Egypt. J. Aquat. Biol. & Fish., Vol. 4, No.2 : 43 -93 (2000) *ISSN 1110-6131*

CHANGES IN THE HAEMATOLOGICAL PARAMETERS OF CARP, *CYPRINUS CARPIO* L., INDUCED BY LAMINARIN AND *NIGELLA SATIVA* OIL DURING THE MOTILE *AEROMONAS* SEPTICEMIA DISEASE

Mostafa A. El-Feki[1]; Hanaa E. Fahim[2]; Mamoon M. Gad[2] and Fatehy A. Abdel-Ghaffar[3]

1- Department of Zoology, Faculty of Science,ElMinia University.
2- Department of Zoology, Faculty of Science, Beni-Suef, Cairo University.
3- Department of Zoology, Faculty of Science, Cairo University.

(Received : January 22, 2000)

Key words: Carp, *Aeromonas hydrophila*, *Nigella sativa*, Laminarin, Blood parameters.

ABSTRACT

Aeromonas hydrophila is responsible for heavy mortality to fish stocks specially under culturing conditions. It is usually associated with the development of fish bacterial haemorrhagic septicemia. Therefore, the need for protection and treatment programmes is increasingly called on to avoid and/or overcome disease outbreaks in fishery populations.

The haematological studies were carried out including the total erythrocytic count, haemoglobin estimation, packed cell volume "PCV" and the total and differential leucocytic counts. Various derived values can be calculated from these results, such as mean corpuscular volume "MCV", mean cell haemoglobin "MCH" and mean corpuscular haemoglobin concentration "MCHC".

The haematological changes in the blood of common carp induced after the i.p. injection with *Aeromonas hydrophila* pathogen were

blood cells, haemoglobin concentration and haematocrit values. These increments were due to the tissue hypoxia resulting from erythropenia caused by *Aeromonas hydrophila* infection. Treatment of the infected fishes with both laminarin and *N. sativa* showed a decrease in the RBCs count and haemoglobin concentration to the range of subnormal level. A marked decrease in the RBCs count beyond the normal value and a noticeable increase in the haemoglobin concentration were noticed at the 5^{th} week and the 4^{th} and 5^{th} weeks, respectively in the *N. sativa* treated group. The PCV values were changed in a fluctuated manner parallel to the changes in the quantities of circulating RBCs and/or modified erythrocyte cellular volume.

In the protected groups, the haematological changes reflected the same effects of the immunopotentiators recorded in the treated ones. Also, following the challenge with bacteria, there was a decrease in the RBCs count, haemoglobin concentration and PCV values. Furthermore, the blood indices were calculated to interrupt and to give valuable support for the haematological results.

The total leucocyte counts was increased after the challenge with bacteria, then a further elevation was recorded during the time of treatment with both of the immunopotentiators. Also, in the protected groups, the leucocyte counts was increased with laminarin and *N. sativa* injection. While it remained nearly unchanged following the infection with bacteria in the laminarin protected group, it was decreased in the *N. sativa* protected one.

The increased leucocyte counts were accompanied by an increase in the neutrophils and a decrease in the lymphocytes following the infection with bacteria and injection with *N. sativa* in both protected and treated groups. Meanwhiles, in the groups injected with laminarin, the neutrophil and lymphocyte counts were nearly fluctuated around the control values. In addition, monocytes were increased in all the studied

control values. In addition, monocytes were increased in all the studied groups except in the laminarin treated one. Also, eosinophils and basophils were slightly changed in most of the fish groups.

From these results, it was obvious that *N. sativa* may have different actions, so the usage of the oil must be within limited dose to avoid its adverse effect. Also, it was found that i.p. injection of laminarin into carp fish induced enhanced resistance against infection by *Aeromonas hydrophila* bacteria. So, laminarin may have the potency for activating early protection against diseases.

INTRODUCTION

In the last years, the Egyptian policy advocated the expansion in fish farming industry and the utilization of the natural resources beside technology to optimize this activity. Concurrently, and due to the fish farming and its intensification, many fish diseases started to appear particularly among the hatchery broods, cage culture and earthen ponds. Some of these diseases are fatal and spread in some cases even among Nile fishes. Some fish diseases were obsereved in Egypt due to importing of some exotic fish species either for farming or ornamental purpose (Easa & Khater, 1996).

Motile aeromonas septicemia (MAS) is considered as one of the bacterial fish diseases, caused by genus *Aeromonas*. The greatest attention is paid by the researchers to *Aeromonas* and biotroph in connection with epizootics in aquaculture which have become more frequent, in particular, under fish breeding (Kompanet *et al.*, 1992). The pathogenesis of *A.hydrophila* is multifactoral. A variety of virulence factors such as extracellular products (haemolysins, proteases and acetylcholinesterase (Nieto *et al.*, 1991 and Angka *et al.*, 1995) and other virulence determinants such as the presence of the S-layer.

Therefore, the research in the field of fish diseases, prophylactics and treatments has to cope with the fast development and progress in fish culture. Medications from natural sources and biological control would be a wise option as a substitute to the chemical remedies in controlling of fish diseases. Problems with present antibiotic drug and chemical treatments to prevent diseases in fish set the stage for a new concept in disease prevention-the immunostimulants. An immunostimulant is a chemical, drug, stressor, or action that elevates the non-specific defense mechanisms or the specific immune response. Immunostimulants may be given by themselves to activate non-specific defense mechanisms, or they may be administered with a vaccine to activate non-specific defense mechanisms as well as heightening a specific immune response.

Laminarin is a β-1,3-glucan, which is a long-chain polysaccharide extracted from yeasts, algae and fungi cell walls, and is a good stimulator of non-specific defense mechanisms in animals, including fish (Yano *et al.*, 1989 and Robertsen *et al.*, 1990). Because these are products of ubiquitous environmental microflora and potential pathogens, the animals may have a predisposition to react to these substances, similar to reactions to LPS from Gram-negative bacteria. Injection of the glucan was followed by an increase in serum lysozyme and complement-mediated haemolytic activity, which may have contributed to the enhancement of non-specific defense mechanism (Engstad *et al.*, 1992).

