EFFECT OF DIETARY BLACK CUMMIN ON PERFORMACE AND MICROBIAL LOAD IN GUT OF KHAKI CMPBELL DUCK

A THESIS

BY

NIPA RANI PAL

REGISTRATION NO.: 1605482

THESIS SEMESTER: JAN-JUNE, 2018

SESSION: 2016-2017

MASTER OF SCIENCE (MS)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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JULY, 2018

DEDICATED
TO MY
BELOVED PARENTS

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The Author

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ABSTRACT

This experiment was aimed to investigate the effect of black cummin seed powder as an antibiotic on the performance of laying duck. In this study Ninety six (96) day old duck of similar body weight was divided into 4 groups and each group contained three replicates group and each treatment group consisted of 24 ducks and the time of experiment was starting from 180 day to 210 day. Treatment groups included T_0 (control), T_1 (1.5% black cummin seed powder), T_2(3% black cummin Powder). T_3 (6% black cummin powder). The result showed during 196 to 210 days of age. The significant higher body weight gain (1247.67gm) was found in duck fed diet containing 6% black cummin powder followed by bird received 3% (1209.33gm) 1.5%, (1201.00gm) and 0% (1170.67gm) black cumin powder. At the age of (180 to 185) days the highest number of egg found at the level of 6% black cummin seed T3 (2.40) at the day of (186 to 190) and (196 to210) days. At the age of 205 to 210 days the number of colony is also highly significant, less number of colony found T3 =30.33 ± 0.33 that treated with 6% black cummin seed.

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LIST OF ABBREVIATIONS AND SYMBOLS

-	= Negative
%	= Percentage
&	= And
+	= Positive
<	= Less-than or equal to
>	= Greater-than or equal to
°C	= Degree Celsius
mg	= Milligram
MR	= Methyl Red
n	= Number of isolates from each source
NA	= Nutrient Agar
NB	= Nutrient Broth
PBS	= Phosphate Buffer Saline
R	= Resistant
S	= Sensitive
Sl. No.	= Serial Number
sp.	= Species
SS	= Salmonella-Shigella Agar
TE	= Tetracycline
V-P	= Voges-Proskauer
WHO	= World Health Organization
µg	= Microgram
µl	= Microlitre
Contd.	= Continuation
E	= Erythromycin
E. coli	= *Escherichia coli*
e.g.	= Example
EMB	= Eosine Methylene Blue
et al.	= Et alia (associates)
etc.	= Etcetra
Fig.	= Figure
gm	= Gram
HSTU	=Hajee Mohammad Danesh Science and Technology
University	
i.e.	=That is
MS	=Master of Science
Prof.	= Professor
PCR	= Polymerase Chain Reaction

CHAPTER I
INTRODUCTION

Black cumin seed *(Nigella sativa)* supplements are consumed in many cultures for their hypolipidemic, antiplatelet and procirculatory effects (Amagase*elal.,* 2001). Black cumin containsmyristic acid palmitoloicacid, oleic acid linoleic acid, arachidonic acid and 'vitamins $B_{1,}$ B_2 B_3, calcium folate, iron copper, zinc and phosphorous. Black cumin seed eliminate harmful bacteria regenerating the body's cells and tissue. Many health expert claim that it is indeed a true panacea. Black cumin seed contain amino acids mostly glulatmicacid, agininenadastartic acid, cystina and methionine (Saleh AI Jassin 1992). Black cumin seed contain 94.29% dry matter. 23.80% CP, 42.08% EE, 7.71% CF, 5.10% CA,0.36% (Ca), 0.65% (P),0.28% (Na), 0.25% Magnesium and 0.79% Potassium. In addition to these benefits, some Black cumin seed preparations have been reported to possess hepatoprotective. Immune-enhancing, anticancer, antibacterial, antifungal and chemopreventive activities. Some preparations appear to be antioxidative, where others may simulate oxidation (Bradford S. weeks md January 6, 2 (15). In animal experiments, Black cumin seed decreased plasma lipid and Cholesterol in rabbits (ARYA Atheroseler, 2012). Black cumin supplementation into diet of the laying hen positively influences egg yield and decreased Cholesterol (Aydin R, *et al.,*poultsci 2008). In human experiments. Black cumin decreased cholesterol level in human (Green med Info LLC, 2014). Top 27 Scientific Health Benefit of the "panacea" Black cumin seed (Josepa M co her 20 17).Black cumin seed have pharmacological anti inflamatory effect (B.H. Ali Gerald Blunden 2003).

Research Objectives

In view of the above discussion, the present research work was undertaken with the following objectives:

- To investigate the effect of black cumin on productive and reproductive performance of laying duck (Khaki Campbell).
- To study the effect of black cumin on microbial load in cloacal content of laying duck.

CHAPTER II

REVIEW OF LITERATURE

Shrivastava *et al.*, (2017) was conduct a study to assess the effect of black cumin (Nigella sativa) oil on egg-yolk lipid profile of adult layer birds. Twenty four 40-weeks-old Jabalpur color birds were fed on 4 dietarytreatments. Birds were caged individually and diets were supplemented with 0 (control), 250, 500 and750 mg black cumin oil/kg of feed in group T_1, T_2, T_3 and T_4 respectively for 56 days. Egg yolk lipidprofile was estimated on 0, 14, 28, 42 and 56th day of feeding. The variation in egg-yolk total lipids,cholesterol and triglycerides between intervals within treatment were highly significant (P<0.01). Thestudy revealed that supplementation of black cumin oil @ 750 mg kg-1 feed is most effective in reducingthe egg yolk lipid profile in Jabalpur color birds fed for a period of 56 days.

Kassu *et al.*, (2016) the proximate analysis of the feed ingredient and additives used in the experimental diets presented. Fenugreek has a better composition of CP (46.70%), than black cumin and turmeric, while black cumin has higher CF (14.73%) and ash (12.3%) than fenugreek and turmeric, while, turmeric contained the lowest percent of CP (8.70%), CF (3.42%) and ash (7.04%), respectively. On the other hand, black cumin powder had the highest calculated metabolizable energy (4148.90 kcal/ kg). Higher energy level of black cumin could be due to higher fat level (36.88%) as compared to fenugreek (7.03%) and turmeric (9.63%), respectively. Values of live BW, BW gain (BWG), average daily gain (ADG) of the chicks fed on the experimental diets are shown. There were no significant differences among treatments in live BW during the starter and finisher phase (P 0.05).

El Bagir *et al.*, (2006)Laying hens were fed diets with 10 or 30 g of the whole seed of black cumin (*Nigella sativa*)/kg. The concentrations of total lipids, total cholesterol, phospholipids and triacylglycerols in serum and egg yolk were measured. Feeding of the diets with 1 and 3% black cumin seeds for a period of three months reduced egg yolk total cholesterol by 34 and 42%, respectively. Serum cholesterol concentrationsaveraged for the whole feeding period were lowered by 15 and 23% after feeding the diets with 1 and 3% black cumin seeds, respectively. Black cumin seeds in the diet of laying hens also caused a lowering of serum and egg-yolk concentrations of triacylglycerols and phospholipids. Inclusion of black cumin seeds in the diet caused a significant reduction in egg production, without any effect on egg

width and length, while there was a significant increase in hen's body weight. The increase in body weight in the hens fed blackcumin seeds is explained by the ingested feed energy not used for egg production. It is concluded that black cumin seeds and the active principle are of interest as potential egg-yolk cholesterol-lowering agents.

Guler *et al.,* (2006)Three hundred and sixty sexed 3-day-old broiler chicks were divided randomly into six treatment groups (control, antibiotic and black cumin at four levels) of 60 birds each. Black cumin seeds at 0.5%, 1%, 2% or 3% and avilamycin at 10 mg/kgt were added to the basal diet and their effects determined on feed intake, daily live weight gain, feed conversion ratio and carcass characteristics. There were no significant differences in daily feed intake at 21 and 42 days ($p>0.05$). Average daily gain was significantly different between the treatments. The birds fed the diet containing 1% black cumin seeds and antibiotic were the highest average daily gain, followed by those the other treatment diets and negative control ($p<0.05$). From 1 to 42 days of age, feed conversion ratios were improved significantly by supplementation with 1% black cumin seeds and with antibiotic ($p<0.05$) by approximately 5% compared to the control group. Similarly, the highest cold carcass, thigh, breast, wing, neck and liver weights were observed in the 1% black cumin and antibiotic groups ($p<0.05$). Accordingly, 1% supplementation of black cumin seeds to diets could be considered as an alternative natural growth promoter for poultry instead of antibiotics.

Mohammed *et al.,* (2016)conducted a studyin the use of herbal medicinal plants as feed additives to animal rations in order improve their productive, reproductive and therapeutic performances. Nigella sativa seeds and their purifed constituents have been shown benefcial effects in several studies on such aforementioned performances. Nigella sativa seed contains more than 100 compounds, some of which have not yet been identifed or explored. Te present review article addresses and discusses the effects of Nigella sativa seeds and their purifed constituents on productive, reproductive and therapeutic performances on mammals.

Hothaify *et al.,* (2016)conducted a study was conducted to investigate the effects of feeding low and high levels of *Nigella sativa* seed (NSS) as a natural substance on growth rate, carcass traits, some serum blood indices and antibody titers of broiler birds under the environmental conditions of Yemen. A total of one hundred eighties unsexed (day-old) chicks were housed into five equal groups and each group contains three replicates with 12 chicks each. There were 5 dietary treatments for feeding birds; first one was the control (T1),

without any natural substances and NSS were added in diets 2, 3, 4 and 5 (T2, T3, T4 and T5) at levels 0.25, 0.5, 1% and 2% respectively. Results at 5 weeks indicated that the addition of NSS up to 1% in the diets enhanced linearly body weight, weight gain and feed conversion ratio. The higher level of the seeds 2% not necessarily caused a higher degree of improvement. Birds fed diets containing 1% NSS (T4) achieved a higher ($P<0.05$) body weight and weight gain compared with the control (T1) and 0.25% NSS (T2) and better ($P<0.05$) feed conversion than other treatments. Similarly, the dietary treatment T4 (1% NSS) recorded the best ($P<0.05$) carcass dressing, breast and thigh percentage compared with the control. However, there were no significant variances in giblets and abdominal fat among all dietary groups. On the other hand, feeding birds' diets supplemented by diverse levels of NSS decreased blood total cholesterol. Dietary group T4 significantly increased ($P<0.05$) serum total protein and albumin as compared to the control group. Additionally, data showed that NSS treatments except T5 enhanced ($P<0.05$) the antibody titers against GD and ND virus.

Tahan *et al.,* (2011) carried out a study of determine the effect of utilization of black cumin *(Nigella sativa)* and dry parsley *(Petroselinum crispum)* in the diets on body weight, feed consumption, feed conversion ratio, egg production, egg quality (Haugh unit, eggshell thickness and, egg yolk cholesterol values) and hatchability in the laying quails. The experiment lasted 8 weeks and was performed on 210 laying quails, 140 females and 70 males at the age of 14 weeks. The quails were randomly allocated with 7 dietary treatments one as the negative control group without any feed additives (C); the others designed as 1.00 % black cumin (G1), 1.00 % parsley (G2), 1.50 % black cumin (G3), 1.50 % parsley (G4), 0.50 % black cumin + 0.50 % parsley (G5) and 0.75% black cumin + 0.75% parsley (G6) respectively. There were not statistically difference in body weight, among the groups, except female body weight ($p<0.05$). While mean feed intakes values did not differ, feed conversion ratio (FCR) values were different among the groups ($p<0.05$). There were no differences in egg production (%) egg weight, egg quality parameters (except the yolk color) and egg yolk cholesterol levels among the groups. Hatchability was found 88.0, 88.0, 68.0, 36.0, 68.0, 56.0, 88.0 %; for C, G1, G2, G3, G4, G5, G6 respectively ($p<0.05$). In conclusion, the use of black cumin and dry parsley as together in the layer quail rations as feed additives have a synergetic effect on body weight gain, egg production and hatchability. Their usage in combination could be profitable to improvement of performance of laying quail as the natural way.

