

Effect Of *Zyzygium aromaticum* (Clove) and *allium sativum* (Garlic) Extracts on Broiler Chickens Infected With *E.Coli*

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Abstract

In these study forty-nine *E. coli* isolates were isolated from 80 broiler experiential chicks collected from broiler farms. These isolates were serotyped in to (01, 08, 018, 078, 086, 0111) and untyped (17 serotypes). The growth inhibitory effect of *Zyzygium aromaticum* (clove) and ***allium sativum* (Garlic)** on isolated strain *E. coli*. Water, 80% ethanol and n-hexan plant extracts were tested against *E. coli*. Gel diffusion method, minimum inhibitory concentration (MIC) values were used in these investigation. The obtained findings indicated that *S. aromaticum* and **garlic** had grown inhibitory effect against tested *E. coli*. Two hundred one day old Ross chicks were divided into 5 equal group (1, 2, 3, 4 and 5), group (1) chicks remained a negative control. Groups (2, 3 and 4) chicks infected by *E. coli* 0111• Group (3) chicks were treated with 25mg/L clove and group (4) treated with 25 mg/L *Garlic* for 3 successive days. The clinical signs, mortality rate, postmortem lesion reisolation of *E. coli*, body weight gain and F.C.R after two week of infection were recorded. In all groups The clove extracts were more effective for controlling the *E. coli* than *Garlic* extracts . It could be concluded that *S. aromaticwn* (clove) and **Garlic** extract had grow inhibitory against tested *E. coli*, and clove has superior activity and efficacy than the we *Garlic* in treatment infection broiler chicks with *E. coli*

Introduction

Bacterial diseases represent an important cause of morbidity and mortality in poultry industry. Antibiotic resistant bacterium is a threat which is becoming increasingly common (**Lessc 1995**).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, the actions of resistance must be taken to reduce this problem, for example, to control the use of antibiotic , moreover the genetic mechanisms of resistance, was studied to continue studies to develop new drugs (**Gislen e et at., 2000 and Cos ct al., 2006**).

Plants are rich source of natural products used for centuries to cure various diseases. The plant-derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness. So, a return to natural substances are an absolute need of our time (**Swayarnjot et al, 2005 and Kumar et al., 2007**).

The inhibitory activity of *clove (Syzygium aromaticum)* is due to the presence of several constituents, mainly eugenol, eugenyl acetate and betacaryophyllene, 2-heptanone (**Chaieb et al , 2007**), acetyl-eugenol, alphahumulene, methyl salicylate and iso-eugenol, methyl-eugenol (**Yang et al., 2003**).

Several studies have demonstrated potent antibacterial effects of *clove (Lopez et al., 2005; Li et al, 2005; Betoni et al, 2006 and Fu ct al, 2007)*. *N. sativa (black cumin)* is an herbaceous indigenous plant in the Mediterranean region. Seeds of this plant have been used for centuries as a spice and food preservative, as well as a traditional medicine for the treatment of various diseases (**Gorcja, 2003**).

Crude extracts and seed constituents of *N sativa*, in particular thymoquinone, have been reported to possess a number of pharmacological properties (**Ali and Blunden, 2003**).

Conuniphora molmol (myrrh) is widely distributed in the Kingdom of Saudi Arabia and it is grown in Jizan area on Red Sea coast. It is also found in Somalia and other coast African countries (**Mugahid, 1981**).

It is used in traditional medicine as antiseptic, carminative, anti-inflammatory, (**Tarig et al., 1985**). The ethanolic extract of *C. molmol* exhibited antimicrobial activity against the Gramnegative organisms (**Omer et al., 2011**).

Several studies have proved that garlic has anti-microbial effects (Lawson, 1988 and Martin and Ernst, 2003). It inhibits the growth of both gram-negative and gram-positive bacteria (**Pai and Platt, 1992 and Ross et al., 2001**).

The present study evaluated the individual and in combination growth inhibitory effect against enterobactrecea.

MATERIALS AND METHODS

Nightly diseased broiler chicken aging one day up to 36 days were used in this investigation. The examined chicks were suffering from sever respiratory manifestation and collected from broiler farms.

Bacteriological examination:

Specimens from heart blood, liver, spleen, unabsorbed yolk sac and air sacs were cultured on MacConkey and blood agar, incubated at 37°C for 24 hrs for detection of *E. coli*. The suspected colonies were identified morphologically as well as biochemically according to **Kuman et al., (2007)**.