Recently, several groups have demonstrated that β-glucans also stimulate the non-specific antibacterial defense of fish (Chen & Ainsworth, 1992 and Jeney & Anderson, 1993). Another study has demonstrated that head kidney macrophages from glucan-treated rainbow trout, *Oncorhynchus mykiss* (walbaum), have an elevated bactericidal capacity (Jørgensen *et al.*, 1993). The increased bacterial killing could be correlated with an increased production of O_2^-. Yeast glucan has also been shown to function as an adjuvant in an intraperitoneal (i.p.)–

administered furunculosis vaccine in Atlantic salmon. (Jørgensen *et al.*, 1993).

Laminarin has been shown to act as an immunomodulator on fish macrophages isolated from the anterior kidney *in vitro* (Dalmo *et al.*, 1994). Moreover, various β-1,3–D–glucans are reported to enhance the non-specific resistance in fish and/or to have a stimulatory effect on fish macrophages *in vivo* and *in vitro* (Anderson, 1992; Jφrgensen & Robertsen, 1994; Sveinbjφrnsson & Seljelid, 1994 and Dalmo & Seljelid, 1995). Engstad & Robertsen (1993) showed that Atlantic salmon macrophages appear to express a specific receptor for yeast glucan, which supports a role for macrophages in the glucan-induced antibacterial defense of fish. In addition to native laminarin, LPS and slightly sulfated laminarin were also examined for their stimulatory effect on head kidney macrophages of Atlantic salmon, *Salmo salar* L., because LPS is a known macrophage activator (Burell, 1990) and sulfated β (1,3)–D–glucans have interesting biological properties such as anti-viral activity (Yoshida *et al.*, 1990) and immunomodulatory effects *in vitro* and *in vivo* (Williams *et al.*, 1991).

Nigella sativa is an annual herbaceous plant belongs to family Ranunculaceae. It is known as black cumin or black seed and is widely distributed in countries bordering the Mediterranean sea, in Western Asia, India, Pakistan, Bangladesh, East Africa and Middle Europe (Al-Jassir, 1992). *Nigella sativa* is considered to have emetic and expectorant potential (Tennekoon *et al.*, 1991). It is used as anticancer and antileukemic (Abdel-Salam *et al.*, 1992 and Zahran *et al.*, 1996), antimicrobial (Hanafy & Hatem, 1991) and as anticestodial drug (Akhtar & Riffat, 1991). Recently, the seeds have been found to possess an immuno-potentiative activity (Zarka, 1993). The volatile oil of *Nigella*

sativa has been found to exhibit an anti-bacterial and anti-microbial activity (Hasan *et al.*, 1989).

The aim of the present work is to look at the potentiating effects of laminarin and *Nigella sativa* on different fish blood parameters against the motile aeromonad septicemia (MAS) fish disease.

MATERIALS AND METHODS

I. Fish:

One hundred and fifty healthy common carp (*Cyprinus carpio* L.) of both sexes (average weight 90 ±10 gm & total length of 15±2 cm) were collected from Gezerat-Abu-Saleh fish farm (18 km away from Beni-Suef). Fish were bathed in 3% NaCl solution, washed three times in sterile tap water and thoroughly examined to ensure that they are free from signs of any microbial or parasitic diseases. The fish were maintained at 24-26°C in well-aerated, chlorine-free tap water glass aquaria (80 × 40 × 50 cm) and fed daily on commercial fish pellets.

II. Bacteria:

Aeromonas hydrophila (A-47) was identified, purified serologically as described by Cruickshank *et al.* (1975) which was kindly provided by the Department of Microbiology, Faculty of Science, University of Al-Azhar. The bacteria were grown on nutrient agar (pH 6.8) and nutrient broth (pH 7.5-7.6). The bacterial numbers used in this work were determined via standard curve constructed according to the matching technique developed by measuring the optical density of both turbidity standards and bacterial cell suspensions at 650 nm (Baron *et al.*, 1994).

III. Immunopotentiators:

III.1. Laminarin:

Laminarin, approx. 95%, from cell walls of *Laminaria digitata*, was purchased from Sigma (England), suspended in 0.65% NaCl (1% w/v) and administered at 0.1 ml / 90gm fish fresh weight (Yano *et al.*, 1989 and Jφrgensen *et al.*, 1993).

III.2. *Nigella sativa* oil:

Nigella sativa seeds were washed with distilled water and sun-dried. Two hundred grams of ground seeds were soaked in 500ml diethyl ether for 6 hrs and filtered. The extract was concentrated by using rotary evaporation (60°C) leaving a clear dark brown oily liquid. The yield of this extract was 11% v/w in terms of dry starting material (Hanafy & Hatem, 1991), stored at 20°C (El-Tahir *et al.*, 1993) and injected at 0.1 ml / 90gm fish fresh weight (Hedaya, 1995).

IV. Experimental design:

One hundred and fifty healthy common carp (*Cyprinus carpio* L.) were divided into five groups, each of thirty fish. Six fishes were sacrificed weekly from each group.

Group I (Control):

This group was injected with an equivalent dose of saline solution (0.65% NaCl).

Group II:

Fish were intraperitoneally (i.p.) daily injected with 0.2 ml of 6×10^6 bacterial cells/ml^3 up to 7 days, and then weekly injected with 0.1 ml of 1% laminarin for 4 weeks.

Group III:

Fish were intraperitoneally (i.p.) weekly injected with 0.1 ml of 1% laminarin for 4 weeks, and then daily injected with 0.2 ml of 6×10^6 bacterial cells/ml^3 for 7 days.

Group IV:

This group of fish were (i.p.) daily injected with 0.2 ml of 6×10^6 bacterial cells/ml^3 for 7 days, then weekly injected with *N. sativa* for 4 weeks at a dose of 0.1 ml/fish.

