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Research Article

Anticoccidial Effects of *Trachyspermum ammi* Essential Oil Against Caecal Coccidiosis in Broiler Chickens

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Abstract

In present study the anticoccidial activity of essential oil of *Trachyspermum ammi* (seeds) was evaluated by *in vivo* methods. For *in vivo* trial, total of 72 (day-old) broiler chicks were randomly divided into six groups A, B, C, D, E and F (each group having 12 chicks). At one week of age groups A, B, and C were treated with essential oil of *Trachyspermum ammi* (seeds) at concentration 1%, 2%, and 3% by supplementing in feed for three consecutive days. Group D served as positive control (infected and medicated with Toltrazuril treatment), group E served as negative control group (infected and non-medicated) and group F as normal (non-infected and non-medicated) control group. All groups except F were infected orally with 50.000 sporulated oocysts of mixed *Eimeria* species. Anticoccidial potential of essential oil was determined by different parameters such as mortality rate, fecal score, oocyst per gram of feces, weight gain, feed conversion ratio. Furthermore, hematological and serum chemistry profile was also evaluated. Hence treatment of *Trachyspermum ammi* (seeds) essential oil at dose rate 1%, 2% and 3% supplementation produces anticoccidial effects in terms of enhanced feed conversion ratio, decreased fecal and OPG count and improved weight gain in infected chickens. These treatment of essential oil showed to put positive effects also on hematological parameters like hemoglobin level, packed cell volume, red blood cells and white blood cells count and also put positive response towards serum biochemistry.

Keywords: *Trachyspermum ammi*, Essential oil, Coccidiosis, Chicken

Introduction

Poultry meat is one of the significant sources of proteins, fats, natural and inorganic constituent's which require to our daily life. The poultry business is increasing day by day however, coccidiosis is major parasitic disease effecting poultry industry overall and causing huge financial loss all over the world [1,2]. In poultry industry coccidiosis is one of the major intestinal parasitic disease of chicken caused by *Eimeria* species. The disease is caused by *Eimeria* protozoa having various species that harm the digestive system of avian host which eventually cause bloody diarrhea, decline feed consumption and reduce weight gain [3]. Anticoccidial drugs are used to control coccidiosis in chickens administered through water and feed, this approach is the main pillar in coccidiosis

control program. Different classes of anticoccidials have been used previously. Chemical drugs like Amprolium, Clopidol, Halofuginone that are extensively used against coccidiosis. Nonetheless, the exorbitant utilization of anticoccidial drugs have brought emergence of resistance in *Eimeria* species in different countries including Pakistan [4]. Moreover, the constant utilization of anticoccidial drugs prompted the poisonous consequences for birds and residual impacts of these medications in poultry items [5]. Furthermore, immunization is also an effective approach in controlling avian coccidiosis. However, immunization may not be successful due to geological varieties of *Eimeria* strain [6]. Among recent approaches, alternative plant drive compounds have shown promising results against coccidiosis.

The plants belonging to family *Asteraceae, Lamiaceae* and *Apiaceae* are well known to possess antiparasitic properties. The different plant components and their essential oils frequently studied due to having positive response in controlling avian Coccidiosis [7]. *Trachyspermum ammi* that is also known as "Ajwain" is well-known medicinal plant that have therapeutic activities. Therefore, keeping in view the anticoccidial potential of various botanicals and their essential oils in the light of previous reports, the current study was planned to evaluate the anticoccidial potential of essential oil of *Trachyspermum ammi* (seeds). The specific goals of this study was: To determine the *in vivo* anticoccidial potential of essential oil of *Trachyspermum ammi* (seeds) in coccidiosis infected broiler chickens.

Material and Methods

Ethical Statement

This research was conducted with the ethical approval of ethics committee on 20/2/18 NO. DGS/3333-36

Trachyspermum ammi Essential Oil

Trachyspermum ammi essential oil was procured from the National® company from Faisalabad Pakistan that was 100% pure form. The essential oil was stored at 4ºC for proper usage in coccidiosis infected groups to monitor the efficiency of essential oil against naturally infected broiler chickens from coccidiosis.