Sohail *et al.*, (2012)evaluated the effect of 3 different levels (1.25, 2.5 or 5.0%) of black cumin seeds (BCS) on five hundred chicks. A basal diet was supplemented with either 0 (negative control), or 0.1% antibiotic (positive control), or 3 levels of BCS. At day 28 and 42 of age, the 2.5 and 5.0% BCS groups had significantly greater body weight gain (BWG) than the 1.25% BCS and the antibiotic group. The same groups had feed efficiency significantly improved ($P<0.05$) compared to the 1.25% BCS group and the controls. At both ages, measurement of the dressing percentage showed no marked variation between BCS supplementation and antibiotic. The 2.5 and 5.0% BCS groups showed anin crease ($P<0.05$) in total protein and higher ($P<0.05$) haematological values than the 1.25%, antibiotic or unsupplemented diet group. The activities of blood enzymes were lower ($P<0.05$) and caecal coliform and Escherichia coli populations decreased ($P<0.05$) in BCS and antibiotic groups. Serum and tissue cholesterol concentration decreased ($P<0.05$) as the levels of BCS increased. The geometric means haemagglutination inhibition (HI) titres of the BCS and the antibiotic group were always higher than the negative control. The mean lymphoid organs weight/body weight ratio of the negative control was significantly ($P<0.05$) lower than BCS and antibiotic groups. In conclusion, including up to 2.5 or 5.0% BSC in the diets of broilers has no deleterious effects on their performance, immunity, serum biochemical constituent's norhaematological indices. In fact, it may lead to the development of low-cholesterol chicken meat.

Shewita *et al.*, (2011)followingthis wayinvestigated the effect of dietary supplementation of different levels of black seed (Nigella sativa L.) on the performance and immune response of broiler chicks. A total 240 day-old broiler chicks were used and randomly allotted equally into six experimental groups designated as 1, 2, 3, 4, 5 and 6 having black seed at the rate of 0, 2, 4, 6, 8 and 10 g /kg diet respectively. The study was lasted for 42 days. Average body weight, weight gain, relative growth rate, feed conversion, antibody titer against Newcastle disease, phagocytic activity and phagocytic index, some blood parameters(GOT, GPT, Glucose, Cholesterol, Triglyceride, Total protein, Albumen, WBCs, RBCs, Hb and PCV), dressing percentage, weight of different body organs, abdominal fat weight, were determined. It was found that, *N. sativa* significantly improved final body weight, total body gain and feed conversion ratio of groups 2 and 3 when compared with the control group. Higher levels of *N. sativa* did not improve growth performance of the chicks. Non significant differences were observed for antibody titer against Newcastle virus, WBCs count, serum GOT, glucose level, dressing %, relative liver, spleenand heart and head percentages. Lymphoid organs (Bursa

and Thymus) improved significantly with increasing *N. sativa* level in all supplemented groups. Serum cholesterol, triglyceride and visible fat % significantly decreased with Nigella sativa supplementation while serum GPT level significantly increased with nigella sativa supplementation.

Longato *et al.*,(2015)reported thatNS is considered one of the most important medicinal plants in the world. Its seeds have many therapeutic effects, including antimicrobial, anticoccidial and anthelminthic activities, most of which are due to the presence of thymoquinone, which is the major bioactive component. NS seeds are also a significant source of proteins, carbohydrates and fatty acids, and thus could be added as an ingredient to formulate balance rations for farm animals. NS had positive effects on productive and reproductive performances, mortality rate, digestibility, blood chemistry parameters, milk yield and composition, compositional characteristics of eggs and carcass traits.

Hossain *et al.*, (2016) investigated the effects of different levels of black cumin seeds *(Nigella sativa L.)* on egg production and cholesterol concentration in egg yolk of laying hens. A total of 60 commercial layer strain day old layer chicks were collected and divided into three groups treated with 1.0%, 1.5% and 2.0% black cumin inclusion. The concentrations of total lipids, total cholesterol, phospholipids and triacylglycerols in serum and egg yolk were measured. Feeding of the diets with 1%, 1.5% and 2% black cumin seeds during the laying period found egg yolk cholesterol by 11.12, 9.88 and 9.83 mg/g respectively. The results found that feed efficiency ratio, egg production, body weight, feed intake and egg weight were nonsignificant between the treatments. However, egg yolk cholesterol concentration was found that 1.5% and 2.0% black cumin in diet were reduced cholesterol concentration insignificance ($P<0.05$). So, dried black cumin supplementation in diets had no any adverse effect on egg production and egg weight. Furthermore, egg yolk cholesterol concentrations were decreased. Hence, it is concluded that black cumin (*Nigella sativa L.*) seeds and/or the active principle are of interest as potential egg-yolk cholesterol-lowering agents.

Azeem *et al.*, (2014)incidences of antibiotic residues and drug resistance against pathogenic organism are common due to inclusion of antibiotics in poultry diet. It is the dire need of the time to use natural and effective alternative to synthetic antibiotics. Nigella sativa (black cumin) seed could be the most suitable alternative to antibiotics in poultry nutrition. Nigella

sativa not only promote bird's health and production performance, but also plays a significant role as a natural antioxidant and immuno-stimulant. The polyunsaturated fatty acids share is almost double than mono-unsaturated fatty acids in oil content of black seed, so it reduces the total cholesterol content. The bioactive compounds in black cumin are anticancerous. The present review describes the natural beneficial effect of Nigella sativa on poultry health and production when used in poultry diet.

Aydin *et al.,* (2008)the objective of this study was to determine the effects of various levels of dietary black cumin seed on egg production, egg weight, feed conversion ratio, egg shell quality, and egg yolk cholesterol. In this study, eighty 27-wk-old laying hens (Hyline-5 White) were randomly assigned into 4 groups with 4 replicates of 5 birds each (20 laying hens per group) and fed diets supplemented with 1, 2, or 3% black cumin. Eggs were collected and weighed daily. Laying performance, egg quality, and feed conversion ratio were evaluated. Laying hens fed the diet supplemented with 3% black cumin had greater egg production than the control. Diets supplemented with 2 or 3% black cumin increased egg weight compared with other groups. Yolk weights of the eggs from hens fed diets containing 1, 2, and 3% black cumin were significantly greater than those from the control group. Shell thickness of the eggs from chickens fed 2 or 3% black cumin seed was significantly greater than those from chickens fed diets supplemented with 0 or 1% black cumin seed. Also, shell strength of the eggs from hens fed diets supplemented with 3% black cumin seed was significantly greater than the control. In addition, diets supplemented with 2 or 3% black cumin significantly decreased egg cholesterol per gram of yolk compared. No level of black cumin seed supplementation had any effect on live weight, feed consumption, feed conversion ratio, organ weights, and abdominal adipose tissue. This study showed that black cumin at the level of 2 or 3% would positively influence egg production, egg weight, and shell quality and decrease the concentration of cholesterol in the egg yolk.

Khan *et al.,* (2013)body condition score (BCS) at all levels revealed greater egg production, egg weight and egg mass than the control group. Similarly, feed conversion was improved by 0.50, 0.55 and 0.36 points compared to controls with the addition of 3%, 4% or 5% levels of BCS, respectively. Haugh units and yolk weights of eggs from hens that were fed diets containing 3%, 4% and 5% BCS were significantly greater than those from the control group. Supplementation of 4% or 5% BCS significantly increased shell thickness and decreased serum LDL cholesterol and egg yolk cholesterol concentration as compared to other groups.

Mean hemagglutination inhibition titers against Newcastle disease virus were higher than in controls.

Rahman *et al.*, (2016)evaluated the effects of *Nigella sativa* seeds (NS) supplementation on meat quality and antioxidants content of chicken meat. Two hundred 1-d-old male broiler chicks were divided into four diet treatment groups: normal control (baseline feed only), NS supplemented (1 and 2%) groups and a standard (200IU/kg vitamin-E) VE group. At the end of this period, 10 birds were randomly selected from each group for examination. Feed conversion ratio was significantly lower in the treatment groups than the control one. There were no significant differences in moisture or crude ash percentage in thigh muscle among groups, but dietary NS powder supplementation resulted in a significant increase in crude protein content and decrease in crude fat content relative to the control group (p<0.05). After 24 h, thigh muscle pH was higher while drip loss, cooking loss and shear force were lower in the NS groups than the control group.

Santamaría *et al.*, (2002)vertebrates are important seed dispersers for many plants. In addition to transport of seeds, ingestion often affects the proportion or rate of seed germination. We present one of the first studies comparing the effects of different waterbird species on the seeds of a subcosmopolitan pondweed, Potamogetonpectinatus. We also present the first comparison of the effects of digestion by ducks (mallard Anasplatyrhynchos, shoveler A. clypeata and wigeon A. penelope) and physical-chemical "simulation of digestion" on pondweed seed germination. In two experiments differing in the length of the preceding stratification period, two to three individuals per duck species were force-fed 150 seeds each. Average retrieval, total germination and germination rate did not differ significantly between duck species. Germination rate was higher for duck ingested seeds, intermediate for scarified seeds (i.e. after mechanical removal of the epicarp+mesocarp) and lowest for the controls and acid treated seeds, independently of the length of the stratification period. Total germination, however, did not differ significantly among duck-ingested, scarified, control and acid treated seeds. Consequently the changes in germination rate after ingestion by ducks seem related to the grinding treatment in the gut and unrelated to exposure to acidic conditions. The co- existence of ingested and uningested seeds within a given seed cohort will increase the diversification of seed germination patterns, which can favour the colonisation of habitats characterised by unpredictable environmental conditions.

El-Bahr *et al.*, (2014)the present findings showed that the control rats (group I) gained weight over the six weeks of the experimental period, with the mean body weight increasing by 60 grams. Moreover, the untreated diabetic rats (group II) lost an average of 30 grams of their weight after six weeks (P0.05). When these diabetic rats treated with Turmeric alone, they did not lost their weight but gained 25 grams of body weight which represented 41.7% of weight gained by normal non diabetic control rats. Also, diabetic rats treated with BTM did not lost their weight but gained 20 grams of body weight which represented 33.3% of weight gained by normal non diabetic control rats. Loss of body weight observed in non-treated diabetic rats was improved by 50% when they treated with Black cumin seed only.

Kokoska *et al.*, (2008) *Nigella sativa L*. seed essential oils obtained by hydrodistillation (HD), dry steam distillation (SD), steam distillation of crude oils obtained by solvent extraction (SE-SD), and supercritical fluid extraction (SFE-SD) were tested for their antibacterial activities, using the broth microdilution method and subsequently analyzed by gas chromatography and gas chromatography– mass spectrometry. The results showed that the essential oils tested differed markedly in their chemical compositions and antimicrobial activities. The oils obtained by HD and SD were dominated by *p*-cymene, whereas the major constituent identified in both volatile fractions obtained by SD of extracted oils was thymoquinone (ranging between 0.36 and 0.38 g/ml, whereas in oils obtained by HD and SD, it constituted only 0.03 and 0.05 g/ml, respectively). Both oils distilled directly from seeds showed lower antimicrobial activity (MIC 256 and 32 g/ml for HD and SD, respectively) than those obtained by SE-SD and SFE-SD (MICs 4 g/ml). All oil samples were significantly more active against gram-positive than against gramnegative bacteria. Thymoquinone exhibited potent growth-inhibiting activity against gram-positive bacteria, with MICs ranging from 8 to 64 g/ml.

