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RESEARCH ARTICLE

Anticoccidial Effects of *Trachyspermum ammi* (Ajwain) in Broiler Chickens

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A B S T R A C T

Anticoccidial drug resistance has been increased due to which alternative protocols are required to control avian coccidiosis. In current study, *Trachyspermum ammi* (Ajwain) was supplemented in crude form in feed (3%) of broiler chickens which were given infection with sporulated oocysts of different *Eimeria* species. Toltrazuril®anticoccidial drug was administered in control group. Anticoccidial activity of *T. ammi* was evaluated on the basis of different parameters such as feed conversion ratio, mortality rate, intestinal lesion, fecal scores, oocyst scores and organ weight. Data on hematological parameters and serum chemistry was also collected. Dietary supplementation of *T. ammi* reduced *Eimeria* infection in terms of better feed conversion ratio, lower lesion and fecal scores in broiler chickens (P>0.05). Supplementation of *T. ammi* also improved hematology and serum chemistry of infected chickens. It was concluded from the experiment that *T. ammi* can serve as alternative candidate against poultry coccidiosis however, further *in vivo* studies are needed to formulate its dose against coccidiosis.

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INTRODUCTION

Coccidiosis is severe and devastating disease infecting intestine of chicken caused by *Eimeria* (protozoa) which have various species (Alzahrani *et al*., 2016). There are seven major *Eimeria* species which cause coccidiosis including *E. tenella, E. necatrix, E.* acervulina, *E. brunetti, E. maxima, E. mitis and E. Praecox*. Among all these species *Eimeria tenella* and *E. necatrix* are considered to be most pathogenic (Blake and Tomley, 2014). Coccidiosis cause economically considerable losses to poultry production in almost all parts of world (Chapman, 2014; Bachaya *et al*., 2015). Disease has various clinical features such as poor weight gain, high mortality and bloody feces. Oocysts of *Eimeria* sporulate rapidly in soil having higher multiplication rate due to which its prevention is difficult once its outbreak has occurred among birds (Abbas *et al.,* 2017a). Anticoccidial drugs are used to control coccidiosis but, their efficacy has been lowered in field due to resistance problems to these drugs and now it is not reliable protocol for its control (Grandi *et al*., 2016).

So, in matter of achieving success in controlling this severe disease other options and protocols are effectively used in different countries of world (Liaqat *et al*., 2016). Plant driven compounds and their products have shown better anticoccidial effects among these options (Blake and Tomley, 2014; Abbas *et al.,* 2017a,b,c). Different Botanicals such as *Bet vulgaris* (Abbas *et al.,* 2017b), *Saccharum officinarum* (Awais *et al*., 2014) and *Ageratum conyzoides* (Nweze and Obiwulu, 2009) have shown excellent activity against coccidiosis.

Use of antioxidant rich plants extracts has gained special importance because of the restriction in use of synthetic compounds against coccidial infections due to emergence of resistance (Masood *et al.,* 2013). Antioxidants (phenols, flavonoids, tannins and saponins) rich plants are being used as an alternative strategy to treat coccidiosis (Alzahrani *et al*., 2016; Awaad *et al.,* 2016).

Trachyspermum ammi commonly known as 'Ajwain' is distributed throughout India and Pakistan (Asif *et al.,* 2014). The fruit is used traditionally as an important remedial agent for abdominal tumors, abdominal pains,

lack of appetite and asthma. It has been proven to possess various therapeutic activities like antiparasitic, antioxidant and immunomodulatory properties (Zarshenas *et al.*, 2014). Based on the various beneficial effects of *Trachyspermum ammi*, current experiment was conducted to check its anticoccidial efficacy against coccidiosis in chickens.

MATERIALS AND METHODS

Plant material: *Trachyspermum ammi* seeds were obtained locally from Faisalabad. Powdered plant material was prepared following method described by Abbas *et al*. (2017a). Briefly, plant material was dried and then was converted to powder using an electric mill.

Parasite: Infected guts with *Eimeria* were taken from outbreak cases and poultry shops. Oocysts of different *Eimeria* species including *E. tenella, E. necatrix, E. brunetii, E. mitis* and *E. maxima* were collected from the intestine of infected chickens. Infective material (*Eimeria* oocysts) were preserved in potassium dichromate solution (2.5%) using the standard guidelines as provided by Ryley *et al*. (1976).