Parasite Collection and Preservation

Poultry guts were collected from different sale points in Faisalabad. Positive guts cecal material were collected in 2.5% potassium dichromate solution. The parasite oocysts were recovered by sedimentation technique and different *Eimeria* species were identified under microscope. To ensure the purity of oocysts washed to remove debris in sodium hypochlorite solution. The washed oocysts were kept in potassium dichromate solution of 2.5% in incubator. To ensure the regular supply of oxygen proper string of solutions were maintained after every four hours. The standard temperature (35-39ºC) and humidity (60-80%) were maintained in the incubator for proper sporulation of coccidian oocysts [8]. After 48 to 72 h overall sporulation was noticed under light microscope at 40X and sporulated oocysts were separated and counted by McMaster technique. Then challenged dose (50.000/bird) of sporulated oocysts were prepared in order to induce coccidiosis in broiler chickens [9].

Birds Management

Day old 72 chicks were purchased from Big Bird Company® in Faisalabad Pakistan. The birds were reared under standard conditions. All chicks were given offered anticoccidial free feed. The first two weeks starter ration was given and next followed to finisher ration till seven weeks of age. The chicks were vaccinated for infectious bronchitis disease, Newcastle disease and infectious bursal disease according to standard vaccination schedule [10]. The temperature was maintained 90-95ºC (32.2 to 35ºC) in first week and maintained 75ºC (23.8ºC) at fourth week. The proper light intensity was maintained during 24 h till 42 days of age.

Experimental Design

At day 7th the all chicks were be divided into six equal groups A, B, C, D, E and F every one including 12 chicks. At 7th day all groups excluding F were orally immunized sporulated oocysts. The first three groups (A, B, C) were treated with the essential oil of *T. ammi* at concentrations of 1%, 2% and 3% respectively. Group D served as infected medicated (IM) and was treated with rational anticoccidial drug Toltrazuril. Group E served as infected non-medicated (INM) and Group F served as normal control group (non-infected, non-medicated) (NINM). The medication continued for three consecutive days with essential oils.

Evaluated Parameters

Following anticoccidial and growth performance parameters were evaluated in study.

Mortality Rate

In this parameter the death rate of chickens was determined. The death of chicken due to environmental stress, nutrition, disease outbreak or any other factor was recorded. The mortality rate was calculated by the overall number of dead birds/ live birds.

Fecal Score

The fecal score of birds from each group were monitored to estimate the disease intensity. Hence the standard fecal score chart was followed to estimate fecal score in chickens. From day 3rd to day 7th of post inoculation fecal score was estimated chart from 1 to 5 numbers in ascending order as described [10].

Oocyst per gram of feces (OPG)

McMaster technique was used to evaluate the number of oocysts per gram of feces. The OPG was performed on the 7th and 14th day of post infection. In this procedure 3 g feces were mixed in 42 mL saturated sodium chloride solution. After sieving the solution was poured in beaker. The 0.3 mL feces sample was poured in each chamber of Master. The McMaster chamber was kept undisturbed for 2 to 3 min. Then oocysts were examined in the chamber microscopically

Feed Conversion Ratio

The feed conversion ratio was determined by following formula

Feed conversion ratio = Average quantity feed consumed (gm)/Average weight gain (gm)

Weight Gain of Chicks

The average weekly weight gain of each group was recorded. The average weight gain was mentioned according to feed consumption. To access the disease Burdon, the average weight gain was calculated in each group in order to identify the medicinal response in controlling disease outbreak.

Hematology and Serum Chemistry

All blood parameters were checked after 35th day infection. Hematological parameters like red blood cells count (RBC), white blood cells (WBC) and hemoglobin (Hb) were estimated by standard protocols. The counting of RBC and WBC were done with the help of Sahlis apparatus. The hematological parameters like hemoglobin determination, monitoring of packed cell volume (PCV), counting of erythrocyte and leukocyte were done according to standard protocol [11]. Serum chemistry was also analyzed by using manufactured Kits.

Statistical Analysis

Different Parameters were statistically analyzed by ANOVA, SAS statistical analysis software (SAS, 2004) and data was considered significant at P≤0.05.

Results

The results of different parameters were discussed to evaluate the potential of plant driven essential oil of *Trachyspermum ammi* at concentration of 1%, 2% and 3% in feed. The mortality rate of different group is given in *Table 1*. *T. ammi* essential oil reduced mortality rate in infected birds and maximum survival rate was observed in birds treated with 3% of *T. ammi* essential oil which was significantly different to (P<0.05) infected group.

