Abbassa Int. J. Aqua., V0l.9 No.(1) 2016. *36-47*

EVALUATION OF THE POTINTIAL EFFECT OF CLOVE (*EUGENIA AROMATIC*) OIL IN COMBATING THE *AEROMONAS SOBRIA* INFECTION IN AFRICAN CATFISH (*CLARIAS GARIEPINUS* L.)

[1]Alshimaa A. Khalil; [1]Abd elhakim, Y. and [2] Haytham A. Ali

[1]*Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Zagazig University.*
[2]*Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University.*

Received 5/ 1/ 2016 *Accepted 26/ 1/ 2016*

Abstract

The present study was conducted to evaluate the pathogenicity of *Aeromonas sobria* and its ability of producing infections and mortality in African catfish *Clarias gariepinus* L (Experiment I) and to estimate the potential effect of clove oil as a prophylactic measure against *A. sobria* infection (Experiment II). One hundred and eighty apparently healthy African catfish (*C. gariepinus*) weighting 30±5g, collected from Abbassa fish farm, Alsharkia governorate, Egypt. Experimental *Clarias gariepinus* exhibited aggressive clinical signs and gross lesions began after 12 hrs. post inoculation (PI) of *A. sobria* that ensure the bacterial capacity to induce the disease. Results suggested the beneficial use of clove oil against *A. sobria* infection in *C. gariepinus*. In which the mortalities of *C. gariepinus* reached 20 % in 1st group which subjected to clove oil by a dose of (1.3µl/L) as a bath for 2 weeks then experimentally infected Intraperitoneally by a dose of 0.2 ml ($2.5x10^8$ $CFU.ml^{-1}$ of *A. sobria*), however the 2nd group showed about 100% mortalities. While the 3rd and 4th groups showed no mortalities. Results of serum biochemical analysis of using of clove oil as a prophylactic treatment (1st group) is relatively improveme the levels of serum IgM and hepatic reduced glutathione levels (GSH), beside decrease the serum levels of ALT, AST, urea, creatinine and serum cortisol levels with reduction in the hepatic MDA when compared with 2nd group.

Keywords: Clove oil, prophylactic measure, serum biochemical assay, *Aeromonas sobria*, *Clarias gariepinus*.

INTRODUCTION

Aquaculture has increased enormously in recent years, making it the fastest growing food producing project in the world (Subasinghe, 2005). The intensity of aquaculture leads to exposure of farmed fishes to a various types of stressors which includes fish diseases particularly the bacterial one. Aeromonas septicemia is a fatal infectious disease of cold-blooded animals including fish, reptiles and amphibians (Austin and Austin, 1987) and in human (Dryden and Munro, 1989) usually caused by motile Aeromonads, particularly *A. hydrphila*, *A. sobria* and *A. caviae* (Das *et al*., 2013).

Treatment of bacterial diseases in aquaculture with different herbs and plant extracts consider a safe method when compared with antibiotics hazards beside its potent antimicrobial properties (Pandey *et al*., 2012). Clove oil has been reported to inhibit the growth of Gram positive and Gram negative bacteria (Burt, 2004; Nanasombat and Lohasupthawee 2005; Chaieb *et al*., 2009). Beside it has a potent invitro antibacterial activity against *A. sobria* (Mansour *et al*., 2014). Application of clove oil in fish farms is safe, cheap, and nontoxic to the fish and environment. Moreover, it does not require a withdrawal period of 21 days for food fish (Weber *et al*., 2009).

Clinco-chemical analysis is a basic tool used in fish diseases diagnosis to monitor the effect of therapeutic, nutritional, and environmental management (Smith and Reynard, 1992) and so, this study was carried out to evaluate the possible effects of clove oil as prophylactic measure on fish health, blood chemistry and ability to antagonize *Aeromonas sobria* infection.

MATERIALS AND METHODS

Experimental fish:

A total number of 180 apparently healthy African catfish (*C. gariepinus*) weighting 30 ± 5 g, collected from Abbassa fish farm, Alsharkia governorate, Egypt. Prior to the experiment, fish were stocked in aquaria of 40 x 80 x 50 cm with dechlorinated tap water and kept for adaptation to laboratory conditions for 10 days at 28-30 ºC using thermostatically controlled heaters

providing adequate feed twice daily with diet contain not less than 30% crude protein and continuous aeration. The experiment was conducted at the Fish diseases and management Department laboratory of veterinary medicine, Zagazig, Egypt.

