential Oil; B: 2% *A. subulatum* Essential Oil; C: 3% *A. subulatum* Essential Oil; D Infected Medicated Control; E: Infected Nonmedicated Control; F: Noninfected Nonmedicated Control. Columns represent the average values obtained from replicates, and the bars represent the standard errors.

Hematological Parameters: The hematological profile of the chicks was estimated. Significant (P<0.05) difference was observed in Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) while the

Table 3. Effects *A. subulatum* essential oil on organ weight ratios in broiler chicks showing that an increase in concentration improved organ weight ratios.

Groups	Kidney	Liver	Spleen	Bursa	Intestine	Heart	Gizzard
A	0.88±0.03^{b}	2.42±0.08^{c}	0.07±0.00^{bc}	0.24±0.01^{c}	4.55±0.16^{de}	0.40±0.02^{c}	1.71±0.07^{b}
B	1.01±0.01^{a}	2.69±0.09^{bc}	0.09±0.00^{a}	0.28±0.00^{ab}	5.72±0.71^{cd}	0.49±0.01^{b}	1.81±0.03^{ab}
C	1.07±0.04^{a}	3.03±0.12^{a}	0.11±0.00^{a}	0.30±0.00^{ab}	7.97±0.23^{a}	0.55±0.02^{a}	1.89±0.10^{ab}
D	1.08±0.01^{a}	2.96±0.08^{ab}	0.11±0.00^{a}	0.28±0.00^{ab}	7.55±0.45^{ab}	0.54±0.01^{ab}	1.94±0.03^{a}
E	0.89±0.00^{b}	2.05±0.07^{d}	0.06±0.00^{b}	0.09±0.00^{d}	3.97±0.23^{e}	0.41±0.00^{c}	1.76±0.07^{ab}
F	1.05±0.04^{a}	2.90±0.12^{ab}	0.10±0.00^{a}	0.25±0.01^{bc}	6.43±0.34^{bc}	0.51±0.01^{ab}	1.93±0.02^{ab}

A: 1% *A. subulatum* Essential Oil; B: 2% *A. subulatum* Essential Oil; C: 3% *A. subulatum* Essential Oil; D Infected Medicated Control; E: Infected Nonmedicated Control; F: Noninfected Nonmedicated Control. The means having similar superscripts in the columns are non-significant (P>0.05)

Table 4. Effects of *A. subulatum* essential oil on hematological profile in broiler chicken shows that essential oil at 3% concentration had the best values of blood profiles.

Treatment	MCH (pg/L)	MCHC (g/dL)	PCV (%)	Hb (g/L)	RBC (x10^{6}/ uL)
A	43.55±1.35^{ab}	29.70±0.67^{ab}	26.74±0.42^{b}	8.03±0.26^{c}	2.60±0.04^{cd}
B	42.56±1.27^{b}	29.11±1.11^{ab}	29.05±0.70^{b}	9.27±0.08^{bc}	2.83±0.08^{bcd}
C	46.36±0.95^{a}	33.69±0.63^{a}	32.85±1.11^{a}	11.02±0.61^{a}	3.30±0.11^{ab}
D	45.35±0.24^{ab}	31.50±3.40^{a}	34.44±0.67^{a}	11.74±0.50^{a}	3.38±0.12^{a}
E	34.35±0.57^{c}	25.84±0.58^{b}	23.53±0.94^{c}	8.15±0.19^{c}	2.40±0.08^{d}
F	43.25±0.68^{a}	31.88±1.18^{a}	32.63±0.99^{a}	10.45±0.77^{ab}	3.10±0.32^{abc}

A: 1% *A. subulatum* Essential Oil; B: 2% *A. subulatum* Essential Oil; C: 3% *A. subulatum* Essential Oil; D Infected Medicated Control; E: Infected Nonmedicated Control; F: Noninfected Nonmedicated Control. The means having similar superscripts in the columns are non-significant (P>0.05).

Table 5. Effects of *A. subulatum* essential oil on differential leukocyte count and total white blood cells in broiler chicks shows that all the groups were significant from the negative control.