Experimental design: 72 day-old broiler chicks were procured and reared according proper management practices. At one week of age chicks were divided in four equal groups (A, B, C, D). There were 18 birds in each group. At two weeks of age each group was treated with 50,000 oocysts (sporulated) of different *Eimeria* species. At the same day, group A was supplemented with *Trachyspermum ammi* powder at the rate of 3% per kg of feed till at the end of experiment (40 days). Group B served as positive Control and was treated with Toltrazuril®. Group C served as infected un-medicated control group. Group D served as un-infected un-medicated control group.

Evaluation of anticoccidial activity: Anticoccidial potential of *Trachyspermum ammi* was evaluated on the basis of parameters such as lesion score (Johnson and Reid, 1970), oocyst score (Hilbrich, 1978), fecal score (Youn *et al.*, 1993), organ weight, mortality rate and feed conversion ratio.

Moratlity rate: Mortality rate of chicks was calculated by the following formula:
Mortality rate = Number of dead chicks / Total number of chicks × 100

Feed conversion ratio: Feed conversion ratio of all treated groups was calculated by following formula.
Feed conversion ratio = Mean Feed Consumption / Mean Weight

Hematology and serum chemistry: Collected blood samples were analyzed for Packed Cell Volume (microhematochrit method), hemoglobin level (Sahli's apparatus). RBCs and WBCs counting were done by method as described Natt and Herrick (1952). Serum chemistry of collected serum was done with the help of different kits (Merck, Germany).

Statistical analysis: Statistical analysis of collected data was done by Duncan's multiple range test. Significance among groups was determined at $P<0.05$.

RESULTS

Lesion, Oocyst and Fecal Score: Mean lesion score values are shown in Table 1. Mean lesion score values of group supplemented with *T. ammi* were comparable to standard medicine, Toltrazuril® ($P>0.05$). Mean oocyst score values of group supplemented with *T. ammi* were comparable to standard medicine, Toltrazuril® ($P>0.05$) (Table 2). *T. ammi* administered group also exhibited lowered fecal score values which were comparable to that of Toltrazuril® treated group (Table 3).

Feed conversion ratio and mortality rate: *T. ammi* administered group showed better feed conversion ratio and less mortality rate as compared to infected un-medicated group ($P<0.05$) as shown in Table 4 and Fig. 1.

Organ Weight: Mean organ weight values of *T. ammi* administered group were also comparable to that of Toltrazuril® treated group as shown in Table 5 ($P>0.05$).

Hematology and Serum Chemistry: Mean hematological values i.e. HB, PCV, RBCs & WBCs and mean serum enzyme evalues i.e. AST, LDH and Creatinine of *T. ammi* administered group were similar to Toltrazuril® treated group as shown in Table 6 and Table 7 respectively ($P>0.05$).

DISCUSSION

Many botanicals and their products are reported to have excellent effect against various diseases and particularly against coccidiosis as proven by different *in vitro* and *in vivo* studies (Abbas *et al.*, 2015, 2017a, 2017b, 2017c, 2018; Idris *et al*., 2017; Khater *et al*., 2018; Mahmood *et al*., 2018). In present study like that of previous studies (Awais *et al.*, 2014; Hong *et al.*, 2016; Gadelhaq *et al.*, 2018), *in vivo* anticoccidial effect of *Trachyspermum ammi* was measured in terms of different parameters such as feed conversion ratio, mortality rate, lesion, fecal and oocyst scores. *Trachyspermum ammi* showed remarkable and similar anticoccidial effects on all of the above parameters when compared with reference drug Toltrazuril® ($P\geq0.05$). *T. ammi* also improved hematology and serum chemistry of infected chickens. Wang *et al*. (2008) has reported similar types of antococcidial effects of grape seed extract in broiler chickens. In another study, *Bidens pilosa* a flowering plant have shown anticoccidial effects against *Eimeria* in chickens (Yang *et al*., 2015).