Group V:

The fish were (i.p.) weekly injected with 0.1 ml of *N. sativa* for 4 weeks, and challenged daily for a week by 0.2 ml of 6×10^6 bacterial cells/ml^3.

V. Blood sampling:

In order to minimize the possible variations in blood values, the technique was standardized as follows. The fish were caught gently in a small net, avoiding stress, as much as possible, and immediately, without anaesthesia, blood was collected into a 3 cm^3 sterile plastic syringe via the caudal vein. The use of plastic syringes is a necessary precaution with fish blood because contact with glass results in shortened coagulation times (Smith *et al.*, 1952). After detaching the needle from the syringe, the blood was mixed well in a vial containing anticoagulant (Potassium salt of ethylene diamine tetra-acetic acid, EDTA) to give a final concentration of 5 mg EDTA per cm^3 blood (Blaxhall & Daisley, 1973).

VI. Total erythrocytic count:

The total red blood corpuscles (RBCs) were counted using the double improved Neubauer chamber as described by Natt and Herrick (1952).

VII. Determination of haemoglobin concentration:

Haemoglobin concentration (gm/dl) was determined using cyanomethemoglobin method (Larsen & Snieszko, 1961).

VIII. Microhaematocrit (Packed cell volume) (PCV):

The well-mixed blood was drawn into a microhaematocrit tube 7.5 cm long, 1mm internal diameter and one end was sealed with clay. The tube was then centrifuged in a microhaematocrit centrifuge for 5 minutes at 15.000 rpm. Readings were made with the aid of a microhaematocrit reader (Dacie & Lewis, 1991).

IX. Wintrobe erythrocyte indices:

Calculations of the absolute values or the erythrocyte indices, namely mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were carried out according to the following equations:

$$MCV\ (fl) = \frac{Hct\ (ml/dl)}{RBC\ (million/\mu l)} \times 10$$

$$MCH\ (pg) = \frac{Hb\ (gm/dl)}{RBC\ (million/\mu l)} \times 10$$

$$MCHC\ (g/dl) = \frac{Hb\ (gm/dl)}{Hct\ (ml/dl)} \times 100$$

Where: -

fl: (femtoliter) = 10^{-15} liter.

pg: (picogram) = 10^{-12} gram.

X. Total leucocytic count:

Direct counting of leucocytes (WBCs) on a Neubauer chamber is the preferred method of determining fish total leucocyte counts, using Natt-Herrick solution.

XI. Differential leucocyte counts:

Percentage of each type of leucocyte in relation to the total number of leucocytes counted was calculated (MacGregor *et al.*, 1940).

XII. Statistical analysis:

Data were analysed using MANOVA for a completely randomized design in a 3×5 factorial treatment arrangement of groups and time. Also, MANOVA for a completely randomized design was performed with 10 treatments to investigate the effect of protection and treatment with either of the two used immunopotentiators inbetween groups on the assayed parameters. If significant differences were found, means were separated using the least significant difference (LSD). Computations were performed using the Statistical Analysis System (Gomez & Gomez, 1983).

RESULTS

The recorded values of control fish (Table 1 and Fig. 1) showed more or less no change in the total red blood cell counts along five weeks of a general mean ($\{1.670\pm0.068\}\times10^6$ cells/mm^3) as compared to that of the normal ones. After the 1st week of infection, both laminarin and *N. sativa* treated groups depicted a marked increase in the total red blood cell counts of ($\{2.063\pm0.069\}\times10^6$ cells/mm^3) and ($\{2.015\pm0.049\}\times10^6$ cells/mm^3) and of the percentage differences 17.22% and 14.49%, respectively. After treatments with laminarin or *N. sativa*, there was a marked continuous decrease in the total red blood cell counts. The maximal effects being recorded at the end of the 5th week and the detected values were of percentage differences -12.39% and -27.06% after treatments with laminarin and *N. sativa*, respectively.

On the other hand, in the laminarin protected group, the total red blood cell counts remained nearly unchanged for two weeks and slightly increased at the 3rd week of the experiment. After four weeks of laminarin injection, the number reached a value of ($\{1.294\pm0.044\}\times 10^6$

cells/mm$^{3)}$ then increased to reach ({1.618 ±0.038} × 10^6 cells/mm^3) after the infection with bacteria. The protection by *N. sativa* exhibited a marked decrease in the total red blood cell count compared with laminarin where the mean value was ({1.318 ±0.061} ×10^6 cells/mm^3) after the first week instead of being ({1.655 ±0.042}×10^6 cells/mm^3) after protection by laminarin. Furthermore, at the end of the 2nd week, the total count was slightly elevated, then showed a continuous decrease up to ({1.289 ±0.063} × 10^6 cells/mm^3) at the 4th week. At the end of the experiment, the total red blood cell counts showed a noticeable decrease ({1.368±0.063}×10^6 cells/mm^3) as compared to that of the control and laminarin protected ones, where their means were ({1.663±0028}×10^6 and {1.618±0.038}×10^6 cells/mm^3), respectively.

The general percentage differences of protection with either laminarin or *N.sativa* were -2.09 % and -12.58 %, respectively, while they were -1.68 % and -7.55 % after treatments with laminarin and *N. sativa*, respectively.

The recorded haemoglobin concentration values of the control animals (Table 1 and Fig.2) were more or less unchanged at all the experimental periods when compared with the normal ones. After the 1st week of injection, infected fish had a haemoglobin concentration of 9.983±0.232 gm/dl and 8.407±0.266 gm/dl in laminarin and *N.sativa* treated groups, respectively. Meanwhile, haemoglobin concentration of laminarin treated group showed a continuous decrease throughout the rest of the tested periods (-10.57%, -5.39%, -1.59% and -3.38%), respectively. *N. sativa* administration induced a marked decrease after the 2nd week (-19.95%) and 3rd week (-5.84%) and a noticeable increase (22.03% and 13.84%) at the 4th and 5th weeks, respectively.