Treatment	Heterophils (x^{10}/ uL)	Lymphocytes (x^{10}/ uL)	Eosinophils (x^{10}/ uL)	Monocytes (x^{10}/ uL)	Basophils (x^{10}/ uL)	White Blood Cells (x^{10}/ uL)
A	773.4±17.43^{ab}	1963.5±88.79^{b}	131.9±2.71^{a}	160.5±3.80^{ab}	14.95±0.92^{a}	3044.5±81.04^{b}
B	704.5±14.87^{bc}	1802.4±53.17^{b}	121.2±2.30^{ab}	145.4±3.23^{abc}	14.91±1.54^{a}	2788.6±68.06^{bc}
C	587.9±93.59^{bc}	1494.8±65.38^{b}	106.0±21.3^{abc}	130.5±17.1^{bc}	16.40±0.74^{a}	2335.7±143.9^{c}
D	573.7±20.07^{c}	1561.2±54.61^{b}	89.1±3.11^{c}	124.9±4.37^{c}	12.00±0.31^{a}	2361.1±81.92^{c}
E	925.1±40.82^{a}	2573.2±307.9^{a}	126.0±6.10^{a}	164.4±10.4^{a}	12.91±2.50^{a}	3801.6±334.4^{a}
F	627.9±86.34^{bc}	1814.3±203.9^{b}	90.8±3.84^{bc}	127.2±5.39^{c}	11.42±2.69^{a}	2671.8±234.6^{bc}

The means having similar superscripts in the columns are non-significant (P>0.05).

Table 6. Effects of *A. subulatum essential* oils on serum chemistry in broiler chicks shows that an increase in concentration of oil improved the serum profile.

Treat.	ALT (IU/L)	AST (IU/L)	Alk.P (IU/L)	Proteins (g/dL)	Albumins (g/dL)	Globulins (g/dL)	Urea (mmol/L)	LDH (IU/L)
A	7.73±0.64^{b}	324.7±15.6^{a}	1990.2±67.0^{a}	3.74±0.09^{a}	1.77±0.08^{ab}	1.96±0.01^{a}	4.30±0.08^{a}	343.4±5.65^{ab}
B	7.98±0.33^{b}	269.3±6.68^{b}	1810.2±44.9^{bc}	3.54±0.14^{a}	1.57±0.07^{b}	1.96±0.14^{a}	4.36±0.15^{a}	298.9±8.19^{bc}
C	9.68±0.16^{a}	250.6±10.5^{b}	1810.9±62.8^{bc}	3.61±0.17^{a}	1.84±0.06^{a}	1.76±0.22^{a}	4.32±0.06^{a}	278.6±4.87^{c}
D	9.53±0.26^{a}	269.0±9.71^{b}	1775.7±35.7^{c}	3.33±0.26^{a}	1.89±0.06^{a}	1.44±0.26^{a}	4.24±0.07^{a}	265.0±8.16^{c}
E	7.19±0.10^{b}	350.9±7.67^{a}	1963.3±18.8^{ab}	3.25±0.23^{a}	1.55±0.08^{b}	1.70±0.19^{a}	4.48±0.18^{a}	371.3±18.1^{a}
F	9.29±0.13^{a}	334.7±23.3^{a}	1798.6±51.4^{bc}	3.34±0.09^{a}	1.85±0.05^{a}	1.49±0.15^{a}	4.41±0.14^{a}	302.3±24.5^{bc}

A: 1% *A. subulatum* Essential Oil; B: 2% *A. subulatum* Essential Oil; C: 3% *A. subulatum* Essential Oil; D Infected Medicated Control; E: Infected Nonmedicated Control; F: Noninfected Nonmedicated Control. The means having similar superscripts in the columns are non-significant (P>0.05). The means having similar superscripts in the columns are non-significant (P>0.05).

Packed cell volume (PCV), total red blood cells (RBC), total white blood cells (WBC), heterophils and Lymphocytes in the 3% AEO treated and Symocox ® treated groups in comparison to the infected nonmedicated control group. The rest of the leukocytes had no specific trend across the groups (Table 4, 5).