Milica *et al.*, (2016)the essential oil content in cumin samples from Serbian market ranged between 2.0 and 4.0%, with 22 identifed compounds, among which the most abundant were cumin aldehyde, pentene, terpinene, terpinene-7-al and p-cymene. Postdistillation cumin seeds waste material that remained after the essential oil extraction contains total polyphenols of between 30.1 and 47.5 mg GAE/g dry extract, as estimated by the FolinCiocalteu method. Hydroxybenzoic and hydroxycinnamic acids, as well as glycosides of avonones and avonoles, are the dominant polyphenols. However, according to DPPH method, the antioxidative potential of cumin postdistillation seeds waste was poor and it ranged between

0.02 and 0.04 mM TE/g. Further research will be focused on agro-food implementation of postdistillation waste material of cumin and other plants which are used for the essential oil production.

Properties of Black Cummin Seed

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Moreover, medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Many researchers are focusing on medicinal plants since only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies.

Fig: 1. Black cummn Plant

Fig: 2. Blank cummin seed powder

Among various medicinal plants, *Nigella sativa*(Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. *N. sativa* is commonly known as black seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia.

The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. And it is an important drug in the Indian traditional system of medicine like Unani and Ayurveda. Among Muslims, it is considered as one of the greatest forms of healing medicine available due to it was mentioned that black seed is the

remedy for all diseases except death in one of the Prophetic hadith. It is also recommended for use on regular basis in Tibb-e-Nabwi (Prophetic Medicine).

N. sativa has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities viz. as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintics, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties. The seeds of *N. sativa* are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. Black seeds are also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity.

The results showed that stimulation of OT-1 (transgenic CD+) T cells with OVA antigen resulted in activation, as shown by a decrease in the surface expression of CD62L which coincided with significant apoptosis measured three and five days after antigen stimulation. Addition of low concentrations of TQ during CD85+ T-cell activation resulted in enhanced survival of the activated T cells and sustained expression of CD62L. These effects coincided with enhancement in the capability of CD8+ T cells to produce the effector cytokine interferon-gamma (IFN gamma). This is concluded that TQ has a beneficial effect in conditioning T cells in vitro for adoptive T-cell therapy against cancer and infectious disease. The cytotoxic effects of different *N. sativa* seed extracts as an adjuvant therapy to doxorubicin on human MCF-7 breast cancer cells was reported. The study showed *N. sativa* lipid extract is cytotoxic to MCF-7 cells with LC50 of 2.720 ± 0.232 mg/mL, while its aqueous extract cytotoxicity exhibited when the applied concentration is high as about 50 mg/mL.

The antitumor and anti-angiogenic effects of TQ on osteosarcoma in vitro and in vivo were investigated. Results showed that TQ induced a higher percentage of growth inhibition and apoptosis in the human osteosarcoma cell line SaOS-2 compared to that of control, and TQ significantly blocked human umbilical vein endothelial cell tube formation in a dose-

dependent manner. It was found that TQ significantly downregulated NF-□B DNA-binding activity, XIAP, survivin and VEGF in SaOS-2 cells. Moreover, the expression of cleaved caspase-3 and Smac were upregulated in SaOS-2 cells after treatment with TQ. It was also found that TQ inhibits tumor angiogenesis and tumor growth through suppressing NF-κB and its regulated molecules. It was concluded that TQ effectively inhibits tumor growth and angiogenesis both in vitro and in vivo. Therefore, inhibition of NF-κB and downstream effector molecules is a possible underlying mechanism of the antitumor and anti-angiogenic activity of TQ in osteosarcoma.

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effector molecules is a possible underlying mechanism of the antitumor and anti-angiogenic activity of TQ in osteosarcoma.

Route from the first day until the end of the study. Proximal colon and ileum were excised for histopathologic, apoptosis (TUNEL) and biochemical evaluation, including xanthine oxidase, SOD, GSH peroxidase (GSH-Px), MDA, and MPO activities. Pups in the NEC+NOS group had better clinical sickness scores and weight gain compared to the NEC group ($P<0.05$). In the macroscopic assessment, histopathologic and apoptosis evaluation (TUNEL), severity of bowel damage was significantly lower in the NEC+NOS group compared to the NEC group ($P<0.05$). Tissue GSH-Px and SOD levels were significantly preserved in the NEC+NSO group ($P<0.05$), whereas, tissue MDA, MPO levels of the NEC+NSO group were significantly lower than those in the NEC group ($P<0.05$). It is concluded that NSO significantly reduced the severity of intestinal damage in NEC. A study was designed to determine whether treatment with TQ prevents and ameliorates colonic inflammation in a mouse model of inflammatory bowel disease. C57BL/6 murine colitis was induced by the administration of dextran sodium sulfate (DSS) (3% W/V) in the drinking water supplied to the mice for 7 consecutive d. The mice with colitis were treated with 5, 10, or 25 mg/kg TQ orally, and changes in body weight and macroscopic and microscopic colitis scores were examined. In addition, biochemical analyses were conducted. The treatment of mice with TQ prevented and significantly reduced the appearance of diarrhea and body weight loss. These results were associated with amelioration of colitis-related damage, as measured by macroscopic and microscopic colitis scores. In addition, there was a significant reduction in colonic myeloperoxidase activity and malondialdehyde levels and an increase in glutathione levels. These results indicate that TQ administration can prevent and improve murine DSS-induced colitis. These findings suggest that TQ could serve as a potential therapeutic agent for the treatment of patients with inflammatory bowel disease.

The cytotoxicity of TQ in human cervical squamous carcinoma cells (SiHa) was investigated. TQ was cytotoxic towards SiHa cells with IC50 values of 10.67±0.12 and 9.33±0.19 µg/mL as determined by MTT assay and trypan blue dye exclusion test, respectively, after 72 h of incubation. TQ was found to be more cytotoxic towards SiHa cells compared to cisplatin. Interestingly, TQ was less cytotoxic towards the normal cells (3T3-L1 and Vero). Cell cycle analysis performed by flowcytometer showed a significant increase in the accumulation of TQ-treated cells at sub-G1 phase, indicating induction of apoptosis by the compound. TQ

was more potent than cisplatin in elimination of SiHa cells via apoptosis with down-regulation of Bcl-2 protein.

The anticancer effects of TQ on breast cancer cells, and its potential effect on the PPAR-□ activation pathway was investigated and it was found that TQ exerted strong anti-proliferative effect in breast cancer cells and when TQ combined with doxorubicin and 5-fluorouracil, cytotoxicity was found to be increased. TQ was found to increase sub-G1 accumulation and annexin-V positive staining, indicating apoptotic induction. In addition, TQ activated caspases 8, 9 and 7 in a dose-dependent manner. Migration and invasive properties of MDA-MB-231 cells were also reduced in the presence of TQ. Interestingly, TQ was found to increase PPAR-γ activity and down-regulate the expression of the genes for Bcl-2, Bcl-xL and survivin in breast cancer cells. More importantly, the increase in PPAR-γ activity was prevented in the presence of PPAR-γ specific inhibitor and PPAR-γ dominant negative plasmid, suggesting that TQ may act as a ligand of PPAR-γ. It was observed by using molecular docking analysis that TQ indeed formed interactions with 7 polar residues and 6 non-polar residues within the ligand-binding pocket of PPAR-γ that are reported to be critical for its activity. Thus, it was concluded that TQ may have potential implication in breast cancer prevention and treatment and anti-tumor effect of TQ may also be mediated through modulation of the PPAR-γ activation pathway. It was also revealed in a study of the assessment of the chemo-preventive potential of crude oils in *N. sativa* on tumor formation using a well-established rat multi-organ carcinogenesis model featuring initial treatment with five different carcinogens that post-initiation administration of 1 000 or 4 000 mg/L *N. sativa* volatile oil in the diet of male Wister rats for 30 weeks significantly reduced malignant and benign colon tumor sizes, incidences and multiplicities. The treatment also significantly decreased the incidences and multiplicities of tumors in the lungs and in different parts of the alimentary canal, particularly the esophagus and fore stomach. It was shown that *N. sativa* administration exerts potent inhibitory effects on rat tumor development and on cellular proliferation in multiple organ sites like colon, lung, esophageal and fore stomach tumors in the post-initiation phase with no evidence of clinical side effects. The potential immuno-modulatory effects of *N. sativa* are investigated in light of splenocyte proliferation, macrophage function, and NK anti-tumor activity using BLAB/c and C57/BL6 primary cells. NK cytotoxic activity against YAC-1 tumor cells was examined by JAM assay. The study demonstrated that the aqueous extract of *N. sativa* significantly enhances

splenocyteproliferation in a dose-responsive manner. It was also evident that the aqueous extract of *N. sativa* significantly enhances NK cytotoxic activity against YAC-1 tumor cells. The effect of TQ on pancreatic cancer cells and its effect on MUC4 expression were investigated. The MUC4-expressing pancreatic cancer cells FG/COLO357 and CD18/HPAF were incubated with TQ, and in vitro functional assays were also done. The results indicated that treatment with TQ down regulated MUC4 expression through the proteasomal pathway and induced apoptosis in pancreattirucallol, 3-O- [β-D-xylopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabino-pyranosyl] -28-O-[α-L-rhamnopyranosyl (1→4) -α-D-glucopyranosyl (1→6) -α-D-gluco-pyranosyl] hederagenin, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol, β-unsaturated hydroxy ketone, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidalsaponin, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamno-pyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methy-lolean-12-enoate, stigma-5, 22-dien-3-β-D-gluco-pyranoside, cycloart-23-methyl-7, 20, 22-triene-3β, 25-diol, nigellidine-4-O-sulfite, N. mines A3, A4, A5, C, N. mines A1, A2, B1, and B2.

2.1 Pharmacognostical Characteristics

2.1.1 Morphology of the plant

N. sativa is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds.

2.1.2 Scientific researches and pharmacological potentials

The extensive researches using modern scientific techniques were carried out by various researchers on *N. sativa* since it is believed to be a miraculous herb that can cure multiple ailments and disorders. A number of pharmacological actions of *N. sativa* have been investigated in the past few decades.

2.1.3 Antibacterial activity

The antibacterial effect of ground black seeds was studied in a modified paper disc diffusion method. A clear inhibition of the growth of Staphylococcus aureus was observed by

concentration of 300 mg/mL with distilled water as control, this inhibition was confirmed by using the positive control Azithromycin. The inhibition obtained was higher with *N. sativa* ground seeds from Hadramout than with *N. sativa* ground seeds from Ethiopia. The positive inhibition may be attributed to the two important active ingredients of N. sativa, TQ and melanin. Different crude extracts of *N. sativa* were tested for antimicrobial effectiveness against different bacterial isolates which comprised of 16 gram negative and 6 gram positive representatives. These isolates showed multiple resistances against antibiotics, specially the gram negative ones. Crude extracts of *N. sativa* showed a promising effect against some of the test organisms. The most effective extracts were the crude alkaloid and water extracts. Gram negative isolates were affected more than the gram positive ones. Antibacterial activity of *N. sativa* against clinical isolates of methicillin resistant Staphylococcus aureus was investigated in 2008 by Hannan et al. All tested strains of methicillin resistant Staphylococcus aureus were sensitive to ethanolic extract of *N. sativa* at a concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/mL. Antibacterial activity of *N. sativa* against and triple therapy in eradication of Helicobacter Pylori in patients with non-ulcer dyspepsia was carried out. It was showed that *N. sativa* seeds possess clinically useful anti H. pylori activity, comparable to triple therapy. The antibacterial activity of TQ and its biofilm inhibition potencies were investigated on 11 human pathogenic bacteria. TQ exhibited a significant bactericidal activity against various human pathogenic bacteria especially Gram positive cocci (Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis CIP 106510). TQ prevented cell adhesion to glassslides surface.