On the other hand, in the laminarin protected fish, the haemoglobin concentration depicatd fluctuated changes during the five

weeks of the experiment with the lowest value (6.156±0.132 gm/dl) at the 4th week and the highest value (8.050±0.231 gm/dl) at the 5th week of the experiment. However, the protection by *N. sativa* had a marked effect on decreasing haemoglobin concentration after one week of *N. sativa* injection (5.660±0.208 gm/dl) with the highest value (8.380±0.107 gm/dl) at the end of the 2nd week. Then the value was gradually decreased (5.667±0.157 gm/dl) after four weeks of *N. saliva* injection. At the end of the experiment, the recorded value showed a marked decrease in the haemoglobin concentration, being 5.960±0.241 gm/dl and the percentage difference -16.45% compared to those of the control and laminarin protected groups with a haemoglobin concentration of 7.133±0.112 gm/dl and 8.050±0.231 gm/dl, respectively

The general percentage differences of protection by either laminarin or *N.sativa* were -0.89% and -9.19%, respectively, while they were 1.94% and 6.30% after treatments with laminarin and *N. sativa*, respectively.

In the control group, the packed cell volume (PCV) remained nearly unchanged during the experimental periods compared with the normal ones (Table 2) and (Fig. 3). Infected fish treated with laminarin depicted a continuous decrease in the packed cell volume from (35.750±1.332 %) after the 1st week of bacterial infection to reach its minimal value (25.417±0.736 %) at the end of 3rd week, then increased to be nearly stable at the 4th and 5th weeks. The packed cell volume values in the *N. sativa* treated group showed fluctuated results with the highest value of (37.583±1.8882 %) at the 4th week, and the lowest value (26.583±0.736 %) after two weeks of the experiment.

On the other hand, for the protected groups, the packed cell volume (PCV) showed fluctuated values during protection to reach the highest value (31.000±1.140 %) with laminarin and (30.917±0.585 %) with *N. sativa* at the 1st and 2nd weeks respectively. While the lowest

values ({28.000±0.837}% and {26.333±0.408}%) were recorded at the 4th week for both laminarin and *N. sativa*, respectively.

The general percentage differences of protection by either laminarin or *N.sativa* were -4.63% and -9.59%, respectively, while they were -2.45% and -2.32% after treatments with laminarin and *N. sativa*, respectively.

The control mean cell volume (MCV) had been given a value of (187.661±7.818 fl) that was nearly equal to that of the normal ones (Table 2 and Fig. 4). Treatment of the infected fish with laminarin showed fluctuated results during the 1st four weeks of the experiment with the lowest value (167.989±1.834 fl) at the 3rd week and the highest value (199.696±4.233 fl) at the 2nd week. At the end of the experiment, mean cell volume (MCV) was slightly increased to 199.146±2.437 fl and of the percentage change was 3.51%.

On the other hand, the mean cell volume was gradually increased after treatment with *N. sativa*, reaching its maximal values at the 4th and 5th weeks of the experiment; being (237.083±4.750 fl) and (235.427±8.733 fl) and the percentage differences were 20.30% and 22.37%, respectively.

The recorded values of laminarin protected group showed a gradual decrease in the mean cell volume, reaching its minimal value (162.965±2.171 fl) and of the percentage difference -14.37% at the end of the 3rd week. The maximal value (216.395±2.963 fl) was detected at the 4th week and the percentage difference was 9.80%. After i.p. injection of bacteria, the value was redecreased again to 190.040±1.979 fl and the percentage difference -1.22%. In case of the protection by *N. sativa*, the mean cell volume depicted a temporary marked increase to record a value of 202.007±6.469 fl and the percentage difference 14.69% at the 1st week post-injection. Then, it was followed by a continuous increase as the

experiment extends to reach the highest value (206.806±6.070 fl) at the end of the experiment. The group protected by laminarin showed -1.22% decrease, while that protected by *N. sativa* exhibited 7.49% increase after the 5th week of the i.p. injection of bacteria.

The general percentage differences of protection by either laminarin or *N.sativa* were -2.09% and -12.58%, respectively, while they were -1.68% and -7.55% after treatments with laminarin and *N. sativa* respectively.

In the control group, the mean cell haemoglobin (MCH) remained nearly unchanged during the experimental periods compared to that of the normal ones (Table 3 and Fig. 5).

Prolonged treatment of the infected fish with each of the tested immunopotentiators for four weeks had different effects on the mean cell haemoglobin. The recorded values were increased gradually after treatment with oil to reach the highest value (67.085±2.594 pg) and the percentage difference of 56.43% at the end of the experiment. It showed fluctuated values during treatment with laminarin reaching its maximal value (47.333±0.827 pg) and the percentage difference was 10.37% at the end of the experiment (i.e at the 5th week).

On the other hand, the mean cell haemoglobin (MCH) in the laminarin protected group showed nearly no detectable change during the 1st three weeks of the experiment then increased gradually reaching its maximal value (49.739±0.717 pg) and the percentage difference was 15.98% at the end of the experiment. The lowest value was obtained at the end of the 2nd week, being 41.233±0.579 pg and the percentage difference was -6.81%. The recorded results of the *N. sativa* protected fish depicted a gradual increase in the values of the mean cell haemoglobin with the maximal value of 49.010±0.521 pg and the percentage difference 9.21% at the 3rd week. Then, they were decreased

till the end of the experiment (i.e at the 4th and 5th weeks). The lowest effect being recorded at the end of the 1st week and the detected value was of percentage difference -2.27%.

The general percentage differences of protection by either laminarin or *N.sativa* were 1.18% and 3.36%, respectively, while they were 3.31% and 14.61% after treatments with laminarin and *N. sativa*, respectively.

Table (3) and Fig. (6) showed no detectable change in the general mean of control mean cell haemoglobin concentration (MCHC) compared with the normal ones.