Bacterial strain:

Stock culture of *A. sobria* used in this study was obtained from Fish Diseases and Management Dept., Fac. of Vet. Med., Zagazig Univ. which was isolated from internal organs of naturally-infected *Oreochromis niloticus*. The isolates were cultured on a Typticase Soya Agar (TSA) at 24-25°C for 24 hours and confirmed identification done using conventional tests specially, Gram staining, motility and catalase tests. The bacterial strain was tested for β-haemolytic activity on blood agar base, supplemented with 5% of sheep erythrocytes to ensure its ability for induction of the disease.

Clove oil:

Clove oil was obtained from local market in Zagazig city, Egypt. Lethal concentration dose (LC_{50}) of clove oil of African catfish *C. gariepinus* was estimated by Mahboub (2011) as 26.5 µl/L. The used dose for prophylactic treatment was 1/20 from LC_{50} as a bath for 2 weeks.

Experimental design:

Determination of the pathogenicity of *A. sobria* in *C. gariepinus* (Experiment I):

The challenge test applied through intraperitoneal (IP) inoculation to know the efficacy of the pathogens in initiating the infection and monitoring the mortality. After acclimatization for 10 days, 60 *C. gariepinus* were randomly distributed into two groups as 10 fish per group with three replicates. The 1st group (infected group) in which fish was IP injected with 0.2 ml of *A. sobria* culture suspension with a concentration of $2.5x10^{8}$ CFU.ml^{-1}. The 2nd group (control non infected group) in which fish IP inoculated with sterile phosphate buffer saline (PBS). Experimented fishes kept for observation and daily

mortalities monitoring for two weeks (Experimental period). Re-isolation of inoculated bacteria was done by collecting samples from kidney, liver and spleen of moribund and freshly dead experimentally infected *C. gariepinus*.

Efficiency of clove oil in combating the *A. sobria* infection in *C. gariepinus* (Experiment II):

One hundred and twenty *C. gariepinus* were used to elucidate the efficiency of clove oil as prophylactic measure against *A. sobria* infection. Fish were divided into four groups each group contain three replicates (10 fish per replicate). 1st group was supplemented with clove oil by a dose of (1.3µl/L) as a bath for 2 weeks then inoculated IP by a dose of 0.2 ml ($2.5x10^8$ CFU.ml^{-1} of *A. sobria*), fish kept for 1 week "observation period"; 2nd group (infected non-treated) in which fish inoculated IP with 0.2 ml ($2.5x10^8$ CFU.ml^{-1} of *A. sobria*) and acts as control positive; 3rd group (non-infected, non-treated), in which fishes inoculated IP with sterile PBS. 4th group (non-infected and treated with clove oil) in which fish subjected to clove oil by a dose of (1.3µl/L) as a bath for 2 weeks. The experimental period is 2 weeks during which all aquaria was completely water change day after day and the dose of clove oil readjusted.

Blood samples:

Blood samples were collected at the end of experiment II, then centrifuged at 3000 rpm for 15 minutes to separate serum. Serum samples were then used to evaluate aspartate aminotransferase (AST) and alanine amino transferase (ALT) enzymes colorimetrically (Reitman and Frankel 1957; Breuer 1996). Serum urea (Chaney and Marbach 1962), creatinine (Husdan and Rapoport 1968) and cortisol levels (Foster and Dunn 1974) were also estimated. Moreover, Serum immunoglobulin (IgM) concentrations were analyzed (Siwicki and Anderson 1993).

Tissue samples:

Small parts from liver (0.5gm) were collected at the end of experiment II, and homogenated in ice by electrical homogenizer then centrifuged at 3000 rpm for 15 minutes to separate homogenate that used to evaluate malondialdehyde

(MDA) concentration as a lipid peroxidation marker (Ohkawa *et al.,* 1979) and reduced glutathione (GSH) content (Moron *et al.,* 1979).

Statistical analysis

Data were analyzed using SPSS Statistics program, where the analysis of variance (ANOVA) and Duncan`s Multiple Range Test (Duncan, 1955) were used to detect the differences between treatments (levels of significance are expressed as $P \leq 0.05$).