2.1.4 Characteristics of the seeds and powder

Macroscopically, seeds are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5mm×1-2 mm, black externally and white inside, odor slightly aromatic and taste bitter. Microscopically, transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick walled rectangular elongated or nearly columnar, elongated cells. Endosperm consists of thin walled, rectangular or polygonal cells mostly filled with oil globules. The powder microscopy of seed powder shows brownish black, parencymatous cells and oil globules.

2.1.5 Chemical composition of black seeds

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpenelongifolene (1%-8%) α-pinene and thymol etc. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; i.e. isoquinoline alkaloids e.g. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent.

Some other compounds e.g. carvone, limonene, citronellol were also found in trace amounts. Most of the pharmacological properties of *N. sativa* are mainly attributed to quinine constituents, of which TQ is the most abundant. On storage, TQ yields dithymoquinone and higher oligocondensation products. The seeds of *N. sativa* contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8 %). The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe etc. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid.

The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. □-sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigmasterol (6.57-20.92% of total sterols).

Examples of various other reported chemical components includes nigellone,avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol,, gramisterol, lophenol, obtusifoliol, stigmastanol, stigmasterol-7-ene, β -amyrin, butyro-spermol, cycloartenol, 24-methylene-cycloartanol, taraxerol.

2.1.6 Antifungal activity

Methanolic extracts of *N. sativa* have the strongest antifungal effect followed by the chloroform extracts against different strains of Candida albicans. Aqueous extracts showed no antifungal activity. An intravenous inoculum of Candida albicans produced colonies of the organism in the liver, spleen and kidneys. Treatment of mice with the plant extract 24 h after the inoculation caused a considerable inhibitory effect on the growth of the organism in all organs studied. Khan et al. in 2003 reported that the aqueous extract of *N. sativa* seeds exhibits inhibitory effect against candidiasis in mice. A 5-fold decrease in Candida in kidneys, 8-fold in liver and 11-fold in spleen was observed in the groups of animals post-treated with the plant extract. These findings were also confirmed by Histopathological examination of the respective organs. Antidermatophyte activity of ether extract of *N. sativa* and TQ was tested against eight species of dermatophytes: four species of Trichophyton rubrum and one each of Trichophyton interdigitale, Trichophyton mentagrophytes, Epidermophytonfloccosum and Microsporumcanis using Agar diffusion method with serial dilutions of ether extract of N. sativa, TQ and griseofulvin. The MICs of the ether extract of *N. sativa* and TQ were between 10-40 and 0.125-0.250 mg/mL, respectively, while those of griseofulvin ranged from 0.00095 to 0.01550 mg/mL. These results denote the potentiality of *N. sativa* as a source for antidermatophyte drugs and support its use in folk medicine for the treatment of fungal skin infections. The antiyeast activity of the black cumin seed quinines, dithymoquinone, thymohydroquinone, and TQ were evaluated in vitro with a broth microdilution method against six dairy spoilage yeast species. It was found that Antifungal effects of the quinones were compared with those of preservatives commonly used in milk products (calcium propionate, natamycin, and potassium sorbate) at two pH levels (4.0 and 5.5), while thymohydroquinone and TQ possessed significant antiyeast activity. Two novel antifungal defensins named Ns-D1 and Ns-D2, were isolated from seeds of *N. sativa* and sequenced. The Ns-D1 and Ns-D2 defensins displayed strong divergent antifungal activity towards a number of phytopathogenic fungi.

2.1.7 Anti-schistosomiasis activity

The effect of NSO against the liver damage induced by Schistosoma mansoni (S. mansoni) infection in mice was studied by Mahmoud et al. When the NSO was given alone, it reduced the number of S. mansoni worms in the liver and decreased the total number of ova deposited in both the liver and the intestine. When NSO was administered in combination with PZQ, the most prominent effect was a further lowering in the dead ova number over that produced

by PZQ alone. Infection of mice with S. mansoni produced a pronounced elevation in the serum activity of ALT, GGT, with a slight increase in AP level, while reduce serum albumin level. Administration of NSO succeeded partially to correct the previous changes in ALT, GGT, AP activity, as well as the Alb content in serum. These results suggest that NSO may play a role against the alterations caused by S. mansoni infection. Results of in vitro testing of *N. sativa* seeds against Schistosoma mansoni, miracidia, cercariae, and adult worms indicate its strong biocidal effects against all stages of the parasite and an inhibitory effect on egg-laying of adult female worms. *N. sativa* seeds also induced an oxidative stress against adult worms which indicated by a decrease in the activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase and enzymes of glucose metabolism, hexokinase and glucose-6-phosphate dehydrogenase. Disturbing of such enzymes of adult worms using *N. sativa* seeds could in turn render the parasite vulnerable to damage by the host and may play a role in the anti-schistosomal potency of the *N. sativa* seed.

2.1.8 Antioxidant activity

The antioxidant and antiarthritic activity of TQ in Wistar rat by collagen induced arthritis was evaluated. TQ was administered at a dose of 5 mg/kg body weight once daily for 21 d. The effects of treatment in the rats were assessed by biochemical (articular elastase, myeloperoxdase (MPO), LPO, glutathione (GSH), catalase (CAT), SOD and NO), inflammatory mediators [IL-1 β, IL-6, TNF-α, IL-10, IFN-γ and PGE(2)] and histological studies in joints. TQ was effective in bringing significant changes on all the parameters (articular elastase, MPO, LPO, GSH, CAT, SOD and NO) studied. Oral administration of TQ resulted in significantly reduced the levels of pro-inflammatory mediators [IL-1β, IL-6, TNF-α, IFN-γ and PGE]and increased level of IL-10. The antioxidant, anti-inflammatory, anticancer and antibacterial activities of the shoots, roots and seeds methanol extracts from *N. sativa* were studied. The three organs exhibited strong antioxidant activity using the oxygen radical absorbance capacity method and a cell-based assay. TQ has been shown to suppress the Fe-NTA-induced oxidative stress, hyperproliferative response and renal carcinogenesis in Wistarrats. It was suggested that dietary supplementation of black seeds powder inhibits the oxidative stress caused by oxidized corn oil in rats. It was also reported that oral feeding of the diet containing black seed powder at 10% level antagonized the oxidative stress effects induced by hepato-carcinogens like dibutylamine and Sodium Nitrate ($NaNO_3$) in Swiss

albino rats by normalizing GSH and NO levels. The black seed oil and TQ by intraperitonial injection were found to shown protective effects on lipid peroxidation process during ischemia-reperfusion injury (IRI) in rat hippocampus. Treating broiler chicks with black seed for 6 weeks prevented the liver from oxidative stress by increasing the activities of enzymes such as myeloperoxidase, glutathione-S-transferase, CAT, adenosine deaminase, myeloperoxidaseandbydecreasing hepatic lipid peroxidation. The crude methanolic extract of black cumin seed cake was found to shown with significant antioxidant properties under in vitro systems.The modulatory effect of TQ on erythrocyte lipid peroxidation and antioxidant status during 1,2-dimethylhydrazine- (DMH-) induced colon carcinogenesis after initiation in male Wistar rats was investigated and The TQ pre-treatment restored the increased level of malondialdehyde and conjugated diene levels, and an augmentation of enzyme activities like CAT, glutathione peroxidase and SOD activities due to exposure to DMH. TQ was a useful compound preventing DMH-induced erythrocyte damages.*L.M. Aziz-Zadeh* (2016).

2.1.9 Anti-inflammatory and analgesic activity

The aqueous extract of *N. sativa* was found to possess anti-inflammatory and analgesic but not antipyretic activities in animal models while anti-inflammatory effect of the alcoholic extracts of *N. sativa* seeds and its callus on mix glial cells of rat with regard to their TQ content was investigated. The mix glial cells, inflamed by lipopolysaccharide, were subjected to anti-inflammatory studies in the presence of various amounts of TQ and the alcoholic extracts. Results confirmed that TQ content of the callus of leaf was 12 times higher than that measured in the seeds extract. Studies on the inflamed rat mix glial cells revealed significant reduction in the nitric oxide production in the presence of 0.2 to 1.6 mg/mL of callus extract and 1.25 to 20 µL/mL of the seed extracts. Osteoporosis has been linked to oxidative stress and inflammation. The studies on the anti-osteoporotic effects of *N. sativa* and TQ were carried out. It was revealed that *N. sativa* and TQ were shown to inhibit inflammatory cytokines such as interleukin-1 and 6 and the transcription factor, nuclear factor κ B. Both NS and TQ have shown potential as anti-osteoporotic agent.

Inflammation has been identified as a significant factor in the development of solid tumour malignancies. Studies show that TQ induced apoptosis and inhibited proliferation in pancreatic ductal adenocarcinoma (PDA) cells. The anti-inflammatory potential of TQ in PDA cells was evaluated in comparison with that of a specific histone deacetylase (HDAC) inhibitor, trichostatin A. The effect of TQ on the expression of different pro-inflammatory

cytokines and chemokines was analyzed by real-time polymerase chain reaction. TQ dose and time-dependently significantly reduced PDA cell synthesis of MCP-1, TNF-alpha, interleukin (IL)-1 β and Cox-2. At 24 h, Tq almost completely abolished the expression of these cytokines. TQ also increased p21 WAF1 expression, inhibited HDAC activity, and induced histone hyperacetylation. HDAC inhibitors have been shown to ameliorate inflammation-associated cancer. TQ as a novel inhibitor of proinflammatory pathways provides a promising strategy that combines anti-inflammatory and proapoptotic modes of action. TQ exhibit a slight inhibitory effect on COX-1 expression and PGE2 production in a mouse model of allergic airway inflammation. This finding suggests that TQ has an anti-inflammatory effect during the allergic response in the lung through the inhibition of PGD2 synthesis and Th2-driven immune response. The antioxidant, anti-inflammatory, anticancer and antibacterial activities of the shoots, roots and seeds methanol extracts from *N. sativa* were studied. The seeds hexane fraction of the methanol extract showed significant anti-inflammatory activity, inhibiting nitric oxide release with an IC50 value of 6.20 μg/mL in lipopolysaccharide-stimulated RAW 264.7 macrophages. A clinical trial study was conducted as prospective and double blind with descriptive analytic to investigate the anti-inflammatory effects of *N. sativa* in patients with allergic rhinitis symptoms. The sample included 66 patients (case and placebo) with allergic rhinitis exposed to *N. sativa* oil. Individual characteristics, including age and sex, and characteristics of the disease, including nasal congestion, runny nose, itchy nose, and sneezing attacks, were evaluated for a period of 30 d The results show that *N. sativa* could reduce the presence of the nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor during the first 2 weeks (day 15). The anti-allergic effects of *N. sativa* components could be attributed to allergic rhinitis. Moreover, *N. sativa* should be considered for treating allergic rhinitis when the effects of other anti-allergic drugs need to be avoided.