Table 2 shows that mean fecal score of *T. ammi* essential oil treated groups was significantly different to (P<0.05)

Table 1. *Mortality rate in in different treatment groups*

Treatment Groups	Mortality Days Post Infection					Total Mortality	Mortality %Age	Survival %Age
	3rd	4th	5th	6th	7th			
T. ammi 1%	1	-	-	-	–	1	0.083	0.92
T. ammi 2%	-	1		-	1	2	0.17	0.83
T. ammi 3%	–		1-	-	-	1	0.083	0.92
IM		-	1	-		1	0.083	0.92
INM	-	1		1	–	2	0.17	0.83
NINM	–	-	-	-	-	-	0	100

IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

Table 2. *Mean fecal score in different treated groups*

Treatment Groups	Fecal Score				
	3rd Day	4th Day	5th Day	6th Day	7th Day
T. ammi 1%	-	3.67 ± 0.33^{AB}	3.00 ± 0.00^{A}	2.67 ± 0.33^{A}	-
T. ammi 2%	-	3.00 ± 0.00^{B}	2.33 ± 0.33^{AB}	2.00 ± 0.00^{AB}	-
T. ammi 3%	-	1.67 ± 0.33^{C}	1.33 ± 0.33^{BC}	1.67 ± 0.33^{AB}	-
IM	-	1.33 ± 0.33^{C}	1.00 ± 0.00^{CD}	1.33 ± 0.33^{B}	-
INM	-	4.33 ± 0.33^{A}	3.33 ± 0.33^{A}	2.67 ± 0.33^{A}	-
NINM	-	0.00 ± 0.00^{D}	0.00 ± 0.00^{D}	0.00 ± 0.00^{C}	-

Means sharing similar letter in a column are statistically non-significant (P>0.05)
IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

Table 3. *Mean oocyst per gram of feces (OPG) in different treated groups*

Treatment	OPG (x 10^3 g-1) at Day 7th	OPG (x 10^3 g-1) at Day 14th
T. ammi 1%	77.37 ± 1.02^{B}	50.85 ± 2.01^{B}
T. ammi 2%	70.40 ± 0.61^{BC}	45.87 ± 0.87^{BC}
T. ammi 3%	65.75 ± 1.02^{C}	39.44 ± 1.17^{C}
IM	64.22 ± 1.01^{C}	39.45 ± 1.58^{C}
INM	95.70 ± 3.41^{A}	75.76 ± 2.52^{A}
NINM	0.00 ± 0.00^{D}	0.00 ± 0.00^{D}

Means sharing similar letter in a column are statistically non-significant (P>0.05)
IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

Table 4. *Feed conversion ratio (FCR) in different treated groups*

Treatment Groups	FCR
T. ammi 1%	2.41
T. ammi 2%	2.32
T. ammi 3%	2.21
IM	2.22
INM	2.61
NINM	2.20

IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

Table 5. *Mean weight gain (weekly basis) in different treated groups*

Treatment	WG (g) 1st WK PI	WG (g) 2nd WK PI	WG (g) 3rd WK PI	Total WG (g)
T. ammi 1%	207.33 ± 4.67^{C}	241.33 ± 5.36^{B}	366.00 ± 3.79^{A}	356.00 ± 5.20^{C}
T. ammi 2%	267.67 ± 4.63^{B}	262.00 ± 5.20^{AB}	351.33 ± 5.49^{A}	378.00 ± 4.93^{BC}
T. ammi 3%	296.00 ± 2.08^{A}	274.00 ± 2.65^{A}	354.00 ± 4.00^{A}	387.67 ± 4.63^{AB}
IM	301.67 ± 5.49^{A}	279.33 ± 5.49^{A}	358.00 ± 4.36^{A}	403.33 ± 2.03^{A}
INM	201.00 ± 5.51^{C}	197.67 ± 2.91^{C}	273.00 ± 2.08^{B}	292.33 ± 5.55^{D}
NINM	285.00 ± 3.21^{AB}	278.00 ± 4.62^{A}	347.67 ± 4.33^{A}	386.00 ± 5.51^{AB}

Means sharing similar letter in a column are statistically non-significant (P>0.05)
WG (g): *Weight in grams,* ***WKPI:*** *Week after Post infection*
IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

