EFFICACY OF OREGANO OIL, CITRUS OIL AND DIGESTAROM® P.E.P. ON NEWCASTLE DISEASE INFECTION IN BROILERS

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ABSTRACT

The present study was carried out to detect efficacy of essential oils (oregano oil, anise oil, citrus oil and Digestarom® P.E.P) as an antiviral on Newcastle disease infection in broilers.One hundred twenty, one-day-old broiler chickens (Copp500) were used. On day twenty, the one hundred chickens were divided into six groups.Each group contained 20 birds. All chicks were challenged with median($10^{8.88}$ $/ml$)virus titer embryo infectious dose (EID50) 1ml per bird intraocular, intranasal and orally of the isolated very virulent Newcastle disease virus that identified by (HA, HI test, PCR and ICPI) and treated with the essential oils blend level used in drinking water for five days after challenge, G1 oregano oil1ml/L , G2 anise oil 1ml/L, G3 citrus oil1ml/L, G4 Digestarom® P.E.P 0.17ml/L, G5 control positive, and G6 control negative without any treatment. The chickens were observed for clinical signs, gross lesion and weight until the end of the experiment.The result indicated that G4showed high protect than other oils with percentage 95% of survival chicks and mean weight 2054.42 gm, then G1 90% survival chickens and mean weight 1971.17gm, G2 85% survival chicks and mean weight 2148.88gm,G3 75% survival chicks and mean weight 2227.07gm while G5 protection was 60% with mean weight 1866.67gm and G6showed 100% mortality rate.The results illustrate that G4 and G1 were the best treatments which recorded significantly (P≤0.01) followed by G2 and G3 respectively which recorded significantly (P≤0.05) in comparison with G5and G6.

Key words: essential oils (EOs), antivirus, broilers, survival, weight .

فعالية زيت المردقوش وزيت اليانسون وزيت الليمون و Digestarom® P.E.P على الاصابة بمرض نيوكاسل في فروج اللحم

رامي اسماعيل ابراهيم **عماد جواد خماس**
باحث **استاذ**
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المستخلص

هذه الدراسة تستبين مدى فعالية الزيوت الاساسية (زيت المردقوش و زيت اليانسون و زيت الليمون و Digestarom® P.E.P) كمضاد فايروسي ضد مرض نيوكاسل في فروج اللحم.مائةً و عشرون طيراً من فروج اللحم (Copp500) بعمر يوم واحد. مائة طير قسمت الى ستة مجاميع, كل مجموعة تضم عشرين طيرا, كل الطيور اعطيت جرعة التحدي بنصف الجرعة القاتلة بجرعة 1 مل لكل طير. (مل/ $10^{8.88}$) معيارية الفايروس بطريقة التقطير بالعين والانف والفم من العترة المعزولة العالية الضراوة والمشخصة بواسطة (HI ,HA test, PCR and ICPI)وعولجت بالزيوت النباتية بخلطها مع الماء لمدة خمسة ايام بإضافة زيت المردقوش للمجموعة الاولىG1 وزيت اليانسون للمجموعة الثانيةG2 وزيت الليمون للمجموعة الثالثة G3 و Digestarom® P.E.Pللمجموعة الرابعة G4 وتركت المجموعة الخامسةG5 والمجموعة السادسة G6 بدون علاج. وتم ملاحظة العلامات السريرية والآفات التشريحية العيانية والوزن للأفراخ حتى نهاية التجربة.النتيجة المتحصلة G4اعطت حماية اعلى من المعاملات الباقية بنسبة بقاء 95% وبمعدل وزن2054.42 غم مقاربة مع G2 التي اعطت نسبة بقاء 90% ومعدل وزن gm1971.17 , G2 85% نسبة بقاء و معدل وزن gm2148 ,G3 75% نسبة بقاء ومعدل وزن gm2227.07 بينما لوحظ على مجموعة G5 60% نسبة حماية ومعدل وزن gm1866.67 و G6 كانت 100% نسبة الوفيات.النتيجة الاحصائية المتمثلة بG4و G1 كانت افضل معاملتين سجلت فرق معنوي (P≤0.01)يتلوها G2 وG3 على التوالي حيث سجلت فرق معنوي (P≤0.05) مقارنة معG5 و. G6

الكلمات المفتاحية: الزيوت الاساسية, مضاد فايروسي, فروج اللحم,البقاء, الوزن.