RESULTS AND DISCUSSION

Clinical and gross pathology of experimental challenged fish (Experiment I):

Fish in Group 1 (infected tested fish) exhibited aggressive clinical signs and gross lesions which begin after 12 hrs. post inoculation (PI) of *A. sobria*. Fish began to die and the clinical signs begin to appear. Fish become listlessness, aggregate to side of aquaria with no movement most time make them easily to be catched. The external lesions could be summarized as severe erythema and hemorrhages on skin, fins, operculum and around burbles in addition to presence of greenish stain on the abdominal skin (Fig.1). With dissection of freshly dead and moribund fish, we found friability and congestion of all internal organs especially liver and gall bladder (Fig.2). At the end of the 3rd day PI fish exhibited highest clinical infection and mortalities, which reached up to 100% in the 1st group whereas no clinical signs or mortality were observed in 2nd group (control negative group).

The general septicemic lesions and high mortality manifested on experimentally infected fish either internally or externally explain how is the *A.sobria* was pathogenic to *C. gariepinus* by producing of cytotoxins that is undergo induce the lesions. This result go hand with hand with that mentioned by Janda and Abbott (2010); Omprakasam and Manohar (1991) who explained the induction of skin lesions by *Aeromonas* spp. is due to cytotoxin produced by this bacterium. Anyanwu *et al*. (2014) stated that higher percentage of fish with

skin lesion in the *A. sobria* groups compared with other infected groups which indicate that *A. sobria* had more damaging effects on fish skin.

High fish mortality observed in infected groups when compared with other groups which prove that the *A. sobria* was pathogenic to the African catfish *C. gariepinus*. This result coordinated with that of Austin and Austin (2012); Anyanwu *et al*. (2014) who reported that the mortality due to experimentally infected *C. gariepinus* may resulted from septicaemia/toxaemia caused by the bacterium strain *A. sobria*. Also Noga (2010) suggested that the mortality may be as a result of the alteration of homeostasis of the fish that manifested as skin damage.

Efficacy of clove oil as prophylactic measure against *A. sobria* infection in *C. gariepinus* (Experiment II):

Our results cleared that, the mortality in the 1st group within 72 hrs. PI which reached 20 %, however the 2nd group showed about 100% mortalities, in which all *C. gariepinus* died within 72 hrs. PI. Whereas the 3rd and 4th groups showed no mortalities. Results suggest the beneficial use of clove oil against *A. sobria* infection in *C. gariepinus*, it may be due to that clove oil has potent antimicrobial agents against bacterial infection as it has two major phynolic components which are eugenol (2-methoxy-4-(2 propenyl) phenol) and eugenyl acetate, these two compounds are responsible for the antibacterial activity of clove oil as explained by Burt (2004).

Assessment of serum parameters and enzyme activities is considered a confirmatory tool for the effect of clove oil as a prophylactic agent against the *A. sobria* infection (Table 1). Serum analysis revealed that the using of clove oil as prophylactic measure (1st group) relatively success to improve the levels of hepatic reduced glutathione levels (GSH) and serum IgM and decrease the serum levels of ALT, AST, urea, creatinine and serum cortisol levels with decrease in the hepatic MDA when compared with 2nd group (control positive) but not reached to normal levels of control negative (3rd group). The results may be due to that clove oil enhance the general antioxidant status, immunological

status, both liver and kidney functions by improving the serum levels of GSH, MDA, IgM, ALT, AST, urea and creatinine specially in the 4th group, but it didn't have the power to face the deleterious effect of *A. sobria* and did not completely protect fish against the infection. Contrary, the 2nd group "control positive" in which the infection with *A. sobria* leads to increases in the ALT, AST, serum urea, creatinine levels, serum cortisol levels and hepatic MDA (marker for lipid peroxidation) levels; with decrease in the levels of hepatic reduced glutathione levels (GSH) and serum IgM values.