2.1.10 Immunomodulatory activity

The potential immunomodulatory effects of *N. sativa* were investigated in light of splenocyte proliferation, macrophage function, and NK anti-tumor activity using BLAB/c and C57/BL6 primary cells. Results demonstrated that the aqueous extract of *N. sativa* significantly enhances splenocyte proliferation in a dose-responsive manner. In addition, the aqueous extract of *N. sativa* favors the secretion of Th2, versus Th1, cytokines by splenocytes. The secretion of IL-6, TNF- α , and NO; key pro-inflammatory mediators, by primary macrophages is significantly suppressed by the aqueous extract of N. sativa, indicating that *N.*

sativa exerts anti-inflammatory effects in vitro. Finally, experimental evidence indicates that the aqueous extract of *N. sativa* significantly enhances NK cytotoxic activity against YAC-1 tumor cells, suggesting that the documented anti-tumor effects of *N. sativa* may be, at least in part, attributed to its ability to serve as a stimulant of NK anti-tumor activity. It was anticipated that *N. sativa* ingredients may be employed as effective therapeutic agents in the regulation of diverse immune reactions implicated in various conditions and diseases such as cancer. A group of medicinal plants including black seed were examined for their immuno-modulatory effect in BALB/c mice. Treatment (intraperitoneal injection) with five doses of methanolic extract for Black seed was found to enhance the total white blood cells count [up to 1.2×104 cells/mm3]. Bone marrow cellularity also increased significantly ($P<0.01$) after the administration of the Black seed extract. Spleen weight of the black seed treated groups was significantly increased ($P<0.01$). Two groups of mice were immunosuppressed with cyclophosphamide, the one which pretreated with the black seed extracts significantly ($P<0.01$) restored their resistance against lethal infection with the predominately granulocyte-dependant Candida albicans. These results confirmed the immunomodulatory activity of black seed, and may have therapeutical implications in prophylactic treatment of opportunistic infections and as supportive treatment in oncogenic cases. The immunomodulating and cytotoxic properties of volatile oil of *N. sativa* seeds was investigated in a Long-Evans rat model designed to examine the effect of *N. sativa* seeds on selected immune components. Long-Evans rats were challenged with a specific antigen (typhoid TH) and treated with *N. sativa* seeds; Treatment with *N. sativa* oil induced about 2-fold decrease in the antibody production in response to typhoid vaccination as compared to the control rats but there was a significant decrease in splenocytes and neutrophils counts, but a rise in peripheral lymphocytes and monocytes in the these animals. These results indicated that the *N. sativa* seeds could be considered as a potential immunosuppressive cytotoxic agent. Chronic administration of oxytetracycline (OXT) (incorporated at a level of 0.05 g/kg of feed for 50 d) to pigeons, significantly decreased total leukocyte and lymphocyte counts, increased heterophil: lymphocyte ratio and lysosomal enzyme activity, and decreased reticuloendothelial system function compared with controls. Coadministration of black seed at a level of 2.5% with OXT completely blocked the effects elicited by OXT and produced immunostimulant effects in pigeons. The addition of black seed to feed of pigeons could act as an immunoprotective agent when chronic administrations of antibiotics are considered[4]. The effect of TQ was tested on experimental autoimmune encephalomyelitis (EAE) animal model that mimic human multiple sclerosis. Myelin oligodendrocyte glycoprotein

subcutaneously was used to induce chronic relapsing EAE. TQ intraperiotoneally was found to be almost 90% preventive and 50% curative in chronic relapsing EAE due to its antioxidant effect. *N. sativa* oil is a promising natural radioprotective agent against immunosuppressive and oxidative.

2.1.11 Cardiovascular activity

The acute (at 4 and 18 h) effects of diesel exhaust particles (DEP) on cardiopulmonary parameters in mice and the protective effect of TQ were investigated. Mice were given, intratracheally, either saline (control) or DEP (30 μg per mouse). At 18 h (but not 4 h) after giving DEP, there was lung inflammation and loss of lung function. At both 4 and 18 h, DEP caused systemic inflammation characterized by leucocytosis, increased IL-6 concentrations and reduced systolic blood pressure. SOD activity was decreased only at 18 h. DEP reduced platelet numbers and aggravated in vivo thrombosis in pial arterioles. In vitro, addition of DEP (0.1-1 μg/mL) to untreated blood-induced platelet aggregation. Pretreatment of mice with TQ prevented DEP-induced decrease of systolic blood pressure and leucocytosis, increased IL-6 concentration and decreased plasma SOD activity. TQ also prevented the decrease in platelet numbers and the prothrombotic events but not platelet aggregation in vitro.

2.1.12 Gastro-protective activity

The mechanism of gastroprotective effect of TQ was assessed. Animals were injected with vehicle, TQ (10, 20 mg/kg), omeprazole (10, 20 mg/kg) or their combination (10 mg/kg). Thirty minutes later, pyloric ligation was carried out and followed consequently with ischemia for another 30 min, abided by reperfusion for 120 min. The ischemia/reperfusion insult increased the gastric acid secretion, acid output, and pepsin, as well as the gastric mucosal content/activity of lipid peroxide, proton pump and myeloperoxidase, along with ulcer index. However, content/activity of gastric mucin, reduced glutathione, total nitric oxide, and SOD were decreased. TQ, especially the high dose level, corrected the altered parameters in a comparable manner to that of the reference drug used, omeprazole. In addition, when the low doses were combined they add to each other to reach the effect of the high dose of either drug. Besides the antioxidant property, TQ has novel gastroprotective mechanisms via inhibiting proton pump, acid secretion and neutrophil infiltration, while enhancing mucin secretion, and nitric oxide production. The anti-ulcer potential of *N. sativa* aqueous suspension on experimentally induced gastric ulcers and basal gastric secretion in

rats was examined to rationalize its use by herbal and Unani medicine practitioners. Acute gastric ulceration was produced by various noxious chemicals (80% ethanol, 0.2 mol/L NaOH, 25% NaCl and indomethacin) in Wistar albino rats. Anti-secretory studies were undertaken in a separate group of rats. Gastric wall mucus contents and non-protein sulfhydryl concentration were estimated, and gastric tissue was examined histopathologically. An aqueous suspension of black seeds significantly prevented gastric ulcer formation induced by necrotizing agents. It also significantly ameliorated the ulcer severity and basal gastric acid secretion in pylorus-ligated Shay rats. Moreover, the suspension significantly replenished the ethanol-induced depleted gastric wall mucus content levels and gastric mucosal non-protein sulfhydryl concentration. The anti-ulcer effect was further confirmed histopathologically. The anti-ulcer effect of *N. sativa* is possibly prostaglandin-mediated and/or through its antioxidant and anti-secretory activities. Both and its constituent, TQ was found to possess Gastro protective activity against gastric mucosal injury induced by ischaemia/reperfusion in rats. Ischaemia/reperfusion (I/R) induced gastric lesion is known to be linked with free radical formation. Male Wistar rats were subjected to I/R and were injected with either NO (2.5 and 5.0 mL/kg, p.o.) or TQ (5, 20, 50 and 100 mg/kg, p.o.). The results showed that I/R elevated the levels of lipid peroxide and lactate dehydrogenase, while decreased those of reduced GSH and SOD. These biochemical changes were accompanied by an increase in the formation of gastric lesions, which was reduced by either treatment. This indicates that both NSO and TQ possess gastroprotective effect against gastric lesions which may be related to the conservation of the gastric mucosal redox state. *N. sativa* prevents alcohol induced increase in lipid peroxidation (i.e. thiobarbituric acid reactive substances) and reduced gastric GSH content, enzyme activities of gastric SOD, GSH-S-Transferase[83]. TQ was found to protect gastric mucosa against the ulcerating effect of alcohol and mitigated most of the biochemical adverse effects induced by alcohol in gastric mucosa, but the effect of TQ was found to be a lesser than black seed whole. Both *N. sativa* and TQ did not affect the CAT activity in gastric tissue. The beneficial effects of NSO on rats with necrotizing enterocolitis (NEC) were studied in newborn Sprague-Dawley rats. NEC was induced by enteral formula feeding, exposure to hypoxia-hyperoxia and cold stress. Pups in the NEC+NSO group were administered NOS at a dose of 2 mL/kg daily by intraperitoneal (i.p.)

2.1.13 Hepato-protective activity

It is reported that *N. sativa* (0.2 mL/kg) intraperitoneally relieves the deleterious effects of ischemia reperfusion injury on liver. Biochemical parameters like the serum aspartate aminotransferase, alanine aminotransferase lactate dehydrogenase levels and total antioxidant capacity (TAC), CAT, total oxidative status (TOS), oxidative stress index (OSI) and MPO were determined in hepatic tissue in rats with hepatic ischemia. Results suggested that *N. sativa* treatment protects the rat liver against hepatic ischemia reperfusion injury. *N. sativa* administration protects hepatic tissue from deleterious effects of toxic metals such as lead, and attenuates hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride. Cadmium (Cd++) causes alteration of the cellular homeostasis and oxidative damage. The protective role of TQ on the hepatotoxicity of Cd++ with special reference to its protection against perturbation of nonenzymatic and enzymatic antioxidants was investigated. The effect of TQ pretreatment was examined in post-nuclear supernatant prepared from liver of Swiss albino mice under in vitro conditions. CdCl2 treatment (5 mmol/L) resulted in a significant increase in antioxidant enzymatic activities. It also caused a significant ($P<0.001$) increase in protein carbonyl and reduced glutathione content. Pretreatment with TQ (10 μmol/L) showed a significant protection as manifested by noticed attenuation of protein oxidation and rejuvenation of the depleted antioxidants of cellular fraction. These results strengthen the hypothesis that TQ exerts modulatory influence on the antioxidant defense system on being subjected to toxic insult.

2.1.14Nephroprotective activity

The nephro-protective effect of vitamin C and *N. sativa* oil was observed against gentamicin (GM) associated nephrotoxicity in rabbits. Serum creatinine, blood urea nitrogen, and antioxidant activity were measured as indicators of nephrotoxicity for all the groups of rabbits. It was revealed that vitamin C and *N. sativa* oil both had nephroprotective effect as they lowered the values of serum creatinine, blood urea nitrogen, and antioxidant activity as compared to GM control group values. When these two antioxidants were given as combination, they proved to have synergistic nephroprotectiveeffect[88]. Recenty, it was observed that there is an inherent lack in regulation of renal organic anion and cation transporters in cisplatin-induced nephrotoxicity. The effect of TQ on alterations in the renal expression of organic anion transporters and organic cation transporters, as well as multidrug resistance-associated proteins in rats treated with cisplatin was reported. Cisplatin-induced MDA and 8-isoprostane increase was found to be markedly reduced in rats treated with TQ.