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INTRODUCTION

Newcastle Disease is an important viral disease of poultry due to its wide distribution and high mortality rate. The disease is highly contagious and deaths can be sudden and whole farm can be lost (13,14), either through 100% fatality rates (22). Outbreaks are caused by virulent strains of Newcastle disease virus, which has been institute to be endemic in several countries.Natural products of the plants origin have been usually used to treat variety of diseases (23). The activity of the EOs is related to their structure, functional collections, and synergistic relations between components (15).Essential oils have antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties (21,24). Also, Essential oils were multifunctional and a wide spectrum of activities, such as immunomodulatory, antiphlogistic, antidiarrheal, spasmolytic, antinociceptive, psychotropic, expectorative, acaricidal, and cancer-suppressing activities. These activities can be mediated by single compounds or groups of compounds (6, 20).

MATERIALS AND METHODS

Experimental design: One hundred twenty, one–day-old broiler chickens (Copp500) from a commercial hatchery, Al-Salam al-hadith hatchery Babylon province, were used. All chickens received the same grower ration with the following percentage of the major components namely, Protein (20%), Fat (5.45%), Fiber (2.7%) and a mean energy value of 3125 Kcal/Kg. The rest of the components in the diet have their concentrations adjusted according to the NRC (1994) values.

Essential oil blend in drinking water

The three essential oils are *origanum marjoram, pimpinellaanisum, and citrus aurantium*oils were extracted by water distillation by University - College of Pharmacy. The essential oil blend level used in drinking water was 1ml\L. The Digestarom® P.E.P is a registered trademark of BIOMIN Holding GmbH (IR-681524) in dosage 0.17 ml/ L drinking water (5).

Vaccination

The vaccination for Newcastle disease virus was accomplished via intraocular, intranasal and orally at 14 day of age in the same room. The vaccine AVI ND LaSota® (Laprovet Ltd) was used.

Challenge: In twenty day old, one hundred twenty chickens were dividedinto six groups each group contained 20 birds. All chicks were challenged with median $(10^{8.88}\backslash ml)$virus titer embryo infective dose (EID50) 1ml per bird intraocular, intranasal and orally, and treatment with the essential oil blend level was used in drinking water, and was supplemented with G1 oregano oil1ml/L , G2 anise oil 1ml/L, G3 citrus oil1ml/L, G4 P.E.P 0.17ml/L G5, and G6 control without any treatment. The chickens were observedfor clinical signs, weight and post mortem lesions until the end of the experiment, 15 days post challenge.

Statistical Analysis

The Statistical Analysis System (26) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

RESULTS AND DISCUSSION

1- Clinical sings

All groups showed mild to severe clinical signs characterized mainly by depression, loss of appetite, edema in the head and around the eyes, conjunctivitis lacrimation in the third – fourth day after challenge. Except the G6 was very severe (Table 1) Respiratory signs: nasal discharge,coughing,sneezin gappeared in the fourth day, observed in G1 and G4 mild signs (+), G2 moderate signs (++), while G3 and G5 severe signs (+++), and G6 signs (++++).Digestive signs: white greenish diarrhea was noticed in G1,G2, and G4 (+), while G3 and G5 (+++)and G6 (++++) in the third-fourthday after infection.Nervous signs: paralysis in legs and wings and torticollis were seen in G2 and G3 mild (+),G5 mild (++), and G6 severe (++++), while G1 and G4 no nervous signs observed. The mean mortality ratein G1 was 10%,andthe average body weight was 1437.89 gminday 28,the weight in the end of the experiment was1971.17gm (35days), G2 15%, and average body weight 28 days 1476.44 gm, the weight in the end of experiment was 2148.88 gm (35 days), G3 25%, and average body weight 1510.76gm in

28 days, the weight in the end of experiment was 2228.07 gm in 35 days), G45%, and average body weight 1475.37gm in 28 days, the weight in the end of experiment was 2054.42gm in 35 days, G5 40%, and average body weight 1333.62gm in day 28, the weight in the end of experiment was 1866.67gm in 35 days and G6 all chickens were died through seven days after challenge, the mortality rate was 100%, this result proved that the isolated strain of NDV was very virulent.