***Serum Parameters*:** The serum parameters Alanine aminotransferases (ALT), Aspartate transferase (AST),

Alkaline phosphatase (ALK.P), total proteins, total albumins, globulins, urea, and lactate dehydrogenase (LDH) were evaluated. All the values remained in the normal range. Urea had non-significant (P>0.05) in all the treated groups. While the 3% AEO treated groups had comparable values (P>0.05) to the infected medicated control group for all other parameters (Table 6).

DISCUSSION

In this research, the essential oils of *A. subulatum* showed anticoccidial effects and showed its best performance at 3% concentration and it was comparable (P>0.05) effects to the Symocox® treated groups while significantly different from the infected non-medicated control group. Multiple researchers have reported the anticoccidial activities of herbals including essential oils in *in-vitro* as well as in *in-vivo* studies (Abbas *et al.*, 2019a; Abbas *et al.*, 2019b; Lee *et al.*, 2020b; Puvaca *et al.*, 2020; Sidiropoulou *et al.*, 2020; Chang *et al.*, 2021).

Similar results have been reported by Sidiropoulou *et al.* (2020) who stated that the essential oils of oregano and garlic reduced the oocyst per gram of the feces caused by *Eimeria* and improved the FCR as well as had positive effects on the intestinal microbiota of the chicken. Lee *et al.* (2020b) also showed similar results on anticoccidial, growth, and serum biochemical parameters while evaluating the performance of the thymol-containing essential oil on the broiler chicks. Essential oil of garlic also efficiently reduced cecal coccidiosis by reducing the lesions, fecal scores, oocyst output, and oocyst per gram of feces in broiler chicks in research conducted by Chang *et al.* (2021). They attributed these results to the antioxidants present in the essential oil. Imran and Alsyeqh (2022) researched the essential oil of *Citrus sinensis* using 1, 2, and 3% concentrations to control coccidiosis in broiler chicks. They reported improvement in performance and coccidial parameters due to the essential oils. They attributed these effects due to the anti-oxidant compounds present in the essential oil. also reported similar results with a slight deviation. Similar results were reported by Mohiti- Asli and Ghanaatparast-Rashtiti (2015) while working on Oregano essential oil.

Essential oils have anticoccidial effects because of antioxidant compounds belonging to terpenes, terpenoids, phenols, and flavonoid families (Aouadi *et al.*, 2021; Chang *et al.*, 2021; Langerudi *et al.*, 2022; Raza *et al.*, 2022). These essential oils are capable to capture the reactive oxygen species produced during the inflammatory response of the immune system to counter the coccidiosis (Hafeez *et al.*, 2020; Kowalczyk *et al.*, 2020; Lee *et al.*, 2020a). Entry of *Eimeria* inside the cells produces lesions and leads to blood in the feces. The repeated cycle of merogony increases the damage leading to increased lesions and oocyst count in the feces. It is well documented that when sporozoites or merozoites of *Eimeria* try to enter the epithelial cells of the gut, they elicit the production of inflammatory cytokines which lead to the inflammatory response against them (Wakelin and Rose, 2019; Soutter *et al.*, 2020; Lee *et al.*, 2022). Immune cells also try to rupture the *Eimeria* sporozoite-containing cells and as a result of necrosis, reactive oxygen species are produced (Dubey *et al.*, 2019; McDougald *et al.*, 2020; Felici *et al.*, 2021). These reactive oxygen species are highly reactive and react with neighboring cells leading to extensive cell damage (Mohsin *et al.*, 2021; Bhutta *et al.*, 2022; Nahed *et al.*, 2022). The active compounds of essential oils can capture these reactive oxygen species (Das *et al.*, 2019; Kaurinovic and Vastag, 2019). *A. subulatum* essential oil has proven antioxidant activities in *in-vivo* and *in vitro* environments (Aftab and Rita, 2018; Alam and Singh, 2021; Mande and Sekar, 2021). Its major components are cineole, terpineol, and terpinene which belong to the monoterpene and terpenoid groups (Sharma *et al.*, 2020; Alam and Singh, 2021). These compounds are great antioxidant substances and multiple essential oils showing anticoccidial activities possess these compounds (Batiha *et al.*, 2020; Abd El-Hack *et al.*, 2022; Raza *et al.*, 2022). Reduced oxidative stress in broilers leads to high metabolism and increased feed intake which leads to improved weight gains and improved FCRs in the essential oil-treated groups (Ding *et al.*, 2022). The extensive cell damage during coccidiosis leads to blood loss causing lowered hematological profiles in broiler chicks, essential oils reduce the damage and lead to reduced blood loss in turn causing improved hematological profiles (Li *et al.*, 2020). The intestinal lesions and oocyst output is reduced and the lesions are recovered due to the anti-inflammatory and antioxidant effects of essential oils.