In cisplatin only treated rats, the induced renal injury increased protein levels of the efflux transporters MRP2 and MRP4 while expression of OAT1, OAT3, OCT1 and OCT2 was reduced. In combination TQ- and cisplatin-treated rats, expression of MRP2 and MRP4 proteins was decreased in the kidneys. Conversely, TQ treatment increased levels of OCT1, OCT2, OAT1 and OAT3 and decreased levels of 8-isoprostane and MDA levels in cisplatin-treated rats. This is concluded that TQ synergizes with its nephroprotective effect against cisplatin-induced acute kidney injury in rats. The protective effects of *N. sativa* oil on methotrexate-induced nephrotoxicity were also studied in albino rats and this study revealed the protective effect of Black cumin in the methotrexate-induced nephrotoxcity. The protective effects of *N. sativa* against ischemia-perfusion damage on kidney tissue were examined. TAC, CAT, TOS, OSI, and MPO in kidney tissue and blood were measured. Serum urea and creatinine levels were also determined. Kidney tissue histopathology was also evaluated. *N. sativa* was effective in reducing serum urea and creatinine levels as well as decreasing the tubular necrosis score. *N. sativa* treatment significantly reduced OSI and TOS levels and increased TAC levels in both kidney tissue and blood. Results revealed the protective effect of *N. sativa* against renal I/R injury in rat kidneys. GM induced nephrotoxicity has been shown to involve the generation of oxygen free radicals. Nephrotoxicity was evaluated histopathologically and by measuring concentrations of urea, creatinine and total antioxidant status (TAS) in plasma and reduced GSH and TAS in kidney cortex. The effect of oral treatment of *N. sativa* oil (0.5, 1.0 or 2.0 mL/kg/day for 10 d) on GM (80 mg/kg/day given intramuscularly) induced nephrotoxicity in rats produced a dose-dependent amelioration of the biochemical and histological indices of GM nephrotoxicity that was statistically significant at the two higher doses used. Treatments of rats with *N. sativa* increased TAS in plasma and reduced GSH concentrations in renal cortex and enhanced growth while it did not cause any over toxicity. The results suggest that *N. sativa* may be useful in ameliorating signs of GM nephrotoxicity in rats[92]. TQ supplementation prevents the development of GM-induced acute renal toxicity in rats. TQ was found to prevent the degenerative changes in kidney tissues against GM induced nephrotoxicity. TQ supplementation resulted in a complete reversal of the GM-induced increase in serum creatinine, blood urea nitrogen, thibarbituric acid reactive substances, total nitrate/nitrite and decrease in GSH, glutathione peroxidase (GPx), CAT and ATP to control values suggesting that TQ prevents GM-induced degenerative changes in kidney tissues[93].The protective effects of NSO in the prevention of chronic cycl

2.1.15 Nephroprotective activity

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2.1.16 Pulmonary-protective activity and anti-asthmatic effects

Wienkotter*et al.,* reported the effect of nigellone and TQ on trachea (antispasmodic effect) and their influence on respiratory clearance. The effects on Ba++ carbachol- and leukotriene-induced trachea contractions and the transport of the fluorescence dye rhodamin B concerning ciliary action in the tracheal area were investigated using a micro dialysis technique. Nigellone and high concentrations of TQ had a concentration-dependent inhibitory effect on the trachea when being contracted by the depolarizing effect of Ba2+. The trachea contractions induced by leukotriene-d (4) LT4 were inhibited by nigellone and by TQ. It was concluded that nigellone possesses an antispasmodic effect and an increase in mucociliary clearance but TQ do not have such effects. Therefore, it is suggested that nigellone but not TQ may be useful in treatment of different respiratory diseases.

The relaxant effects of four cumulative concentrations of n-hexane, dichloromethane, methanol and aqueous fractions of *N. sativa* (0.8, 1.2, 1.6 and 2.0 g%) in comparison with saline as negative control and four cumulative concentrations of theophylline (0.2, 0.4, 0.6 and 0.8 mmol/L) were examined by their relaxant effects on precontracted tracheal chains of guinea pig by 60 mmol/L KCl (group 1) and 10 microM methacholine (group 2). The results showed relaxant effect of most fractions from *N. sativa* on tracheal chains of guinea pigs which was more potent for methanol and dichloromethane fractions[98]. The protective effect of *N. sativa* on tracheal responsiveness (TR) and lung inflammation of sulfur mustard gas exposed guinea pigs was examined. Guinea pigs were exposed to diluent's solution (ethanol,

control group), 100 mg/m3 inhaled sulfur mustard (SME group), and SME treated with N. sativa, 0.08 g daily (SME+N), n=6 for each group. TR to methacholine, total white blood cell count of lung lavage, and differential white blood cell were done 14 d post exposure. The results showed a preventive effect of *N. sativa* on TR of sulfur mustard gas-exposed guinea pigs[99]. The possible beneficial effects of the seeds of *N. sativa* L. on experimental lung injury in male Wistar rats after pulmonary aspiration of different materials was investigated. Results showed that *N. sativa* treatment inhibits the inflammatory pulmonary responses, reducing significantly ($P<0.05$) peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudate, alveolar macrophages, interstitial fibrosis, granuloma and necrosis formation in different pulmonary aspiration models. Data indicated a significant reduction in the activity of inducible nitric oxide synthase and a rise in surfactant protein D in lung tissue of different pulmonary aspiration models after *N. sativa* therapy. It was concluded that *N. sativa* treatment might be beneficial in lung injury and have potential clinical use[100]. The beneficial effects of NSO on rats with hyperoxia-induced lung injury were evaluated since oxygen-induced lung injury is believed to lead to the development of broncho-pulmonary dysplasia in premature infants. NSO significantly reduced the severity of lung damage due to hyperoxia. The prophylactic effect of boiled extract of *N. sativa* on asthmatic disease was examined. Twenty-nine asthmatic adults were randomly divided into control group (14 patients) and study group (15 patients), and they were studied for 3 months. In the study group 15 mL/kg of 0.1 g% boiled extract and in the control group a placebo solution was administrated daily throughout the study. Asthma symptom score.

2.1.17 Drugs-nigella interaction

There is a possibility that *N. sativa* may interact with co-administered drugs and affect their intestinal availability and pharmacological effect. In vitro studies have shown that *N. sativa* extracts inhibit cDNA-expressed human cytochrome P-450 3A4, 2C9, 3A5 and 3A7-mediated metabolism of marker substrates therefore may affect and/or inhibit the metabolism of a wide range of drugs (117). Further, the effect of *N. sativa* on bioavailability of amoxicillin was investigated in everted rat intestinal sacs. The in vitro studies both with methanol and hexane extracts of Nigella increased the permeation of amoxicillin significantly ($P<0.001$) as compared to control. Permeation was also found to be significantly higher for the hexane extract ($P<0.001$) in comparison to methanol extract at the same dose levels. in vivo experiments revealed that Cmax of amoxicillin in rat plasma when administered orally alone and in combination with hexane extract increased correspondingly from 4

138.251±156.930 to 5 995.045±196.280 ng/mL while as AUC 0→t increased from 8 890.40±143.33 to 13 483.46±152.45 ng/mL/h. Nigella enhanced amoxicillin availability in both in vivo and in vitro studies.*L.M. Aziz-Zadeh* (2016).

CHAPTER III
MATERIALS AND METHODS

3.1 Statement of the experiment

The experiment was conducted for a period of 1 year from1st January to 31th December, 2017 to investigate the dietary effect of black cumin seed on productive performance and microbial load in cloacal content in Khaki Campbell.

3.2 Venue of the experiment

The rearing of duck trial was conducted at Nandoir, Basherhat, Dinajpur and egg quality determination was performed at the poultry science laboratory of the department of doing and poultry science and microbial parameter determination was conduct at microbiology laboratory of the department of microbiology, HSTU.

3.3 Collection of experimental materials

Black cumin seed was collected from local market of Dinajpur District of Bangladeh

3.4 Experimental Layers

A total ninetysix laying duck of uniform body weight from a high yielding egg laying duck breed Khaki Campbell of 65 weeks of age were selected and duck are divided into four groups having 3 replication containing 8 duck in each replication. Four ducks kept in each cage were considered as an experimental unit. Ducks were randomly distributed to cages. All of the group of duck marked by different colour of thread.

3.5 Layout of the experiment

The layout of the experiment is shown in table 1 there were three replications in each dietary phase treatment. Thus total number of replicate was 12.

Table 1.Showing the distribution of layer to different dietary black cumin seed levels in age from 180 to 210days of age.

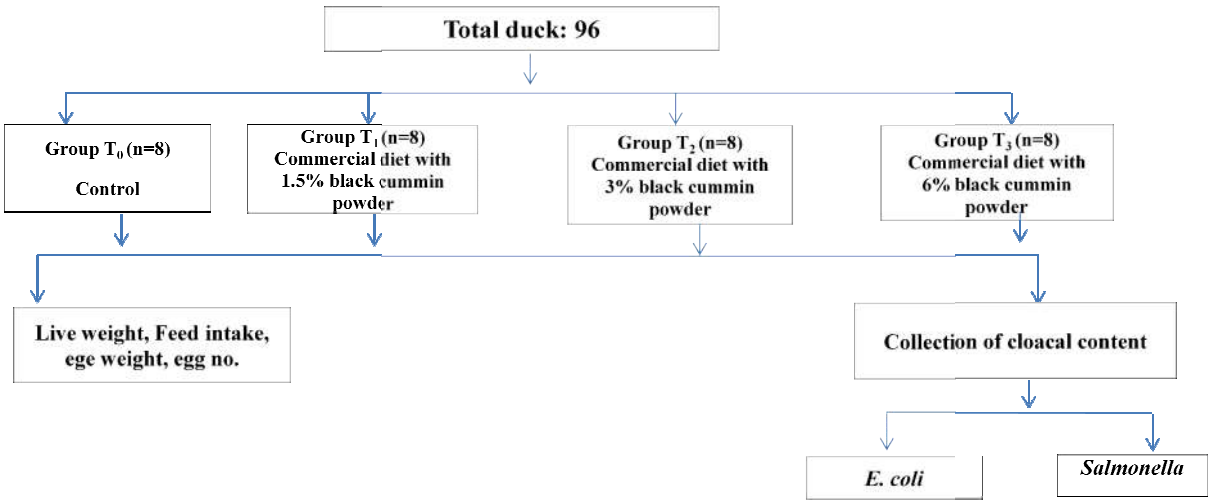
Replication (R)	Treatment			
	T_0	T_1	T_2	T_3
R_1	8	8	8	8
R_2	8	8	8	8
R_3	8	8	8	8

T_0 = control (Basal diet)

T_1 = control + 1.5% black cumin

T_2 = control + 3% black cumin

T_3 = control + 6% black cumin



3.6Procurement of feed Ingredients.

Feed ingredients are purchase from local market of Dinajpurtown. During purchase Ingredients are evaluated carefully for their freshness by observing its color with naked eye and smell with nose. During initial stage I supply, laying stage I supply layer feed.

3.7Collecting, Processing & storage of black cummin powder for duck:

Dried black cummin seed (*Niglla sativa*) purchased from local spices market, Dinajpur Bangladesh the Samples were further ground into powder by grinding machine at Dinajpur, The Obtained powder was packed in a polyethylene bag and preserved in the feed formulationand proper care was taken in the feed storage room to avoid Spoilage.

3.8 Preparation of diet:

Ready feed was used throughout the experimental Study. The black cummin Seed were mixed with small amount of control feed and then thoroughly mixed with total amount of feed according to black cumminseed Level. However four diets were randomly distributed to four groups in a completely randomized design (CRD). Dietary treatments consist. Of basal feed with Supplementing black cummin feed was used at the rate of1.5%,3%,6% black cummin powder/kg of fed in T_0,T_1,T_2&T_3 respectively.

3.9Composition of basal diet

CP%	CF%	ME(kcal/kg)	Fiber	Ca%	P%	Moisture%
18.00%	3-4%	2800	3-5%	3.90%	0.38%	11.00%

Lisenceno: 137, Power layer feed guaranteed by Animal Health Division.

The black cummin seed powder was used at the age of 180 days to 210 days. During this experimental period the required amount of ready feed ingredients were weighed by digital weighing machine. Then different Level of black cummin powder was mixed with different treatment. During the time of mixing cross mixing was applied. Mixing was done manually and no coccidiostate. This prepared feed was supplied at the morning & night. In the day time they take scavangingly from the out Side of the Shed for their swimming.

3.10Lighting.

During the whole experimental period. all ducks were exposed to a 16 hours continuous photo period (natural light + artificial Light) in an open Sided house. Electrical bulbs were used for additional lihgt at right.

3.11Routine Management

Ducks were provided with similar care and management.T_0,T_1,T_2&T_3 groups used black cummin seed throughout the study period. Adequate hygiene and Sanitation were maintained properly.

3.12Management of the experimental duck

At first experimental duck were identified with different color thread such as red. Yellow, Green with tag number.Similar care and management in all the talent groups throughout the experimental period was practiced. At the recital of the experiment. Body weight of duck was

recorded as initial body weight. Identical Management of duck practices with different colour thread.