Table 1. The clinical sings in all groups

Treatment / Clinical sings	G1 Oregano oil	G2 Anise oil	G3 Citrus oil	G4 P.E.P	G5 control +ve	G6 control -ve	P-value
Depression, loss of appetite,	++	+ +	+ +	++	+ + +	+ + + +	0.0281 *
Edema around the eyes and the head, conjunctivitis, lacrimation,	+ +	+ +	+ + +	+ +	+ + +	+ + + +	0.0296 *
Nasal discharge, coughing, sneezing	+	+ +	+ + +	+	+ + +	+ + + +	0.0074 **
Greenish diarrhea	+	+	+ + +	+	+ + +	+ + + +	0.0069 **
Paralysis in legs & wings, torticollis	-	+	+	-	+ +	+ + + +	0.0001 **
No. chicken death	2	3	5	1	8	20	
* (P≤0.05), ** (P≤0.01).							

+ mean mild, + + mean moderate, + + + severe, + + + + vary severe

Table 2. Mortality rate and mean weight in different groups in (28 days old)

Group	Mortality rate (%)	Mean ± SE of body weight/28 day (gm)
G1: Oregano	10% cd	1437.89 ± 37.12 ab
G2: Anise	10% cd	1476.44 ± 39.91 a
G3: Citrus	15% bc	1510.76 ± 40.21 a
G4: P.E.P	5% d	1475.37 ± 44.45 a
G5: Control +ve	35% a	1333.62 ± 40.51 b
G6: Control -ev	100% a	/
LSD value	8.031 **	106.84 **
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

Table 3. Mortality rate and weight mean in different groups in (35 days old)

Group	Mortality rate (%)	Mean ± SE of body weight/35 day (gm)
G1: Oregano	10% de	1971.17 ± 82.51 b
G2: Anise	15% d	2148.88 ± 83.21 ab
G3: Citrus	25% c	2227.07 ± 54.99 a
G4: P.E.P	5% e	2054.42 ± 51.55 b
G5: Control +ve	40% b	1866.67 ± 73.05 b
G6: Control -ev	100% a	/
LSD value	9.028 **	178.52 **
Means having with the different letters in same column differed significantly. ** (P≤0.01).		

Gross lesions

G1and G2 showed moderate lesions, (Table 4), but in G2 was more severe than G1, the macroscopic examination include: conjunctivitis, head swelling,congestion in the trachea,lungs,spleen, pinpoint hemorrhages at the tips of the proventricular glands,mild hemorrhagic ulcers in the intestinal wall and enlarged cecal tonsils that appeared mild edematous,bloody and necrotized, moderate fibrinous pricarditis and prihipatitis (airsacculitis) as a secondary complication. G3 showed severe lesions involved conjunctivitis,congestion in the spleen,petechial hemorrhages in pancreas and kidney.Postmortem examinations of infected birds showed pinpoint hemorrhages at the tips of the proventricular glands,hemorrhagic

ulcers in the intestinal wall,enlarged cecal tonsils that appeared edematous,bloody,and necrotized,congestion in the trachea,and fibrinous pericarditis and perihepatitis (airsacculitis) assecondaryinfectionatfirstweek after challenge. G4 showed conjunctivitis, mild congestion in the trachea, very mild hemorrhagic ulcers in the intestinal wall,and enlarged cecaltonsils. G5showed severe lesions including head swelling, severe conjunctival edema and hemorrhage, petechial hemorrhage in thymus, splenomegaly, petechial hemorrhage in pancreas, uratesinkidneys, congestion and mucoid exudate seen in trachea extended to lung with opacity and thickening of the air sac as secondary bacterial complication,liver congestion,hemorrhage ulcers in the intestinal wall,hemorrhage at the proventricular glands enlarged cecal tonsils and Payer's patches that were edematous, bloody and necrotized. G6 showed conjunctivitis, severe congestion in the liver, lung, congestion and mucoid exudates seen in the trachea, enlargement and necrosis of spleen and petechial hemorrhages in pancreas, hemorrhages of the proventricular mucosa extended to esophagus and gizzard, necrotic hemorrhagic areas on the mucosal surface of the intestine, especially lymphoid foci such as Payer'spatches and cecal tonsils filled with greenish material,hemorrhage and edema around the thymusmay also be seen. Birds were markedly dehydrated and showed multifocal accumulation of urates in the kidney.