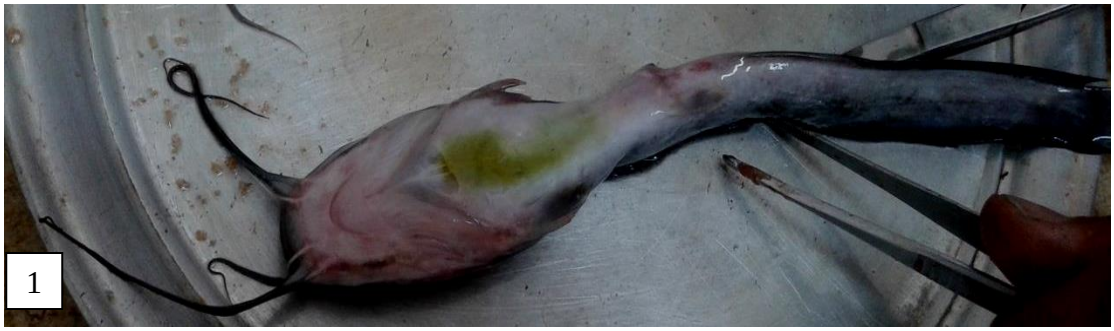
The fish liver plays an important role in vital functions in basic metabolism and it is the major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo-Fernandes *et al*., 2006). The measurement of suitable biomarkers in liver becomes useful and can give an idea about the health state of fish. Among the biochemical profiles, liver enzymes monitoring the liver status and has been proved to be a very useful tool in liver toxicological studies (Osman *et al*., 2010). The transaminases, AST and ALT are two key enzymes considered as a sensitive measure to evaluate hepatocellular damage and some hepatic diseases (Ibrahim and Mahmoud, 2005). So, in the present study, the increase in AST and ALT transaminases might be attributed to tissue damage; particularly liver as a result of bacterial infection.

CONCLUSION

It was confirmed that *Aeromonas sobria* were highly pathogenic and able to induce infection as well as severe mortality in the experimentally infected African catfish *C. gariepinus*. In addition, the use of essential oils as clove oil *Eugenia aromatic* by a dose of 1.3µl/L as a prophylactic measure for 2 weeks can help in combating and minimizing the *A .sobria* infection. Further studies are recommended to clarify the effect of prolonged exposure to clove oil in water or addition in diets on the fish resistance to *A. sobria* infection in African catfish.

Acknowledgement:

Our sincere thanks and appreciation to Dr. Shaimaa Abdul Karim Ali Assistant Lecturer, Department of fish diseases and management - Faculty of Veterinary Medicine-Zagazig University to provide well identified *Aeromonas sobria* strain.



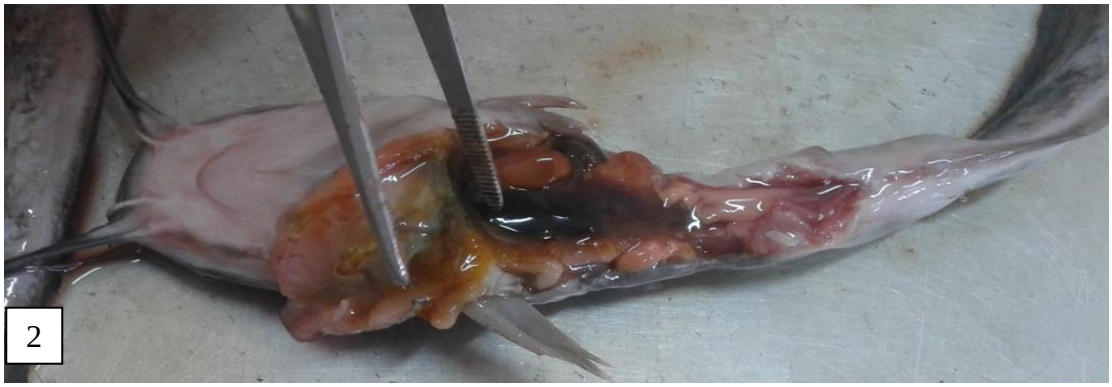


Fig. 1. *C. gariepinus* experimentally infected I/P with *A. sobria* 12 hrs. post inoculation showed erythema at base of operculum, mouth and around burbles beside greenish coloration of abdominal skin.

Fig. 2. *C. gariepinus* experimentally infected with I/P *A.sobria* 12 hrs. post inoculation showed enlarged friable gall bladder and kidney.

Table 1. Serum parameters and enzyme activities of *C.gariepinus* in experiment II.