Treatment of the infected fish with laminarin or *N.sativa* depicted fluctuated results of the mean cell haemoglobin concentration along the experimental time; the maximal effects being recorded at the 3rd week and at the end of the experiment, respectively. The detected values were of the percentage differences 11.69% and 27.82% after the continuous administration of laminarin and *N. sativa*, respectively.

In the *N. sativa* protected group, the results revealed increased values of mean cell haemoglobin concentration at the 2nd and 3rd weeks as compared to that of the control with the percentage differences of 11.75% and 8.88%, respectively. The lowest value was recorded at the last week of the experiment and showed the percentage difference of -5.39%.

On the other hand, the group protected by laminarin depicted the highest record at the end of the experiment, at which the value was 26.174±0.393 gm/dl and of the percentage difference 17.41%. The lowest value was 21.991±0.235 gm/dl and of the percentage difference 0.47% at the 4th week of the experiment.

The general percentage differences of protection by either laminarin or *N.sativa* were 3.26% and -0.27% respectively, while they

were 4.26% and 6.12% after treatments with laminarin and *N. sativa* respectively.

The total leucocyte counts of the control fish was about 7.462×10^3 cells/mm^3 to 8.983×10^3 cells/mm^3 with an average of $(8.012\pm0.805)\times10^3$ cells/mm^3, while it was $(7.498\pm0.596)\times10^3$ cells/mm^3 in the normal ones (Table 4 and Fig. 7).

After the 1st week of the i.p. injection of bacteria, infected fish had total leucocyte counts of $(14.465\pm0.854)\times10^3$ cells/mm^3 and $(13.310\pm1.846)\times10^3$ cells/mm^3 in both laminarin and *N. sativa* treated groups, respectively. After treatments with laminarin or *N. sativa*, the leucocyte counts were raised progressively reaching their maximal value at the 3rd week of the experiment; the values recorded were $(20.295\pm1.301)\times10^3$ cells/mm^3 and $(30.030\pm2.115)\times10^3$ cells/mm^3, respectively.

On the other hand, laminarin and *N. sativa* protection exerted a marked and continuous increased effect on the total leucocyte counts throughout the tested periods. The maximal values being detected at the 4th and 2nd weeks and of the percentage difference 177.49% and 274.65% after the continuous weekly protection by laminarin or *N. sativa* respectively.

The general percentage differences of protection by either laminarin or *N.sativa* were 141.10% and 178.93%, respectively, while they were 121.18% and 167.29% after treatments with laminarin and *N. sativa* respectively.

Collected data for the differential leucocytic counts of control and laminarin and *N. sativa* injected fishes were given in Tables (4, 5 and 6) and illustrated in(Figs 8 , 9 , 10 , 11 and 12).

experiment. On the other hand, fish treated with *N. sativa* depicted a gradual increase in the neutrophil percentage during the 1st three weeks of the experiment then the number was decreased up to the end of the experiment, but still higher than that of the control ones.

Meanwhile, group protected by *N. sativa* showed a marked increase in the neutrophil percentage during the time of protection with a maximal value of (56.000±6.693 %) at the 2nd week. group protected by laminarin revealed a single elevated value (39.333±5.125 %) after two weeks of laminarin injection. After the infection with bacteria, the neutrophil percentage was markedly increased in fish protected by either of the tested immunopotentiators. The effect observed with such potentiators was quite similar following the infection; the recorded values were 52.500±5.167 % and 57.667±4.633 % and of the percentage differences were 162.50 % and 188.34 % after the protection by *N. sativa* and laminarin, respectively.

The percentage of lymphocytes showed an opposite behaviour to that of neutrophils (lymphopenia) in all groups with the highest values at the 4th week for laminarin treated group and the 5th week for *N. sativa* treated ones, whereas they were recorded at the 1st week for both laminarin and *N. sativa* protected groups, respectively.

While monocytes in the *N. sativa* treated group were markedly increased throughout the experimental periods to reach the highest value of (11.667±2.160 %) at the 4th week, treatment with laminarin exhibited fluctuated results with a maximal record (4.000±0.894 %) after the 1st week of bacterial injection. Protection by either of the two used immunopotentiators had a marked increased effect on the number of monocytes. The highest values were detected at the 4th and 5th weeks of the experiment and the recorded values were 12.500±2.739 % and

14.000±2.366 % after the administration of *N. sativa* and laminarin respectively.

In group protected by laminarin and *N. sativa*, eosinophil percentage revealed fluctuated results throughout the experimental periods. The highest values were recorded at the 5th week and of the percentage differences were 266.67 % and 88.87 %, respectively. Also, treatments of the infected fish with laminarin and *N. sativa* showed fluctuated values with the maximal records at the 2nd and 3rd weeks respectively.

Concerning the basophils percentage, there were no noticeable changes in all the tested groups during the time of the experiment.

The values of the least significant difference of the studied haematological parameters were recorded in Table (7).

DISCUSSION

The use of haematological parameters on fish blood and the need for establishing normal values in fish with a view to the aid in the diagnosis of disease and their connection with immunopotentiators and its effects has been emphasized.

The haematological changes in the blood of common carp (*Cyprinus carpio*) induced after the i.p. injection with *Aeromonas hydrophila* pathogen were characterized by a temporary increase in the total number of red blood cells, haemoglobin concentration and haematocrit values at the end of the 1st week post-infection in both laminarin and *N.sativa* treated groups. The temporary increase in the RBCs count may be due to the tissue hypoxia resultant from erythropenia caused by *Aeromonas hydrophila* infection, which characterized by the level of haemolytic anaemia that related to its high requirement for iron and which could be obtained by different ways: 1) the ability of the strain