Fig: 3. Identification of duck with thread

3.13 Date collection and record keeping

The following records were kept during 180 days of rearing period:

i. Live weight.
ii. Feed consumption.
iii. Egg number and egg weight.
iv. Cloacal parameter (salmonella &*E.coli*).

3.14Live weight gain

The average body weight gain of each replication was calculated by deducting initial body weight the final body weight of the birds.

Live weight gain= Final weight- Inital weight

3.15 Feed intake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

$$\text{Feed Intake (g/bird)} = \frac{\text{Fed intake in a replication}}{\text{No. of birds in a replication}}$$

Figure 4: Egg weight

Figure 5: Live weight of duck

3.16 Statistical analysis

Date on different variables were subjected to analysis of variance (ANOVA) in a Completely Randomized Design (CRD) (Steel and Torre, 1980). The significant differences between the treatment means were calculated from analysis of variance (ANOVA) table. All analyses were performed by using ``IBM SPSS statistics 20'' program.

3.16 Isolation of E. coli and salmonella from feces sample

3.16.1 Fecal sample collection, transportation and preparation

Fecal samples of duck were collected from healthy duck at the last week of experiment. All samples were collected with the help of sterile cotton buds and transferring the buds immediately to sterile nutrient agar. All the samples were transferred carefully to appropriate container. These were kept in box, wrapped with ice and transferred to laboratory for subsequent bacteriological examination.

3.16.2 Bacteriological media

a. **Cultural Media**

Commercially available media were used during this study. The commercial media were prepared according to the direction of the manufacturer's. The composition and the procedure for the preparation of media are presented in the Methods. The media used for bacteriological culture were Nutrient Agar (NA; HiMedia), Nutrient Broth (NB; HiMedia), Eosin-Methylene-Blue (EIMB. Hi Media) Agar, MacConkey(MC; HiMedia) Agar.

b.Biochemical media

The following biochemical media were used for the bacteriological analysis: Triple Sugar Iron (TSI) medium, Methyl Red-Vogel Proskauer Broth (MR-VP Broth; HiMedia), Motility Indole Urea medium (MIU, HiMedia), Indole test.

3.16.3 Bacteriological reagents

The reagents used were phenol red, phosphate buffered saline (PBS), mineral oil, normal physiological saline solution, peptone water, 3% tri sodium citrate solution and other common laboratory chemicals and reagents as and when required during the experiment.

3.16.4 Sugars

- Dextrose
- Sucrose
- Lactose

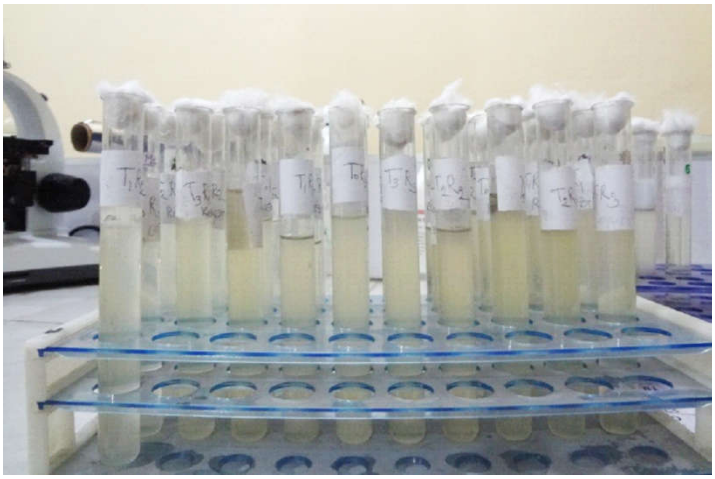


Fig: 6.Ten fold dilution

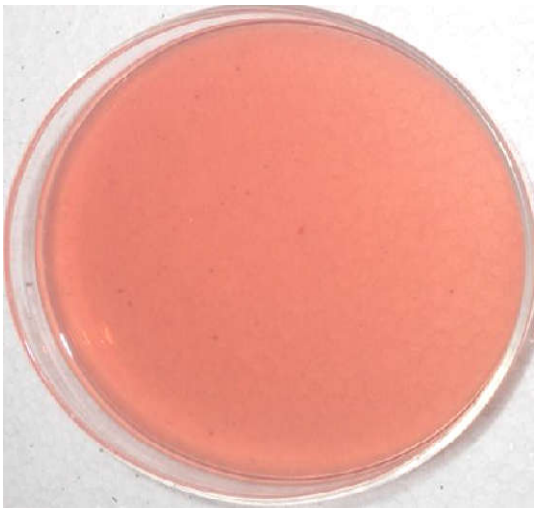


Fig: 7. EMB agar of *E. coli*

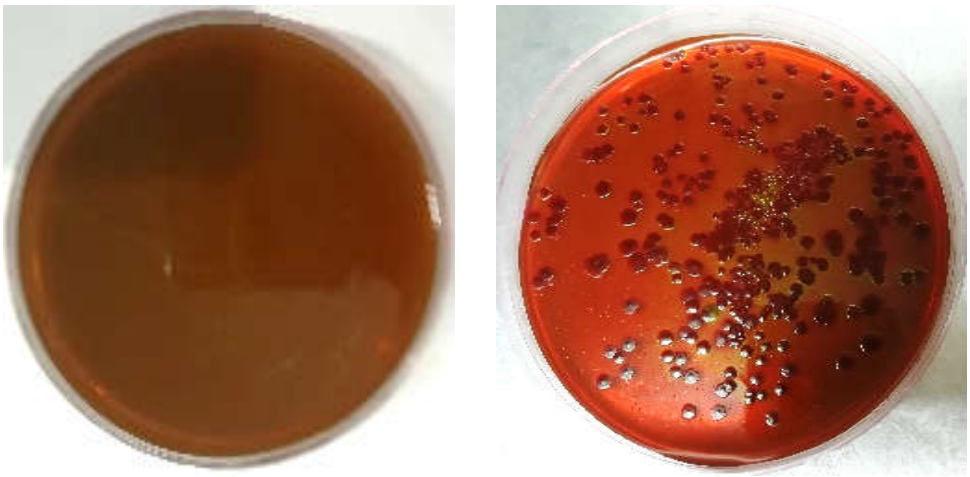
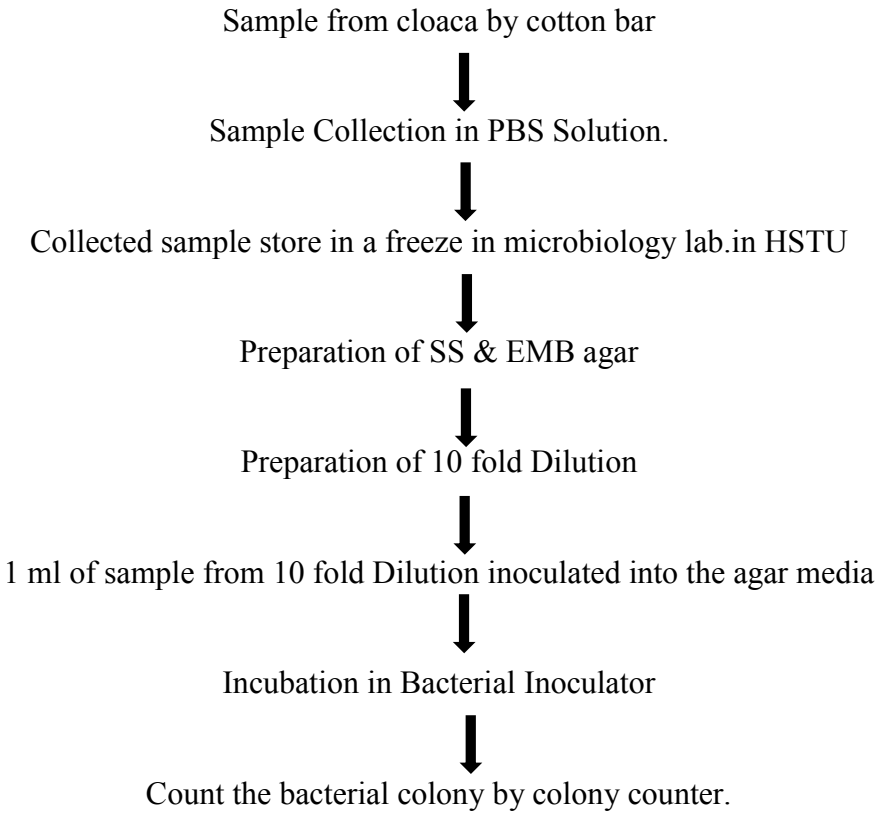


Fig:8. Colony of *E.coli*

3.16.5 Method of bacterial Counting



3.16.6 Bacteriological media preparation

a) Nutrient broth (NB)

Nutrient broth was prepared by dissolving 13 grams of dehydrated nutrient broth (Himedia, India) into 1000 ml of distilled water and was sterilized by autoclaving, at 121°C tinder 15 Ib pressure per square inch for 15 minutes. Then the broth was dispensed into tubes (10 mUtube) and stored at 4°C in the refrigerator until used.

b) MacConkey (MC) agar media

51.50 grams powder of MC agar base (HITIVIedia, India) was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at I21°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45() C to decrease its temperature. After solidification of the medium in the petridishes, the petridishes were

allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

c) Eosin Methylene Blue (EMB) agar media

Thirty six (36) grams of EMB agar base (HiMedia, India) was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 12 FC maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its, temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

d) Triple Sugar Iron (TSI) media.

A quantity of 65.0 gm of Bacto TSI medium (HiMedia) was dissolved in 1000 mlt1- of distilled water dispensed in 5 ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its temperature. After solidification of the medium in the test tubes, the test tubes were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

e) Methyl-Red Voges-Proskauer (MR-VP) broth

A quantity of 17.0 gm of Bacto MR-VP medium (HiMedia) was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

f) Motility Indole Urea (MIU) broth

18.00 grams powder of MIU agar base (HiMedia, India) was added to 950 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45(' C to decrease its

temperature. After this the medium in the test tubes were allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

3.16.7 Isolation of *E. coli* in pure culture

All the samples were cultured primarily in nutrient agar at 37°C for 24 h, and then subcultured onto the MacConkey and EMB agar and S-S agar by streak plate method to observe the morphology. The organism showing, characteristic colony morphology of *E. coli* was repeatedly subcultured onto EMB agar until the pure culture with homogenous colonies was obtained.