***Conclusion*:** Results of the present research prove that the essential oil of *A. subulatum* has anticoccidial effects in the broiler chicks and has positive effects on the performance parameters, and hematological parameters without disturbing serum biochemical parameters. These results show that the essential oil of *A. subulatum* can be used as an alternative to synthetic anticoccidial drugs. The essential oil of *A. subulatum* is a medicine of herbal origin that is easily available and eco-friendly. So, this can be an optimum anticoccidial to be used in commercial broiler regimes.

***Animal Ethics*:** Humane handling of the birds was performed during the experiment and no other stress was given to the animals except for the research-related parameters. Animals were humanely slaughtered to observe lesion scores and organ weights. All the animals were handled following animal ethics.

***Conflict of Interest*:** The authors declare no conflict of interest regarding any part of this research.

***Author's Contribution*:** ZS conducted the research and wrote the manuscript, RZA supervised and designed the plan, MKK assisted in lab analysis and MKS assisted in statistical analysis.

REFERENCES

Abbas, A., R.Z. Abbas, M.K. Khan, M.A. Raza, M.S. Mahmood, M.K. Saleemi, T. Hussain, J.A. Khan and Z.U.D. Sindhu. 2019a. Anticoccidial effects of Trachyspermum ammi (Ajwain) in broiler chickens. Pakistan Veterinary Journal 39:301-304.

Abbas, R.Z., A. Abbas, M.A. Raza, M.K. Khan, M.K. Saleemi and Z. Saeed. 2019b. In vitro anticoccidial activity of Trachyspermum ammi (Ajwain) extract on oocysts of Eimeria species of Chicken. Advancements in Life Sciences 7:44-47.

Abd El-Hack, M.E., M.T. El-Saadony, A.M. Saad, H.M. Salem, N.M. Ashry, M.M.A. Ghanima, M. Shukry, A.A. Swelum, A.E. Taha and A.M. El-Tahan. 2022. Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. Poultry Science 101:101584.

Aftab, A. and S.M. Rita. 2018. Antioxidant activity of essential oil of three cultivars of Amomum subulatum and standardization of high performance thin layer chromatography (HPTLC) method for the estimation of 1, 8-cineole. African Journal of Biotechnology 17:1129-1137.

Alam, A. and V. Singh. 2021. Composition and pharmacological activity of essential oils from two imported Amomum subulatum fruit samples. Journal of Taibah University Medical Sciences 16:231-239.

Aouadi, M., E. Sebai, A. Saratsis, V. Kantzoura, K. Saratsi, K. Msaada, S. Sotiraki and H. Akkari. 2021. Essential oil of Rosmarinus officinalis induces in vitro anthelmintic and anticoccidial effects against Haemonchus contortus and Eimeria spp. in small ruminants. Veterinární medicína 66:146-155.

Balkrishna, A., B. Joshi, A. Srivastava and S. Bisht. 2023. An Insight on Nutraceutical Influences of Spice and Condiments: Serve as a Flavor and Immunity Enhancer from Ages, Herbs and Spices-New Advances. Herbs and Spices-New Advances. IntechOpen

Batiha, G.E.-S., A. Olatunde, A. El-Mleeh, H.F. Hetta, S. Al-Rejaie, S. Alghamdi, M. Zahoor, A. Magdy Beshbishy, T. Murata and A. Zaragoza-Bastida. 2020. Bioactive compounds, pharmacological actions, and pharmacokinetics of wormwood (Artemisia absinthium). Antibiotics 9:353.