to produce potent cytolysin capable of lysing RBCs (Lee & Ellis, 1989,1990), 2) increased haemolytic and proteolytic damage of host tissue by *Aeromonas hydrophila* which caused the liberation of intracellular iron stores for use by the organism during *in vivo* growth (Hirst *et al.*, 1994), 3) the ability of the strain to utilize variety of haem sources representative of the differing physiological functions of haem, in addition to its ability to use haem sources complexed to the serum proteins albumin and haptoglobin like haemoglobin-haptoglobin and haem-albumin (Stoebner & Payne,1988), 4) bacteria can utilize transferrin-bound iron through the proteolytic degradation of transferrin (TF) and disruption of the iron binding site to release Fe^{3+} , it can also use lactoferrin (LF) as a source of iron throughout a direct interaction between lactoferrin and the bacterial cell surface (Hirst& Ellis,1996), 5) the ability of the strain to develop an iron sequestering mechanism based on the secretion of a siderophore, which induces separation of plasma and tissue iron from its transferrin or ferritin binding proteins. These complexes with siderophore are attached to specific complex transporting outer cell membrane proteins for absorption into the bacterial cells (Hirst & Ellis, 1994 and Hirst *et al.*, 1994), 6) the 70-kd serine protease formed by bacteria was able to cause an alteration in membrane lysophospholipid content which can affect the cell shape, charge, distribution and activity of membrane bound enzymes which in turn could damage RBCs and cause their lysis (Rosjo *et al.*, 1993 and Esteve *et al.*, 1995) and 7) nutritional diseases, since during the infection time, the fish suffered from starvation and so erythropoietic impairment (Wedemeyer & Mcleay, 1981).

So, if the amount of the lost blood was large, the blood volume was decreased and the homeostatic mechanisms tend to restore the original blood volume by absorption of tissue fluid. This led to blood dilution accompanied with the reduction of the number of RBCs; hence

the tissues were subjected to deficient oxygenation. Such oxygen deficiency triggered the production of erythropoietin hormone which in turn stimulates erythropoiesis that involved an increase in the number of developing cells, rate of maturation due to shortening of mitotic cycle, and cell release from the haemopoietic organs (Benjamin, 1984).

Also, the increase in haemoglobin concentration and haematocrit values of the infected fishes were in agreement with Yamamoto & Itazawa (1988) reported an elevation in the haemoglobin concentration and haematocrit values of mirror carp exercised for an hour. They explained that as a result of increased supply of RBCs from the spleen; erythrocytes swelling due to severe stress of antigen injection which increase haemoglobin level producing a reverse of oxygen capacity, and water shifting out of the vasculature (Awad, 1992 and El-Feki *et al.*, 1993). In addition, Klontz (1972) found that the low tension of CO_2 could cause an increase of approximately 25% in the red cell volume. The mechanism of RBCs swelling appeared to reside on the stimulation of inward ion flux and water contransport by circulating catecholamine during stress (Dheer *et al.*, 1987).

Treatment of the infected fishes with *N. sativa* and laminarin showed a decrease in the RBCs count to the range of subnormal level, except a marked decrease beyond the normal value at the 5^{th} week in the group treated with *N. sativa*. The decrease in the total RBCs count in the *N. sativa* injected fishes was referred to the enhanced red blood cell haemolysis by *N. sativa* (Sallal & Alkofahi, 1996). For the depletion of erythrocytes in the fishes treated with laminarin, this may be attributed to the direct effect of laminarin on the haemopoietic organs by increasing the production of leucocytes by these haemopoietic tissues (Baulny *et al.*, 1996) so homeostasis of total blood volume was maintained by the reduction of red cell mass. In addition, such reduction in erythrocyte

count may be as a result of the prolonged effect of *Aeromonas hydrophila* infection, which can utilize haem by lysing the RBCs.

Cessation or impairment of feeding coupled with stressful effects of infection may be a reason for such depletion of erythrocyte count. Also, the skin haemorrhage caused by *Aeromonas* infection decreases the absorption of vitamin B_{12}, which mainly used in the erythropoiesis, leading finally to a megaloblastic anaemia as declared by Robbins *et al.* (1984).

In the laminarin and *N. sativa* treated fishes, the large drop in the haemoglobin concentration may be due to the decrease in the erythrocytes count or may be due to the failure in the blood osmoregulation and plasma osmolarity (Wong & Davidson, 1983).

The changes in the PCV values in both laminarin and *N. sativa* treated groups may be caused by alteration in the quantities of circulating red cells, altered plasma volume and/or modified erythrocyte cellular volume resulting from osmotic stress and distributed ion exchange during osmoregulation (Awad, 1992).

In the *N.sativa* protected group the haematological changes reflected the same effects of *N. sativa* recorded in the treated group, since there were marked decreases in the total RBCs count, haemoglobin concentrations and PCV values at the 1st and 4th weeks of the experiment. These decreases were interpreted previously on the basis of enhanced RBCs lysis (Sallal & Alkofahi, 1996). Also, the temporary slight increase in the haemoglobin concentration at the 2nd week may be attributed to the beneficial effect of the oil on the injected fishes (El-Tahir *et al.*, 1993), or may be due to erythrocytes swelling (El-Feki *et al.*, 1993). The decrease in the RBCs count, haemoglobin concentration and PCV value following the challenge with bacteria may be as a result of the direct effect of antigen injection, and also may be due to the prolonged effect of the

injected oil. These different results of *N. sativa* may be attributed to the adverse effect of the oil, since the prolonged administration of *N. sativa* was toxic (Kandil *et al.*, 1993).

For the laminarin protected group, the total number of RBCs was nearly normal along the experimental period except a temporary slight increase at the 3rd week of protection followed by a reduction in the erythrocytes count at the next week (i.e. 4th week) of the experiment. To explain these results it was postulated that fish recognize polysaccharide constituents in glucan, products of environmental microflora and potential pathogens, as foreign agent because of their similarity to bacterial gram-negative polysaccharides (Anderson & Siwicki, 1994). So, results of such group parallel the results of the laminarin treated one, since the latter depicted a temporary more elevated red cell counts after the injection with bacteria followed by a decrease in the number of RBCs during the treatment with laminarin. Also, the reduction in the erythrocyte counts may be due to the decrease in the penultimate stages of RBCs in the haemopoietic tissues (El-Feki, 1987), or may be as a result of the haemopoietic differentation directed to the production of leucocyte cells under the effect of laminarin (Baulny *et al.*, 1996). The slight decrease in the haemoglobin concentration and PCV value at the 4th week may be attributed to the reduction in the red cell volume (Klontz, 1972). The slight reincrease in the haemoglobin concentration at the last week of the experiment (i.e. after the i.p. injection of bacteria) may be due to the increased erythrocytes count or may be a result of RBCs swelling due to stress of infection (Wood *et al.*, 1983).