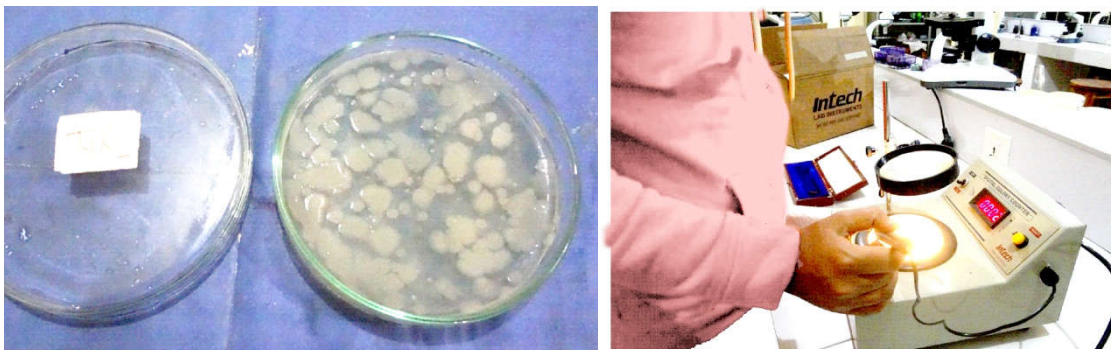


Fig: 9. Bacterial colony counting

3.16.8 Examination of Plates (Identification of the isolates)

a) Gross colony study

Morphological characteristics (shape. size, surface texture, edge, elevation, colour, opacity etc.) developed after 24 h of incubation were carefully studied as described by Marchant and Packer (1967) and recorded.

b) Microscopic study by staining method

Gram's staining method was done to study their morphology and staining character. Suspected colony from EMB agar were stained using Gram's stain as described by manual of Veterinary Investigation Laboratory Technique, 1984 (OIE, 2000).

c) The procedure was as follows:

A small colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violet solution was then applied on the smear to stain for two minutes and then washed with running water. Lugol's iodine was then added to act as

mordant for one minute and then again washed with running water. Acetone alcohol was then added, which act as a decolourizer, for few seconds. After washing with water, safranine was added as counter stain and allowed to stain for two minutes. The slide was then washed with water, blotted and dried in air and then examined under microscope Is' with 10 X objectives and then with 100X objective using immersion oil. Gram negative rod shaped organisms were suspected for *E. coll.*

3.16.9Procedure for total salmonella and *E. coli* count:

We were used Nutrient agar media for total viable count and Eosin Methylene Blue (EMB) agar media for *E. coli* count in this study. The procedure was as follows-

At first 10% suspension of the collected fecal sample was prepared in 0.1% peptone water. Then serial 10 fold dilution of the suspension was prepared in 10 sterile test tubes using 0.1% peptone water as diluent. Then 1 ml of diluted sample from each test tube was taken and poured into a sterile petri dish. Three different petridishes were used for each dilution. Then 10ml of melted Glucose tryptone yeast agar was poured into each petridish when the temperature was reduced at 45 C. Then the petridishes were rotated clockwise and anticlockwise gently to mix the sample with the culture media. Then the petridishes were allowed for solidification of the media. After solidification of the media the petridishes were marked and incubated at 30^{o}C for 72 hours. Then the colonies of each petridish were counted. The petridishes containing 30 to 300 colonies were taken in consideration. Then average numbers of colonies were counted.Then the result was obtained by using the following formula:

The number of total viable organisms per ml of sample = Average number of colonies x dilution factor. Therefore, The number of total viable organisms per gm of fecal sample = Average number of colonies x dilution factor x 10.

3.17 Statistical analyses

Data were analyzed by analysis of variance using Completely Randomized Design with factorial arrangement (Steel and Torrie, 1986). The significance differences between the treatment means were calculated by the Duncan's Multiple Range Test (Duncan, 1955). All analyses were performed by SPSS Program.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of black cummin powder on body Wight gain

Initial body weight of 179 day laying ducks feed on all treatments were similar. From 180 to 195 days of age,the body weight was not significant in different treatment groups. Significant different ($P<0.05$) were found at 196-200 days, 201-205 days & 206-210 days of age on body weight of birds treated with at the level of 1.5 %, 3% and 6% black cummin powder. During 196 to 210 days, the body weight (g) was significantly ($P<0.05$) higher in T_3(1197.00 ± 1.53) followed by T_2 (1182.00±1.20),T_1 (1179.33±0.33) and T_0 (1164.00±2.00) respectively. During 196 to 210 days of age, the body weight gain (1247.67gm) in duck feed diet containing 6%black cummin powder was significantly ($P<0.05$) followed by bird received 3% (1209.33gm) 1.5%, (1201.00gm) and 0% (1170.67gm) black cummin powder. The significantly increased in body weight in treatment T_3(1247.67gm) Jordan *et al.,*(2008) used black cummin seed with 1%, 1.5% and found significantly increased the body weight gain.

Table 2: Effect black on live weight of laying duck

Days	Body weight (g)				Level of significance
	T_0	T_1	T_2	T_3	
Days 180-185	1150.00±0.00	1150.00±0.00	1152.00±0.00	1151.00±0.00	NS
Days 186-190	1159.00±2.08	1161.00±0.58	1166.00±0.577	1173.00±2.08	NS
Days 191-185	1161.66±1.67	1167.33±1.20	1176.67±0.333	1177.33±1.33	NS
Days 196-200	$1164.00±2.00^{c}$	$1179.33±0.33^{b}$	$1182.00±1.20^{b}$	$1197.00±1.53^{a}$	*
Days 201-205	$1174.00±1.00^{c}$	$1194.33±1.20^{b}$	$1194.67±0.33^{b}$	$1212.67±2.08^{a}$	*
Days 206-210	$1170.67±0.67^{c}$	$1201.00±2.08^{b}$	$1209.33±0.88^{b}$	$1247.67±1.20^{a}$	*

* means statistically significantly at 5% level of significance ($P<0.05$)
** means statistically significantly at 1% level of significance ($P<0.01$)
*** means statistically highly significant ($P<0.001$)
NS means statistically not significant ($P>0.05$)

4.2 Effect of black cummin of Egg number

The difference of Egg number per duck significantly effects (P<0.05)at 180 to 185 days and 191 to 195 days. At the age of (180 to 185) days the highest number of egg found at the level of 6% black cumminseed T_3(2.40) at the day of (186 to 190) and (196 to210) days.The egg production was not significantly (P>0.05) differed among the treatment groups during 196 to 200 days, 201 to 205 days and 206 to 210 days of age but significantly (P<0.05) varied during 180to 185 days and 191 to 195 days. At 180 to 185 days the higher a production was found in T_3(2.40±0.2) which was followed by T_2(2.33±0.130), T_1(1.53±0.67) and T_0(1.73±0.24)respectively.

Table 3: Effect of black cummin on egg production

Days	Egg number/ duck				Level of significance
	T_0	T_1	T_2	T3	
Days 180-185	1.73±0.24[b]	1.53±0.67[b]	2.33±0.13[a]	2.40±0.20[a]	*
Days 186-190	2.53±0.13	2.60±0.13	2.53±0.67	2.53±0.24	NS
Days 191-185	2.87±0.67[a]	2.73±0.067[ab]	2.40±0.11[b]	2.47±0.13[b]	*
Days 196-200	2.600±0.20	3.00±0.00	2.80±0.12	2.80±0.12	NS
Days 201-205	2.800±0.12	2.67±0.24	2.80±0.11	2.73±018	NS
Days 206-210	2.933±0.07	2.73±0.13	2.80±0.67	2.83±0.05	NS

* means statistically significantly at 5% level of significance (P<0.05)
** means statistically significantly at 1% level of significance (P<0.01)
*** means statistically highly significant (P<0.001)
NS means statistically not significant (P>0.05)

4.3 Effect of Egg weight

There is no significant effect of black cummin seed on egg weight in laying duck. Akhtar *et al.,*(2003) found that duck treated with 1.5% black cummin seed increased egg weight.

Table 4: Effect of black cummin on egg weight

Days	Egg weight (g)				Level of significance
	T_0	T_1	T_2	T_3	
Days 180-185	62.26±0.4	62.64±080.1	62.52±0.5	64.15±0.5	NS
Days 186-190	64.44±0.2	64.22±0.7	65.33±0.5	64.78±0.9	NS
Days 191-185	66.44±0.2	65.33±0.5	64.67±0.3	66.66±0.3	NS
Days 196-200	65.66±0.8	65.11±0.1	65.33±0.6	66.55±0.2	NS
Days 201-205	75.66±0.3	83.58±8.0	76.07±0.5	76.69±0.6	NS
Days 206-210	77.59±0.2	76.40±0.3	77.66±0.9	78.07±0.0	NS

* means statistically significantly at 5% level of significance (P<0.05)
** means statistically significantly at 1% level of significance (P<0.01)
*** means statistically highly significant (P<0.001)
NS means statistically not significant (P>0.05)

4.4 Effect of feed consumption

There is no significant effect of black cummin seed on feed consumption. But Abdul karim*et al.,* (2013) in his study result indicate that duck received 2.5% & 3.5% black cummin seed in the diet increased significantly average daily intake.

Table 5: Effect on black cummin on fed consumption/duck/day

Days	Feed consumption/duck/day				Level of significance
	T_0	T_1	T_2	T_3	
Days 180-185	125.79±0.8	123.66±2.0	125.68±1.1	126.01±0.4	NS
Days 186-190	122.91±1.1	124.89±0.5	124.52±0.5	128.05±0.7	NS
Days 191-185	125.40±1.7	123.27±0.8	126.32±0.9	126.54±0.2	NS
Days 196-200	126.62±0.9	125.74±0.5	128.22±0.3	126.58±0.6	NS
Days 201-205	125.09±1.6	124.51±0.5	127.64±1.3	127.67±0.8	NS

NS means statistically not significant (P>0.05)

4.5 Effect of black cummin Seed on cloacal content

Presence of *E. coli* is highly significant at the age of 180-185 days. The number of colony is has number at T_3 =31.00+0.58, T_2= 32.00±0.67, T_1=35.67±0.67 and the number of colony is more in number at T_0 =2.86.67± 4.41. The less number of colony the treatment group T_3that treated with 6% black cummin powder. At the age of 205 to 210 days the number of colony is also highly significant, less number of colony found T_3 =30.33 ± 0.33 that treated with 6% black cummin seed. At age of 186-190 days, the presence of *E.coli* is highly significant (P<0.001) which is followed that T_3(31.00±0.58), T_2 (32.00±2.89) and T_1 (31.00±0.58) respectively. At the age of 201-205 days the presence of *Salmonella* sp. T_3 (176.00±13.01),T_2 (162.33±27.17) and T_1 (172.33±31.86) respectively.

The presence of *salmonella* sp, 5% level of significant (P<0.05) at the age of 180-185 days that is T_3= 163.00 ± 37 that treated with 6% black cummin seed. At the age of 205-210 days the presence of *salmonella* sp. 1% level of significant (P<0.01).The number of colony found T_2= 162.33± 27 that treated with 3% blackcummin in powder. Akhtar*et al.,* (2003) found that 6% black cummin seed decreased the bacterial loads. Again Bokaj, *et al.,*(2014) found that 2% black cummin seed is the best intestinal health indices.

Table 6: Effect of black cummin seed on bacterial load in cloacal content

Bacterial species	Day	Bacterial load				Level of significance
		T_0	T_1	T_2	T_3	
E. coli	186-190 days	286.67±4.41	35.67±0.67	32.00±2.89	31.00±0.58	***
E. coli	201-205 days	281.00±5.57	34.67±0.33	31.67±0.88	30.33±0.33	***
Salmonella sp.	186-190 days	295.67±2.85	171.67±33.46	168.67±33.86	163.00±37.00	*
Salmonella sp.	201-205 days	292.00±3.06	172.33±31.86	162.33±27.17	176.00±13.01	**

* means statistically significantly at 5% level of significance (P<0.05)
** means statistically significantly at 1% level of significance (P<0.01)
*** means statistically highly significant (P<0.001)
NS means statistically not significant (P>0.05)

CHAPTER V
CONCLUSION

Considering the result obtained in the current study, it could be concluded that dietary inclusion of black cummin seed powder may effect on cloacal content that decreased the *salmonella* and *E. coli* load in the cloacal sample. Dietary black cummin seed may effect on body weight. That means 6% level of black cummin seed may significantlyaffect on the body weight. Black cummin seed also significantly effect on egg number at the level of 6% black cummin seed. It also found that black cummin seed has no significantly effect on feed consumption and egg weight. So, 6% black cummin seed can be added in duck diet for their better performance. Further studies are needed to analysis the effect of black cummin seed powder on cholesterol concentration of egg yolk.

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