Table 6. *Mean Heamatological values in different treated Groups*

Treatment	PCV%	Hb g/dL	RBC 10*6/µL	WBC 10*3/µL
T. ammi 1%	27.63 ± 0.20^{AB}	12.83 ± 0.09^{A}	4.04 ± 0.04^{A}	24.84 ± 0.10^{B}
T. ammi 2%	28.23 ± 0.17^{A}	11.80 ± 0.10^{B}	3.28 ± 0.04^{C}	25.67 ± 0.41^{B}
T. ammi 3%	28.42 ± 0.27^{A}	12.77 ± 0.19^{A}	3.66 ± 0.01^{B}	22.16 ± 0.10^{C}
IM	27.25 ± 0.19^{B}	11.33 ± 0.17^{B}	2.82 ± 0.06^{D}	22.53 ± 0.17^{C}
INM	19.20 ± 0.10^{C}	11.17 ± 0.12^{B}	1.87 ± 0.04^{E}	34.00 ± 0.58^{A}
NINM	27.55 ± 0.23^{AB}	7.34 ± 0.10^{C}	3.36 ± 0.04^{C}	21.33 ± 0.33^{C}

Means sharing similar letter in a column are statistically non-significant (P>0.05)
IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

Table 7. *Mean serum enzymes values in different treated groups*

Treatment	ALT	AST	LDH	Urea	Creatinine
T. ammi 1%	10.23±0.15^{B}	174.37±2.99^{B}	478.00±6.08^{B}	5.32±0.10^{B}	0.16±0.01^{B}
T. ammi 2%	9.87±0.11B^{C}	168.00±4.36^{B}	462.00±7.23^{B}	5.50±0.14^{B}	0.15±0.01^{B}
T. ammi 3%	9.29±0.14B^{C}	174.00±3.51^{B}	468.00±9.50^{B}	5.42±0.11^{B}	0.16±0.00^{B}
IM	9.61±0.25B^{C}	181.67±2.73^{B}	477.00±5.51^{B}	5.29±0.10^{B}	0.17±0.01^{B}
INM	23.67±0.44^{A}	277.00±4.36^{A}	891.33±5.81^{A}	19.68±0.30^{A}	0.57±0.01^{A}
NINM	8.92±0.03^{C}	184.00±2.65^{B}	468.42±9.03^{B}	5.37±0.18^{B}	0.16±0.01^{B}

Means sharing similar letters in a column are statistically non-significant (P>0.05).
IM: *Infected medicated,* ***INM:*** *Infected non medicated,* ***NINM:*** *Non infected non medicated*

infected group. Mean fecal score was reduced in *T. ammi* essential oil treated groups at different days of post *Eimeria* infection in birds.

Table 3 shows that mean Oocyst per gram (OPG) of *T. ammi* essential oil of treated group was significantly different to (P<0.05) infected group. OPG was significantly reduced and minimum OPG was observed groups treated with 3% of *T. ammi* essential oil.

The *Table 4* clearly showed that essential oil treated groups exhibited improved feed conversion ratio (FCR) in chickens infected with mixed *Eimeria* species. Groups treated with *T. ammi* essential oil shown to improve FCR and better FCR was observed at higher dose of *T. ammi* essential oil.

T. ammi essential oil treated groups also improved mean weight gain as compared to infected group (P<0.05) *Table 5*. *Table 5* shows that weight gain was significantly improved in birds treated with *T. ammi* essential oil in dose dependent manner.

Table 6 shows that mean hematological parameters of natural oil treated group were improved was significantly different (P<0.05) from infected group. Mean PCV, HB, RBCs and WBCs values were improved which shows positive impact on hematological parameters in phase of infection.

Similarly, *T. ammi* essential oil improved serum chemistry parameters (ALT, AST, Urea, Creatinine) in infected birds which shows that it has no toxic effects on birds and these values which were significantly different (P<0.05) from infected group as shown in *Table 7*.

Discussion

Recent investigations about treating the coccidiosis showed that using herbal medicine having antioxidant compounds like flavonoids and phenols are batter choice to avoid the resistance [7]. Furthermore, botanical driven essential oils redirect the consideration of numerous analysts toward poultry industry for their helpful potential and therapeutic effects. There are a few reports that demonstrated the positive effect of natural compounds like essential oils for use them against different diseases and role in improvement of parameters like intestinal performance and feed conversion ratio in animals [12-14]. The *Trachyspermum ammi* and *Anethum graveolens* are both popular plants having medicinal values in controlling different infectious diseases and hence found in all over the world. The different studies proved that *T. ammi* (seeds) is an excellent remedy for the gastrointestinal problems including antimicrobial, antiviral, antifungal and antioxidant potential [15,16].