Group parameters	1st group	2nd group	3rd group	4th group
ALT (IU/dl)	56.32±2.34[b]	86.37±3.22[a]	41.45±1.36[c]	40.23±2.21[c]
AST (IU/dl)	37.14±2.13[b]	56.24±2.95[a]	24.65±1.53[c]	23.84±1.21[c]
Hepatic GSH (ng/ml)	14.06±0.92[b]	8.46±0.23[c]	16.89±1.02[a]	17.2±1.06[a]
Hepatic MDA (nmol/l)	24.3±1.16[b]	32.31±2.14[a]	18.4±1.3[c]	19.1±1.93[c]
Serum Cortisol value (µg /ml)	5.82 ±0.52[b]	9.23 ±0.27[a]	4.33 ±0.2[c]	4.21 ±0.43[c]
Serum creatinine (mg/dl)	0.81±0.02[b]	1.85±0.08[a]	0.46±0.03[c]	0.47±0.04[c]
Urea (mg/dl)	0.94±0.06[b]	1.72±0.02[a]	0.62±0.04[c]	0.6±0.03[c]
IgM value(µg /ml)	16.32±0.21[b]	9.38±0.54[c]	22.42±0.09[a]	23.18±0.1[a]

Means ± S.E in the same row carrying different superscript were significantly different (P<0.05).

REFERENCES

Anyanwu, M.U; K.F. Chah and V.S. Shoyinka, 2014. Evaluation of pathogenicity of motile *Aeromonas* species in African catfish. ISSN: 2347-5129 IJFAS 2015, 2 (3): 93-98.

Austin, B. and D.A. Austin, 1987. Bacterial fish pathogens: disease in farmed and wild fish. Halsted Press, New York, N.Y.

Austin, B. and D.A. Austin, 2012. Bacterial fish pathogen of farm and wild fish, Edn 5, Springer, United Kingdom, 19-22.

Breuer, J., 1996. Report on the symposium "Drug effects in Clinical Chemistry Methods". *Eur J Clin Chem Clin Biochem* 34, 385-386.

Burt, S., 2004. Int. J food Microbiol, 94. 223-253.

Chaieb, K; T. Hajlaoui Hzmantar; A.B. Kahla- Nakabi; M. Rouabhia; K. Mahdouani and A. Bakhrouf, 2009. Phytother. Res., 6, 501-506.

Chaney, A.L. and E.P. Marbach, 1962. Modified reagents for determination of urea and ammonia. *Clin Chem*, 8: 130-132.

Das, A; A. Rathore; C. Janani1; C. Hemanth and R. Arvind Balakrishnan, 2013. Diagnosis Of Motile *Aeromonas Sobria* From Cat Fishes With

Septicemia By PCR. Journal of Agriculture and Veterinary Science (IOSR-JAVS). ISSN: 2319-2380, Volume 2, Issue 6: PP 87-91.

Dryden, M. and R. Munro, 1989. Aeromonas septicemia: relationship of species and clinical features, Pathology, 21 (2): 111-4.

Duncan, B. 1955. Multiple range and multiple (F) tests. Biometrics, 11: 1-2.

Figueiredo-Fernandes, A; A. Fontainhas-Fernandes; E. Rocha and M.A. Reis-Henriques, 2006. The effect of paraquat on hepatic EROD activity, liver and gonadal histology in males and females of Nile Tilapia, *Oreochromis niloticus*, exposed at different temperatures. Archives of Environmental Contamination and Toxicology, 51 (4): 626-632.

Foster, L.B. and R.T. Dunn, 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clin Chem*, 20: 365-368.

Husdan, H. and A. Rapoport, 1968. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. *Clin Chem*, 14: 222-238.

Ibrahim, S.A. and S.A. Mahmoud, 2005. Effect of heavy metals accumulation on enzyme activity and histology in liver of some Nile fish in Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 9 (1): 203-219.

Janda, J.M. and S.L. Abbott, 2010. The genus *Aeromonas*: taxonomy, pathogenicity and infection. Clinical Microbiology Reviews, 23:35-73.

Mahboub, H.H., 2011. Studies on some mycotic diseases in cultured fishes. Thesis submitted to Zagazig Univ. Fish Diseases and Management Dept.

Mansour, A.M.A; H.M. Zaki; N.A. Hassan and N.A.M. El-Nashar, 2014. Phenotyping, virulence characteristics of *Aeromonas* species and the effects of essential plant oils as antimicrobial agents against pathogenic isolates from different sources. *American Journal of Infectious Diseases* 10, 21-35. DOI: 10.3844/ajidsp. 21.35.

Moron, M.S; J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta*, 582: 67-78.

Nanasombat, S. and P. Lohasupthawee 2005. KMITL, Sci. Tech. J., 5: 527-538.

Noga, E.J., 2010. Fish disease: Diagnosis and treatment, 2nd edn. pp. 519. Ames, Iowa: Wiley-Blackwell.

Ohkawa, H.; N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95: 351-358.