Serological identification:

Antisera of *E. coli* used serological identification of somatic antigen (O) using slide agglutination test according to **Edward and Ewing (1972)**. Antisera of *E. coli* were obtained from Denka Sicken Co. Ltd, Tokyo, Japan.

Experimental chicks:

Two hundred, one day-old Ross broiler chicks were obtained from Socary Hatchery and feed on balanced ration free from medication.

Plant material and its extraction:

The two natural plant samples used in this study were flowers of *Syzygium aromaticum* (clove) and unorganized part of *Garlic*. Samples were purchased from market, distributed in Zagazig region during April 2014, then dried in an incubator at 37°C, the samples were ground into fine powders using electric blender and extracts were by soaking 125mg of each samples separately in 500ml solvents, (distilled water 80% ethanol and n-hexan) using conical flasks plugged with cotton plugs, the mixture were kept at room temperature for 72hrs under discontinuous shaking. The crude extracts were filtered through sintered glass funnel (500 ml) under vacuum. The filtrate were evaporate to dryness by rota-vapour (Buchi, R-215, Switzerland), the rotary water bath was adjusted to 37°C, then the extract were kept over-night under vacuum fume hood to obtained a constant dry weight and extracts stored at 4°C for further use. The extracts weighted and dissolved at concentration of 50mg/ml.

Antibacterial tests:

Independent and in combination watery, 80% ethanol and n- hexan extracts of two plants were tested against *E. coli* 0111 • The growth inhibitory affect were determined by agar well diffusion method as previously described by **Collins et al., (1995)**. Active cultured for experiments were prepared by transferring loopful from *E. coli* 01 11 to test tubes of Mueller-Hinton broth (Oxoid –England). The test tube were incubated at 37°C for 24 hrs., the cultured were diluted with broth to achieve an optical density corresponding to 2.0×10^6 (cfu/ml) after agar solidification. Muller Hinton agar were swabbed with suspension of *E. coli* 0111 using sterile cotton swab. The medium was punched with six mill i meters diameter well, and filled 100

ml or test sample and allowed to diffuse at room temperature for 20 mint.

The minimum inhibitory concentration (MIC) of individual and in combination extract were determined by microdilution technique as described by National Conunittee for Clinical Laboratory Standard (NCCL 2000).

Experimental infection :

Two hundred day old Ross chicks were divided into five equal groups (1, 2, 3, 4 and 5) of 40 chicks of each were reared in separate

Unite and fed on ration without any medication . Group(1) chicks were kept as negative control (non infect and non treated). At 7 day-old group 2, 3 and 4 chicks were injected with *E. coli* O11 1 in a dose 0.25 ml of 2×10^6 cfu/ml intratracheal injection according to **Stipkovits (1988)**. Group (2) chicks, were remained as positive control, group (3) chicks were treated with a dose 25mg/l of Aromaticurn in drinking water for 3 successive days. Group (4) chicks were treated with a dose 25mg/l of Garlic extract in drinking water for 3 successive days.Group (5) chicks were treated with aromaticum and Garlic extract in drinking water for 3 successive days.

Two weeks post treatment all remind chicken weighted and determine. The body weight gain, FCR and sacrificed 5 chicken each group for P.M and bacteriological examination .

Results and Discussion

Bacteriological and serological identification:

The results of bacteriological examination of 80 diseased chickens for detection of *E. coli* showed 49 strain of *E. coli* were isolated. Serogrouping of the isolates indicates presence 6 different serotype and 17 were untyped.

The inhibitory effect of water 80% ethanol and n-hexane plant extracts:

(alone or in combination) was evaluated on *E. coli*. The result of inhibition zone diameter (IZD) and MIC of independent plant extracts against *E. coli*. Water extract of Aromaticum exhibited inhibitory effect against *E. coli* with IZD 14.3 n.l.m and MIC 3.12 mg/ml, wh i le *E. coli* were susceptible to ethanolic and hexanic extract of Garlic 17-mrn and MIC (6 -12).

The ethalonic extract of Garlic inhibit show that growth of *E. coli* with IZD was 14.33 imll and MIC was 6.52 while water extract of Garlic did not inhibit *E. coli*.