Bhutta, Z.A., M. Kulyar, I.S. Jahanzaib, S. Shabbir, P. Boruah and A. Bello. 2022. Evaluation of hematological, antioxidant enzymes and oxidative stress parameters in buffaloes infected with babesiosis. Continental Veterinary Journal 2:29-34.

Chang, L.Y., K.Q. Di, J. Xu, Y.F. Chen, J.Z. Xi, D.H. Wang, E.Y. Hao, L.J. Xu, H. Chen and R.Y. Zhou. 2021. Effect of natural garlic essential oil on chickens with artificially infected Eimeria tenella. Veterinary Parasitology 300:109614.

Das, S., Z. Gazdag, L. Szente, M. Meggyes, G. Horváth, B. Lemli, S. Kunsági-Máté, M. Kuzma and T. Kőszegi. 2019. Antioxidant and antimicrobial properties of randomly methylated β cyclodextrin–captured essential oils. Food Chemistry 278:305-313.

Ding, Y., Y. Hu, X. Yao, Y. He, J. Chen, J. Wu, S. Wu, H. Zhang, X. He and Z. Song. 2022. Dietary essential oils improves the growth performance, antioxidant properties and intestinal permeability by inhibiting bacterial proliferation, and altering the gut microbiota of yellow-feather broilers. Poultry Science 101:102087.

Dubey, J.P., D.S. Lindsay, M.C. Jenkins and C. Bauer. 2019. Biology of intestinal coccidia. Coccidiosis in livestock, poultry, companion animals, and humans.1-36. CRC Press.

Felici, M., B. Tugnoli, A. Piva and E. Grilli. 2021. In vitro assessment of anticoccidials: methods and molecules. Animals 11:1962.

Gavahian, M., A. Farahnaky, K. Javidnia and M. Majzoobi. 2012. Comparison of ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from Thymus vulgaris L. Innovative Food Science & Emerging Technologies 14:85-91.

Gul, S.T. and A.F. Alsayeqh. 2023. Probiotics Improve Physiological Parameters and Meat Production in Broiler Chicks. International Journal of Veterinary Science 12:182-191.

Hafeez, A., Z. Ullah, R.U. Khan, Q. Ullah and S. Naz. 2020. Effect of diet supplemented with coconut essential oil on performance and villus histomorphology in broiler exposed to avian coccidiosis. Tropical Animal Health and Production 52:2499-2504.

Imran, A. and A. Alsayeqh. 2022. Anticoccidial Efficacy of Citrus sinensis Essential Oil in Broiler Chicken. Pakistan Veterinary Journal 42:461-466.

Johnson, J. and W.M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Experimental Parasitology 28:30-36.

Joshi, N.P. and L. Piya. 2019. Large cardamom (Amomum subulatum Roxb.) production, marketing and trade in the Indian sub-continent. Journal of Contemporary India Studies: Space and Society 9:1-13.

Kaurinovic, B. and D. Vastag. 2019. Flavonoids and phenolic acids as potential natural antioxidants. Antioxidants. IntechOpen London, UK.

Khallaf, M., L.O. Elmajdoub, A. Awad, A. El-Bahrawy and A. Elkhatam. 2022. Prevalence of Monogenean

Trematodes of Tilapia species from River Nile in in Beheira Province, Egypt. Continental Veterinary Journal 2:59-66.

Khan, M., N. Chand, S.Naz and R.U. Khan. 2023. Dietary tea tree (Melaleuca alternifolia) essential oil as alternative to antibiotics alleviates experimentally induced Eimeria tenella challenge in Japanese quails. Journal of Animal Physiology and Animal Nutrition 107:643-649.

Khater, H.F., H. Ziam, A. Abbas, R.Z. Abbas, M.A. Raza, K. Hussain, E.Z. Younis, I.T. Radwan and A. Selim. 2020. Avian coccidiosis: Recent advances in alternative control strategies and vaccine development. Agrobiological Records 1:11-25.

Kowalczyk, A., M. Przychodna, S. Sopata, A. Bodalska and I. Fecka. 2020. Thymol and thyme essential oil—new insights into selected therapeutic applications. Molecules 25:4125.