Omprakasam, M. and L. Manohar, 1991. Experimental infection of some bacterial fish pathogens in the cichlid fish, *Oreochromis mossambicus*. Indian Journal of Fisheries, 38:106-110.

Osman, A.G.M; M. Koutb and A.E.H. Sayed, 2010. Use of hematological parameters to assess the efficiency of quince (Cydonia oblonga Miller) leaf extract in alleviation of the effect of ultraviolet- A radiation on African catfish *Clarias gariepinus* (Burchell, 1822). Journal of Photochemistry and Photobiology B: Biology, 99: 1-8.

Pandey, G; M. Sharma; A.K. Mandloi and Y.P. Shani, 2012. Antimicrobial activity of some medicinal plants against fish pathogens. Int. Res. J. Pharmacy, 3 (4): 28-30.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*, 28: 56-63.

Siwicki, A.K. and D.P. Anderson, 1993. Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. In Fish disease diagnosis and prevention methods ed. Siwicki, A.K., Anderson, D.P. and Waluga, J. pp. 105-112. Olsztyn, Poland: FAO-Project GCP/INT/526/JPN, Wyd. IRS.

Smith, C. M. and A.M. Reynard, 1992. "Text Book of Pharmacology." W. B. Saunders Company Philadephia, London, Toronto, 878pp.

Subasinghe, R. 2005. State of world aquaculture FAO, Fisheries and Aquaculture Department, Rome.

Weber, R.A; J.B. Peleteriro; L.O. Garcia Martin; B. Aldegunde, 2009. The efficacy of 2 – phenoxy ethanol, metomidate clove oil and MS 222 as anaesthetics agents in the Senegalese sole. Aqua, 288: 147-150.

تقييم التأثير المحتمل لزيت القرنفل (يوجينيا العطرية) في مكافحة العدوى بالإيروموناس سوبريا في القرموط الأفريقي

الشيماء خليل[١] ، ياسر عبد الحكيم[١] ، هيثم علي جاد[٢]

[١] قسم أمراض ورعاية الأسماك – كلية الطب البيطري– جامعة الزقازيق.
[٢] قسم الكمياء الحيوية – كلية الطب البيطري – جامعة الزقازيق.

الملخص العربي

أجريت الدراسة الحالية لتقييم القدرة المرضية للأيروموناس سوبريا وذلك من خلال القدرة علي احداث عدوي ونفوق القرموط الأفريقي (التجربة الأولي) ولتقدير التأثير المحتمل لزيت القرنفل كإجراء وقائي ضد العدوىبالأيروموناس سوبريال (التجربة الثانية). تم العمل علي ١٨٠ قرموط افريقي والتي تزن ٣٠ ± ٥جم، والتي تم الحصول عليها من المزارع السمكية بالعباسة، محافظة الشرقية، مصر. أظهرت القراميط المعداه تجريبيا أعراض ظاهرية عنيفة واصابات خارجية جسيمة التي تبدأ بعد ١٢ ساعة بعد الحقن و التي تؤكد القدرة البكتيرية لإحداث المرض. وتشير النتائج الاستخدام المفيد من زيت القرنفل ضد عدوى الأيروموناس سوبريا في القرموط الأفريقي. حيث بلغت نسبة النفوق ٢٠% في المجموعة الأولي اوالتي تعرضت لزيت القرنفل بجرعة من (١٠.٣μl/ L) كحمام علاجي لمدة أسبوعين ثم حقنت تجريبيا بجرعة ٠.٢ مل (٢.٥ x ١٠^{٨} وحدة بكتيرية في الملي) في حين أن نسبة نفوق الأسماك في المجموعة الثانية كانت ١٠٠% بينما لم تظهر المجموعات الثالثة والرابعة أية نسبة للنفوق. نتائج تحليل الكيمياء الحيوية في الدم تؤكد وتدعم النتائج حيث أن إستخدام زيت القرنفل كعلاج وقائي (المجموعة الأولي) ساعد نسبيا في تحسين مستويات الجلوتاثيون المختزل (GSH) في الكبد ومعدل الغلوبولين المناعي، بجانب خفض مستويات ALT، AST، واليوريا، والكرياتينين والكورتيزول في الدم وأيضا انخفاض في MDA بالمقارنة مع المجموعة الثانية . لذا يوصي باستخدام زيت القرنفل كوقايه وتقليل من التاثير المرضي لميكروب الايروموناس سوبريا في القرموط الأفريقي.