Plants remain one of the main sources of the natural products for new therapies particularly in poor countries because most of them are cost less,

affect a wide range of antibiotic resistant microorganism and another reason is there is an erroneous impression that herbal medicines have fewer adverse effects (**Ozoula et al., 2010**). In these study extraction was done using water 80% ethanol and n-hexan. Chemical content of plant extracts differ depending on the nature of the solvent used in extraction procedure (**Jules et al., 2011**).

In the present study individual ethalonic and n - hexan extracts of clove showed inhibitory activity against *E. coli*. Our finding agree with other observations (Sulieman et al., 2007 and Ram et al., 2010) who demonstrated the clove ethalonic extracts exhibited the maximum zone of inhibition against tested bacteria. Variable grow inhibitory effect of individual water, ethalonic and hexanic extracts of Garlic on tested bacteria was detected with IZD ranged from 0.0 - 14.3mln. This finding was supported by Omer et al., (2011).

Result of experimental infection:

Clinical signs, mortality rate and lesion score in infected non treated Birds (group 2) showed sign of depression off food, diarrhea with sever P.M. lesion (air saculits, pericarditis and perihepatitis) and high mortality reach 60%. These finding was subsided post treatment with both extracts and the mortality rate was 15% with mild lesion score with cohphora while the morality rate was 20% in chicken treated with Garlic.

All chicks of group (1 and 5) were negative culture for *E. coli* (O111) however, a higher frequency of resolution of the pathogen from liver and heart of group 2 compared with those groups 3 and 4 for *E. coli* symptoms were observed.

Experimentally infected chicks with isolated *E. coli* 011 1 were showed clinical signs in form of sever respiratory signs with securities, peri card itis and perihepatitis on postmortem examinati on. Experimentally infected chickens showed high mortality rate (60%). The mortality rate infected chickens was reduced to 15 and 20% after treatment with Aromticum and Garlic extracts respectively.

The treatment with Aromticum was inore effective than Garlic for controll ing infection *E. coli* supporting it is still effective lead to decreasing mortality from 60% (infected and non- treated) to 15%, this 1nay be due to Aromticum extract active against *E. coli*. The salne result of Ram et al., (2010) who demonstrated clove extract exhibited the maximum zone of inhibition against tested bacteria.

Garlic extracts has low efficacy for controlling *E. coli* where highest lesion scores with high mortality rate of infected and treated with Garlic

The influence of infections by *E. coli* body weight, food consumption (F.C) and feed conversion rate (FCR). The mean body weight of infected, untreated chicks was significantly ($p>0.05$) decrease than infected and treated chicks (table 3).

Also improvement of general condition body weight gain and feed conversion rate caused by treated with Aromti cun1 and Garlic were recorded after use of both extracts in this study **Ferendez et al., (1998)** stated that the improvement of body weight gain in infected bacteriological effect of antibiotics on the infection and consequently improvement of general health condition.

Table (1): Serological identification of pathogenic Strain *E. coli* (49 strain) isolate from broiler chicks suffering from respiratory disease

Scrogroup	01	08	018	078	086	0111	Untyped strains
Number of isolates	8	2	3	6	8	11	17
6/6	10	4.9	6.1	12.2	10	22.4	46

Table (2): Mortality rate lesion score and reisolation of *E. coli* 0111 isolates following the treatment with Aromticum and Garlic extracts 25mg/ml for 3dyes

group	Mortality %	Lesion score			Frequency reisolation
		Pericarditis	Air Saculities	Pcrihepatitis	
1	0	0	0	0	0
2	60	70	70	70	75
3	15	20	20	20	20
4	20	25	25	25	25
5	0	0	0	0	0

Table (3): The influence of Aromticum and Garlic extract 25mg/ml for 3 day on experimental chicken performance

Group	Befor treatment 10th day infection				Two week post treatment			
	Body weight (g)	Body weight gain	FC	FCR	Body weight (g)	Body weight gain	FC	FCR
1	230 ± 10	190 ± 6	340 ± 11	1.57	110 ± 21	870 ± 18	1449 ± 35	1.7
2	236 ± 12	195 ± 7	307 ± 15	1.57	82± 38	584 ± 32	122 ± 42	2.1
3	238 ± 12	195 ± 8	303 ± 12	1.55	97 ± 20	732 ± 21	131 ± 40	1.8
4	236 ± 16	195 ± 12	308 ± 8	1.58	940 ± 25	704 ± 26	130 ± 38	1.85
5	232	191	299.9	1.57	1200 ± 30	960 ± 32	159 ± 41	1.65

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