Langerudi, M.T., M.R. Youssefi and M.A. Tabari. 2022. Ameliorative effect of Psidium guajava essential oil supplemented feed on chicken experimental coccidiosis. Tropical Animal Health and Production 54:120.

Lee, J.E., S.M. Seo, M.J. Huh, S.C. Lee and I.K. Park. 2020a. Reactive oxygen species mediated-antifungal activity of cinnamon bark (Cinnamomum verum) and lemongrass (*Cymbopogon citratus*) essential oils and their constituents against two phytopathogenic fungi. Pesticide Biochemistry and Physiology 168:104644.

Lee, J.W., D.H. Kim, Y.B. Kim, S.B. Jeong, S.T. Oh, S.Y. Cho and K.W. Lee. 2020b. Dietary encapsulated essential oils improve production performance of coccidiosis-vaccine-challenged broiler chickens. Animals 10:481.

Lee, Y., M. Lu and H.S. Lillehoj. 2022. Coccidiosis: Recent progress in host immunity and alternatives to antibiotic strategies. Vaccines 10:215.

Li, A.L., W.W. Ni, Q.M. Zhang, Y. Li, X. Zhang, H.Y. Wu, P. Du, J.C. Hou and Y. Zhang. 2020. Effect of cinnamon essential oil on gut microbiota in the mouse model of dextran sodium sulfate-induced colitis. Microbiology and Immunology 64:23-32.

Mahmoud, D.H., H.B. Mahmoud, F.A. EL-Nawawi and H.H. Abdel-Naeem. 2021. Impact of Thawing Methods on the Bacteriological Quality of Chicken Meat. International Journal of Veterinary Science 10:214-219.

Makhija, P., H.K. Handral, G. Mahadevan, H. Kathuria, G. Sethi and B. Grobben. 2022. Black cardamom (*Amomum subulatum* Roxb.) fruit extracts exhibit apoptotic activity against lung cancer cells. Journal of Ethnopharmacology 287:114953.

Mande, P. and N. Sekar. 2021. Comparative study of chemical composition, antibacterial and antioxidant activity of essential oils isolated from the seeds of Amomum subulatum by using microwave extraction and hydro-distillation methods. Journal of the Indian Chemical Society 98:100201.

Mande, P. and N. Sekar. 2021. Comparative study of chemical composition, antibacterial and antioxidant activity of essential oils isolated from the seeds of Amomum subulatum by using microwave extraction and hydro-distillation methods. Journal of the Indian Chemical Society 98:100201.

McDougald, L.R., H.M. Cervantes, M.C. Jenkins, M. Hess and R. Beckstead. 2020. Protozoal infections. Diseases of poultry 1192-1254.

Mohiti-Asli, M., and M. Ghanaatparast-Rashti. 2015. Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers. Preventive veterinary medicine 120:195-202.

Mohsin, M., R.Z. Abbas, G. Yin, Z.-U.-D. Sindhu, A. Abbas, Z. Huang, M.T. Aleem, Z. Saeed, M.Z. Afzal and A. Ejaz. 2021. Probiotics as therapeutic, antioxidant and immunomodulatory agents against poultry coccidiosis. World's Poultry Science Journal 77:331-345.

Moryani, A.A., N. Rajput, M. Naeem, A.H. Shah and A.R. Jahejo. 2021. Screening of the Herbs and Evaluation of their Combined Effects on the Health and Immunity of Coccidiosis Challenged Broiler Chickens. Pakistan Veterinary Journal 41:228-234.

Nahed, A., M.E. Abd El-Hack, N.M. Albaqami, A.F. Khafaga, A.E. Taha, A.A. Swelum, M.T. El-Saadony, H.M. Salem, A.M. El-Tahan and S.F. AbuQamar. 2022. Phytochemical control of poultry coccidiosis: a review. Poultry Science 101:101542.