The *T. ammi* plant belong the *Apiaceae* family. The *T. ammi* is one of the most traditional medicinal plants known due to its wide therapeutic properties like antifungal, antibacterial, antihypertensive, antitussive, antioxidant, antispasmodic, bronchodilation, antifilarial, antiseptic, and anthelmintic potential [17]. The essential constituents found in the *T. ammi* seed are thymol and carvacol as well other fractions including p-cymene, g-terpiene and β-pinene [18]. The scientist proved that therapeutic and antiparasitic potential of essential oil of *T. ammi* is due to the presence of essential component like thyumolm and carvacol [19]. The essential oils are more effective as compare to synthetic drugs and are less toxic, no drug residues in poultry meat and can be used as multipurpose against various infectious disease like coccidiosis [20].

In an experimental trial the ethanolic extract of *T. ammi* was utilized to show the inhibitory action on different enzymes like lactate dehydrogenase that is the regulatory key of a parasitic nematode *H. contortus*. The ethanolic extract found to oppose the enzyme activity in favour of worm *H. contortus* in fovour to inhibit to the energy metabolic pathway [21]. Therefore, it was concluded that the prohibition of LDH enzyme, inhibition of protein synthesis and ATP prohibition potential of essential oil put a positive behavior toward the anthelmintic potential of essential oil of *T. ammi* oil. Hence the inhibition of LDH enzyme in maintenance of *H. contortus* proved that *T. ammi* has a main role to restrict the development of the worm [22]. The active constituents present in the essential

oil of *T. ammi* are thymol and carvacol have been used in different therapeutic potential to monitor the various infectious diseases of viral, bacterial, parasitic and to boost up immune potential of the host. The *T. ammi* essential oil components have the potential to fight against the various infectious agents of parasitic origin including filarial worms like *Setaria digitate*. Hence it was concluded that the methanolic extract of *T. ammi* having the therapeutic potential against the filarial worms [23,24].

The current study was planned by keeping in view the antiprotozoal potential of essential oil of *T. Ammi* in broiler chicken preciously infected from coccidiosis. The *in vivo* potential was measured in terms of improved weight gain, effective feed conversion ratio, decrease oocyst per gram of feces, reduced bloody diarrhea and better feed consumption ratio. The *T. ammi* treatment results in favorable anticoccidial potential in terms of substantial efficacy ($P<0.05$) from diseased non medicinal group and comparable to diseased medicinal group (G, D). The intake of essential oil of *T. ammi* favored a positive response toward the hematological values. The *T. ammi* having positive response in measuring serum biochemical values like AST, ALT, urea creatinine, LDH proved to be hepatoprotective and nephroprotective effect [25,26]

Hence, this study concludes that the supplementation of essential oil in broiler feed has given similar results as in the previous studies [27-29]. In this experimental trial the supplementation of essential oil of *T. ammi* was given to the broiler chickens infected from coccidiosis at a concentration of 1%, 2% and 3% in feed. The intake of essential oil of 3% in feed showed comparable results to treated groups with Toltrazuril medicine in controlling coccidiosis in terms of increased feed intake, improve weight gain, maintenance of intestinal gut health, decreased bloody diarrhea, decreased fecal scoring, and improved lesion scoring. The *T. ammi* treatment also put positive response in controlling coccidiosis in terms of hematological monitoring and serum biochemical values (ALT, AST Urea and Creatinine). Hence it was demonstrated that the treatment of coccidiosis with the essential oils of *T. ammi* oil is a likeable approach for the future prospective in poultry industry. Therefore the *T. ammi* essential oil 3% in feed can be used as best alternative for controlling coccidiosis disease in broiler chickens [30].

Declarations

Availability of Data and Materials: Data will be offered by the author (R. Fayyaz) on demand.

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Author Contributions: MRF intended the study; KH, AA, SS, SI, AR, SAR, MUW, RZA, HS, MK, WAH, MMM helped in methodology, work plan, and statistical analysis.

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