Table 4. The gross lesion on different organs in all chickens groups

Treatment Grosslesion	G1Oregan ooil	G2Aniseo il	G3Citrusoi l	G4P.E. P	G5control +ve	G6contro l-ve	P-value
conjunctivalHemorrhagesine yelid,	+++	+++	+++	+++	+++	++++	0.092 NS
Congestion in trachea	+++	+++	+++	+++	+++	++++	0.092 NS
congestioninthelungs	+	++	+++	+	+++	++++	0.0001 **
airsacculitis,	+	++	+++	_	+++	++++	0.0001 **
hemorrhagesatthetipsofthepr oventricularglands	_	+	+++	_	+++	++++	0.0001 **
enteritis,hemorrhages inPeyer'spatchesandenlarged cecaltonsils	+	++	+++	+	+++	++++	0.0001 **
** (P≤0.01).							

+ meanmild , + + meanmoderate, + + + severe, + + + + vary severe

Bird showing clinical signs, including sneezing, rales, swollen head, nasal and ocular discharge, greenish diarrhea, torticollis, and recumbency, as well as dead birds were examined for post mortem lesions, such as proventricular haemorrhage, intestinal button-like ulcers, ileocaecal tonsil haemorrhage, and congested pectoral muscles and tracheal mucosa (29).Though the obtained result for vaccinated and EOs treated (of each group) , that were supported previously by Lang and Buchbauer (20) when he proved an improvement of clinical protection (mortality, clinical signs, PM lesions and viral shedding titre) This could be due to stimulating effect on cell-mediated and local immunity, therefore eliminating some of the viruses from tissues (3).EOs have limitations as antimicrobial properties because of their variable composition, which is dependent on species, part of the plant used, and method of extraction (10,16). In addition, high concentrations of botanicals are required to reach minimum inhibitory concentrations (11).The use of ND vaccines, regardless Eos supplementation has improved overall body weight gain in all challenged groups compared to the control positive group, similar results were obtained by Sedeik et al. (27).The increasing weight in groups that were treated with Eos in this study was related to the Eos enhance digestibility, balance the gastrointestinal tract (GIT) microflora and stimulate the secretion of endogenous digestive enzymes, thus improve growth performance in poultry (8,28).The acquired results of body weight gain in this study were similar to the findings of (21,25). The various results of the effect of different types of EOs on bird performance in several studies could be related to variations of dose, active components of the EOs mixture, the period of

administration, and the environmental and nutritional factors (12).This high mean survival rate in treatment groups with EOs is indicative of the safety of NDV vaccination by the live strain and of the administered essential oil blend. It is documented in experiment that the essential oils are devoid of any toxicity effects (5). Thiscurrent result indicates the positive effect of the inclusion of essential oil treatment with the NDV vaccination in increasing the survival percentage of birds against a virulent challenge by the vNDV (PEP 95%),(Oregano 90%),(Anise 85%),(Citrus 75%) actually, the NDV vaccination alone did result only a 60% survival rate against the vNDV used in this experimental. This is in agreement with previous documented studies on the inability of classical vaccines or vectored vaccines to provide protection against the vNDV (7,18). G2 (anise group) and G3 (citrus group) showed high feed intake and body weight compared with G4 (Digestrom P.E.P group) related to acrimonious taste of Digestrom P.E.P at concentration 0.17 ml / L led to decrease of feed intake (27).The ability of Digestrom P.E.P for solubility and miscible in water is related to manufactured acquired it good efficiency as antivirus led to good survival rate, decreasing mortality, clinical signs and gross lesion than another treated groups.Study of Miller and Koch (22) and OIE (29) have indicated same result , that the vaccine alone is not sufficient to give complete protection when the strain of virus was very virulent. This explain increase the mortality rate in G5. Non vaccinated chickens (G6) showed sudden death without clinical signs was happened in the first three days after challenge, very severe clinical signs (+ + + +) appeared in other chicken. This result is similar to study of Adwar and Lukesova (1), when showed that ND is a major constraint to poultry production throughout developing countries, frequently causing mortality rates 100 % in unvaccinated flocks.The finding in control negative(G6) is the same in the study ofAlazawy and Ajeeli (nge of NDV.

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