These results confirmed those reported by Robertsen *et al.* (1990) which showed that the i.p. injection of yeast glucan into Atlantic salmon (*Salmo salar* L.) induced enhanced resistance against infection by several pathogenic bacteria. So, laminarin may have the potency for activating early protection against diseases.

The decrease in the MCV of the infected fishes was in agreement with the result of Abu El-Saad (1996) which can be due to the failure in blood osmoregulation and plasma osmolarity (Wong & Davidson, 1983) or to the depression of the fish immune system as a result of the stressful effect of antigen injection (Solanki & Blackburn, 1985). Also, the reduced MCV in both treated and protected groups may be attributed to iron deficiency and some deficiencies of haemopoietic factors (Benjamin, 1984). The elevated MCV may be due to the increased activity of the haemopoietic tissues; for example, the increase in cell release from such tissues under erythropoietin stimulation which associated with an increase in mean corpuscular volume (Benjamin, 1984).

The MCHC was temporary increased during the time of infection, while MCV was decreased. This difference may be attributed to the dysfunction occurred in the red cell membrane which become inflexible (England *et al.*, 1982). The increased MCHC may be due to the increase in the weight of haemoglobin in the erythrocyte and not to an increase in the concentration of haemoglobin per unit volume. So, the decreased MCHC may be referred to the reduction in haemoglobin which relatively greater than the average decrease in erythrocyte volume (Benjamin, 1984). In addition, the decrease in MCH and MCHC may be due to that the *Aeromonas sp.* can utilize various haem sources such as haem, haemin and haemoglobin besides the haem complexes such as haemoglobin-haptoglobin and haem-albumin (Hirst *et al.*, 1994).

The increase of the total leucocytes count following the infection with bacteria can be considered as an inflammatory response induced as a defense mechanism against the injected antigen (Said, 1990; El-Bakry, 1992; Zahran *et al.*, 1992 and El-Shahawy *et al.*, 1995). Ansari & Williams (1976) explained the absolute increase in circulating neutrophil polymorphs as responsible for the onset of leucocytosis. This explanation

was supportedas the stained blood films showed a marked increase in neutrophil in the peripheral blood. In addition, Stoskopf (1993) mentioned that the bacterial diseases routinely caused a marked leucocytosis that was most commonly neutrophilia and lymphopenia with a reversed ratio from base line.

On the other hand, the increase of white blood corpuscle counts during the time of treatment may be attributed to the stimulatory effect of the immunopotentiators on the haemopoietic tissues to produce extensive number of effective functional cells as a defense mechanism. Since these substances stimulate the humoral agent, granulopoietin to carry signals to the haemopoietic tissues in order to accelerate cell proliferation and consequently increases the ratio of granulopoiesis (Fawcett, 1986). Otherwise, this large number of produced granulocytes precursors supply the circulation before their fully maturation because of the impairment of the protein synthesis in the cytoplasm of the developed cells by the immunopotentiators as deduced by Erslev (1983) and Gilman *et al.* (1991). Such immature granulocytes can not perform the original functions of the mature granulocytes. Therefore, the humoral agent, granulopoietin acting back upon the haemopoietic tissues in an attempt to produce functional granulocytes, and so on, resulting in a gradual increase of the total leucocyte counts.

The increase of tissue damage after the i.p. injection of bacterial pathogen probably caused the increase of neutrophils in the studied blood films. Ellsaesser *et al.* (1985) depicted that infection and stressful conditions elevate the number of neutrophils (heterophils) in fish blood. These results agree with those of Harding & Hogland (1984); El-Feki (1987); Awad (1992) and El-Fayoumi (1996) on *Salmo salar* L.; *Cyprinus carpio*; *Oreochromis niloticus* and *Clarias lazera* respectively. The heterophilia seen in response to infection with bacteria is a relatively rapid response in fish (within 24 hour) and appeared to be independent of

temperature (Stoskopf, 1993). The heterophilic activity in bacterial infection has shown a high phagocytic response in goldfish (Watson *et al.*, 1963) and rainbow trout (Finn & Nielson, 1971).

Also, the fish heterophils were noticed in infiltrate injured tissues early in the inflammatory aeromonad response (Thrope & Robert, 1972 and Joy & Jones, 1973). In addition, El-Feki *et al.* (1993) reported that neutrophils are phagocytic cells and concluded that the increase of neutrophils may be due to their phagocytic activity. Furthermore, Bly *et al.* (1990) recorded that the increase of neutrophils immediately post-injection was due to rapid cell proliferation and/or differentiation account for the peripheral blood. Also, they noticed that the neutrophils are harbored elsewhere in the body pre-injection, which empties post-injection.

For the group treated and protected with *N. sativa*, the increase in the number of neutrophils with the continuous administration of *N. sativa* and then its gradual decrease along the experimental period confirmed the previous work carried by Abd Rabou (1996) who suggested that *N. sativa* may accelerate the developmental stages of polymorphonuclear leucocytes and hence the number of mature neutrophils may be increased in the blood. Since it decreases the release of histamine from mast cells, which induce vasodilation as deduced by Chakravarty (1993). This in turn provokes traversing of neutrophils from the entire blood to the tissue.

In fish, heterophilia due to stress responses is most frequently associated with lymphopenia (Slicher, 1961). Sövényi *et al.* (1990) reported a case of lymphocyte arrest in response to stressful conditions after antigen injection, i.e. an inhibition of their release into the blood for two reasons: (1) density of lymphocyte-like cells increased, (2) a massive proliferation that could justify the increase in their percentage.