Nair, K.P. 2020. Large Cardamom (Amomum subulatum Roxb.), The Geography of Cardamom (*Elettaria cardamomum* M.). Springer.253-280

Nethi, S. and P. Mande. 2020. Chemical Composition antibacterial and antioxidant activity of the essential oils of Amomum subulatum by using Microwave extraction comparison with hydro distillation. Authorea Preprints.

Parveen, U., M. Maaz, M. Mujeeb and U. Jahangir. 2018. Biological And Therapeutic Uses Of Amomum Subulatum Roxb: A. European Journal of Biomedical and Pharmaceutical Sciences 5:167-176.

Parveen, U., M. Maaz, M. Mujeeb and U. Jahangir. 2018. Biological And Therapeutic Uses Of Amomum Subulatum Roxb: A. European Journal of Biomedical 5:167-176.

Puvaca, N., D. Horvatek Tomić, S. Popović, S. Đorđević, I. Brkić, N. Lalić, T. Shtylla Kika and E. Lika. 2020. Influence of tea tree (Melaleuca alternifolia) essential oil as feed supplement on production traits, blood oxidative status and treatment of coccidiosis in laying hens. Veterinarski Arhiv 90:331-340.

Raza, Q.S., M.K. Saleemi, S. Gul, H. Irshad, A. Fayyaz, I. Zaheer, M.W. Tahir, Z. Fatima, T.Z. Chohan and M. Imran. 2022. Role of essential oils/volatile oils in poultry

production—A review on present, past and future contemplations. Agrobiological Records 7:40-56.

Rizwan, H.M., M.K. Khan, M.A.S. Mughal, Z. Abbas, R.Z. Abbas, Z.u.D. Sindhu, M.S. Sajid, Q.u. Ain, A. Abbas and A. Zafar. 2022. A new insight in immunomodulatory impact of botanicals in treating avian coccidiosis. Journal of Parasitic Diseases 46:1164-1175.

Ryley, J.F., R. Meade, J. Hazelhurst and T.E. Robinson. 1976. Methods in coccidiosis research: separation of oocysts from faeces. Parasitology 73:311-326.

Sharma, B., N. Vasudeva and S. Sharma. 2020. Essential oil composition and anti-scabies potential of Amomum subulatum Roxb. leaves. Anti-Infective Agents 18:261-267.

Sidiropoulou, E., I. Skoufos, V. Marugan-Hernandez, I. Giannenas, E. Bonos, K. Aguiar-Martins, D. Lazari, D.P. Blake and A. Tzora. 2020. In vitro anticoccidial study of oregano and garlic essential oils and effects on growth performance, fecal oocyst output, and intestinal microbiota in vivo. Frontiers in Veterinary Science 7:420.

Sorour, S.S., S. Abou Asa, N.M. Elhawary, E.W. Ghazy, A. Abd El Latif, M.A. El-Abasy and H.O. Khalifa. 2018. Anticoccidial and hepatoprotective effects of artemisinin liquid extract, cinnamon essential oil and clove essential oil against Eimeria stiedae infection in rabbits. Tropical Biomedicine 35:926-943.

Soutter, F., D. Werling, F.M. Tomley and D.P. Blake. 2020. Poultry coccidiosis: design and interpretation of vaccine studies. Frontiers in Veterinary Science 7:101.

Thinh, B.B., R.V. Doudkin and V.Q. Thanh. 2021. Chemical composition of essential oil of Amomum xanthioides Wall. ex Baker from Northern Vietnam. Biointerface Research in Applied Chemistry 11:12275-12284.

Van, H.T. 2021. Chemical constituents and biological activities of essential oils of Amomum genus (Zingiberaceae). Asian Pacific Journal of Tropical Biomedicine 11:519.

Wakelin, D. and M.E. Rose. 2019. Immunity to coccidiosis, Coccidiosis of man and domestic animals. CRC Press 281-306

Youn, H.J., Y.B. Kang and D.M. Jang. 1993. Effects of γ-irradiation from cobalt-60 on pathogenicity of Eimeria tenella. Korean Journal of Veterinary Research 33:649-655.

Yu-Wen, J.T., 2008. Orego-Stim: the proven and researched alternative to infeed antibiotic growth promoters and coccidiostats. Analele IBNA 24:48-55.