Anticoccidial effect of *T. ammi* may be attributed due to the action of its antioxidant compounds and its essential oils such as thymol and carvacrol derivatives which act as antiparasitic, immunomodulatory and therapeutic effects against various diseases of livestock importance (Mathew *et al*., 2008). Essential oils such as thymol and carvacrol have also demonstrated *in vitro* anticoccidial effects against *Eimeria* oocysts (Remmal *et al.*, 2013).

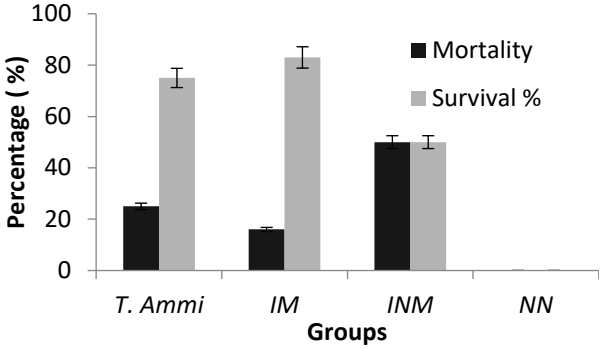

Fig. 1: Effect of *T. ammi* treatment on mortality and survival %; IM; Infected medicated; INM; infected non medicated NN; non infected non medicated

Table 1: Mean lesion score values in different treated groups (n=6)

Groups	Day 4th	Day 5th	Day 6th
A	1.83±0.98^{B}	1.66±0.81^{B}	1.50±0.51^{B}
B	1.33±0.40^{B}	1.50±0.40^{B}	1.60±0.40^{B}
C	3.83±0.60^{A}	3.30±0.45^{A}	2.60±0.50^{A}
D	0.00±0.00^{C}	0.00±0.00^{C}	0.00±0.00^{C}

Means having the different superscripts are significant different; A; *T. ammi* treated; B; Toltrazuril®; C; infected un-medicated; D; Un-infected un-medicated.

Table 2: Mean oocyst score values in different treated groups (n=6)

Groups	0	+1	+2	+3	+4	+5	Mean
A	1	1	3	1	–	–	1.66±0.51^{A}
B	2	1	2	1	-		1.33±0.75^{A}
C	–	1	–	2	2	1	3.33±0.40^{B}
D	–	–	–	–	–	–	–

Means having the different superscripts are significant different; A; *T. ammi* treated; B; Toltrazuril®; C; infected un-medicated; D; Un-infected un-medicated.

Table 3: Mean fecal score values in different treated groups (n=6)

Groups	Day 3rd	Day 4th	day 5th	Day 6th
A	–	2.83±0.98^{B}	1.66±0.81^{B}	1.50±0.51^{B}
B	–	0.33±0.70^{B}	1.50±0.70^{B}	1.66±0.52^{B}
C	–	3.83±0.70^{A}	3.30±0.40^{A}	2.66±0.60^{A}
D	–	0.00±0.00^{C}	0.00±0.00^{C}	0.00±0.00^{C}

Means having the different superscripts are significant different; A; *T. ammi* treated; B; Toltrazuril®; C; infected un-medicated; D; Un-infected un-medicated.

Table 4: Feed conversion ratio of different treated groups

Groups	Feed consumed	Weight gain	FCR
A	1400.01	700.10	2.00
B	1594.15	780.12	2.04
C	1690.14	581.70	2.90
D	1801.12	900.42	2.00

*Because of group feeding statistical analysis was not achievable.

Table 5: Mean organ weight values in different treated groups

Treatment	Liver	Spleen	Intestine	Gizzard	Proventriculus
A	4.13±0.40^{B}	0.12±0.02^{B}	11.87±0.6^{B}	1.10±0.2^{B}	5.47±0.22^{B}
B	4.21±0.28^{B}	0.11±0.03^{B}	13.01±0.5^{B}	1.13±0.1^{B}	5.69±0.18^{B}
C	5.17±0.63^{A}	0.14±0.02^{A}	14.31±1.1^{A}	1.02±0.4^{A}	5.70±0.08^{A}
D	2.91±0.38^{C}	0.13±0.02^{C}	12.11±1.1^{C}	1.90±0.2^{C}	5.61±1.88^{C}

Means having the different superscripts are significant different; A; *T. ammi* treated; B; Toltrazuril®; C; infected un-medicated; D; Un-infected un-medicated.

Table 6: Mean hematological values in different treated groups

Groups	PCV %	HB g/dl	RBC 10^6/ul	WBC10^3/ul
A	25.33±1.36^{B}	11.63±1.30^{B}	3.25±0.53^{B}	30.50^{B}
B	27.16±1.34^{B}	11.33±0.90^{B}	3.36±0.65^{B}	20.66^{B}
C	22.14±1.47^{A}	08.52±0.55^{A}	1.94±0.24^{A}	32.64^{A}
D	30.16±1.47^{C}	14±0.55^{C}	3.00±0.24^{C}	35.66^{C}

Means having the different superscripts are significant different; A; *T. ammi* treated; B; Toltrazuril®; C; infected un-medicated; D; Un-infected un-medicated.

Table 7: Mean serum enzymes values in different treated groups

Groups	ALT	AST	LDH	Creatinine
A	10.50±0.60^{B}	170.46±50.96^{A}	465.60±22.09^{B}	0.19±0.07^{B}
B	10.51±0.84^{B}	179.59±10.71^{B}	473.47±20.36^{C}	0.17±0.02^{B}
C	24.57±1.51^{A}	263.10±56.60^{A}	887.89±26.25^{A}	0.52±0.02^{A}
D	7.08±.82^{C}	182.07±12.24^{C}	462.40±13.64^{C}	0.16±0.03^{C}

Means having the different superscripts are significant different; A; *T. ammi* treated; B; Toltrazuril®; C; infected un-medicated; D; Un-infected un-medicated.

Plants and their products have provided better results in terms of controlling coccidiosis in chicken as compared to commercially available anticoccidial drugs (Gandi *et al.*, 2016). Antioxidant compounds in different plants have played important role in increasing the immunity and protection level against coccidiosis (Awaad *et al*., 2016). Leaves of *Carthamus tinctorius* commonly known as sunflower as have shown to enhance cellular and humoral immunity against poultry coccidiosis (Lee *et al*., 2009).

In another study, *Ageratum conyzoides* (billy goat weed) extract was administered orally at the dose rate of 250-3000 mg/kg in broiler chicks. Oral administration of *Ageratum conyzoides* extract showed positive effect in terms of improved hematological parameters such as red and white blood cell count (Nweze and Obiwulu, 2009). In one study *Triticum aestivum* or wheat bran polysaccharides (arabinoxylans) have shown immunomodulatory effects against *Eimeria* infection in chicken (Akhtar *et al*., 2012). Furthermore, they had better effect on organ weight gain and reduction in severity of *Eimeria* infection. *Saccharum officinarum* extract shown to improve immune response and act as biological modifier in broiler chickens (Awais *et al*., 2014). In a latest study, Abbas *et al.* (2017) has reported the immunomodulatory effects of *Camellia sinensis* crude powder. *Camelliasinensis* crude powder showed positive effects against coccidiosis.

Zaman *et al.* (2012) reported the anticoccidial effects of herbal complex comprising of *Trachyspermum ammi* (seeds), *Nicotiana tabacum* (leaves), *Azadirachta indica* (leaves) and *Calotropis procera* (flowers) in broiler chicken. The results of study were in similar to that of present study.

In another recent study, Abbas *et al.* (2017b) reported anticoccidial effects of *Bet vulgaris* extract in broiler chicken. *Beta vulgaris* demonstrated similar type of anticoccidial potential in terms of reduced lesion, oocyst score, also improved feed conversion ratio and serum chemistry of infected chicks.

Conclusions: Results of current study have confirmed that supplementation of crude powder of *Trachyspermum ammi* showed anticoccidial effects in broiler chickens and also improved hematological parameters and serum chemistry of infected chickens. *Trachyspermum ammi* can serve as alternative to synthetic anticoccidal drugs. An advantages of using natural plants like *Trachyspermum ammi* is to lower risk of developing resistance, also the residues of such natural products in meat are friendly to human consumers and have no adverse effects on their health.

Authors contribution: AA, RZA, MKK planned, designed and performed the experiment. MAR and TH analyzed the data. ZDS helped in preparation of plant

material. MSM, MKS and JAK assisted in data analysis and approving the final draft of manuscript.

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