The increase in monocytes may be due to the increase in haemopoietic activity after the exocytosis done by monocytes after the discharge of its granules to lyse the antigen(s) extracellulary (Roitt *et al.*, 1982).

The change in the eosinophil number may be due to the engagement of these cells in phagocytic processes against the injected antigen, since they are capable of phagocytosing and killing ingested micro-organisms. Also, eosinophils release histaminase which inactivate cell products histamine secreted as a result of antigen administration. The net effect of this factor is to dampen down the inflammatory response induced by the injection of antigen (Roitt *et al.*, 1985).

Also, the small change in basophils comes from the allergy induced by the injected antigen. These cells have randomly distributed granules in their cytoplasm, surrounded by membranes, after initiation with appropriate stimulus, pharmacological mediators released following degranulation which react against antigen injection allergy (Roitt *et al.*, 1985).

REFERENCES

Abdel-Salam, I.M.; Abdel-Wahab, S.; El-Aaser, A.A. and El-Merzabani, M.M. (1992): Biochemical and cytotoxic effect of *Nigella sativa*. Egypt. J. Biochem., *10* (2): 348-55.

Abd Rabou, H.E. (1996): The anticarcinogenic effect of black seed (*Nigella sativa*) on leukemia. M.Sc.Thesis, Fac. Sci., Minia Univ.

Abu El-Saad, A.S.A. (1996): Pathogenicity of *Aeromonas hydrophila* against common carp *Cyprinus carpio*. M.Sc. Thesis, Fac. Sci., Minia Univ., Egypt.

Akhtar, M.S. and Riffat, S. (1991): Field trial of *Saussurea lappa* roots against nematodes and *Nigella sativa* against cestodes in children. JPMA. J. Pak. Med. Assoc., *41* (8): 185-7.

Al-Jassir, M.S. (1992): Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food chem., *45* (4): 239-42.

Anderson, D.P. (1992): Immunostimulants, adjuvants, and vaccine carriers in fish: Application to aquaculture. Ann. Rev. Fish Dis., 281-307.

Anderson, D.P. and Siwicki, A.K. (1994): Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. Prog. Fish-Culturist., *56*: 258-261.

Angka, S.L.; Lam, T.J. and Sin, Y.M. (1995): Some virulence characteristics of *Aeromonas hydrophila* in walking catfish (*Clarias gariepinus*). Aquaculture., *130*: 103-112.

Ansari, A. and Williams, J.F. (1976): The eosinophilic response of the rat to infection with *Taenia* taeniae-formis. J. Parasitol., *62*: 728-736.

Awad, E.M.M. (1992): Immunohistopathological changes of *Oreochromis niloticus* induced by *Saprolegnia* sp. infection. M.Sc. Thesis, Fac. Sci. Minia Univ.

Baron, E.J.; Peterson, L.R. and Finegold, S.M. (1994): Diagnostic Microbiology. Baily and Scott (eds.): 9th Ed., C.V. Mosby, Co. London, U.K.

Baulny, Mo-de.; Quentel, C.; Fournier, V.; Lamour, F.; Gourello, R-Le.; De-Baulny, Mo. and Le-gouvello, R. (1996): Effect of long-term oral administration of BETA-glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of trout *Scophthalmus maximus*. Dis. Aquat. Org., *26* (2): 139-147.

Benjamin, M.M. (1984): Outline of veterinary clinical pathology. 4th printing. U.S.A. Iowa State Univ. Press. 123-129.

Blaxhall, P.C. and Daisley, K.W. (1973): Routine haematological methods for use with fish blood. J. Fish Biol., *5*: 771-781.

Bly, J.E.; Miller, N.W. and Cleam, L.W. (1990): A monoclonal antibody specific for neutrophils in normal and stressed channel catfish. Develop. Comp. Immunol., *14*: 211-221.

Burell, R. (1990): Immunomodulation by bacterial endotoxin. Critical Rev. Microbiol., *17*: 189-208.

Chakravarty, N. (1993): Inhibition of histamine release from mast cells by nigellone. Ann. Allergy., *70* (3): 237-42.

Chen, D. and Ainsworth, A.J. (1992): Glucan administration potentiates immune defense mechanisms of channel catfish, *Ictalurus punctatus* Rafinesque. J. Fish Dis.,*15*: 295-304.

Dacie, S.J. and Lewis, S.M. (1991): Practical haematology. 7th Ed. Churchill Livingstone.

Dalmo, R.A.; Ingebrigsten, K.; Horsberg, T.E. and Seljelid, R. (1994): Intestinal absorption of immunomodulatory laminarin and derivatives in Atlantic salmon, *Salmo salar* L. J. Fish Dis., *17*: 579-589.

Dalmo, R.A. and Seljelid, R. (1995): The immunomodulatory effect of LPS, *laminarin* and sulfated *laminarin* {B (1,3)-D-glucan} on Atlantic salmon, *Salmo salar* L., macrophages *in vitro*. J. Fish Dis., *18*: 175-185.

Dheer, M.S.; Dheer, T.R. and Mahajan, C.L. (1987): Haematological and haemopoietic response to acid stress in an air-breathing freshwater fish. *Channa Punctatus* bloch. J. Fish Biol., *30*: 577-588.

Easa, M.E. and Khater, A.A. (1996): Fish Diseases: Prophylaxis, therapy and their role in fish production. Fish diseases and their effects in the development of fish resources. A seminar on fish culture in Egypt. Academy of Scientific research and technology., 17 January, 1-19.

El-Bakry, H.A. (1992): Effect of the rodenticide (brodifacoum) on the lymphatic system of the Egyptian mouse *Gerbillus gerbillus* M.Sc.Thesis, Fac. Sci., Minia Univ.

El-Fayoumi, H.M.K. (1996): Immunological responses induced against different antigens by catfish (*Clarias lazera*). M.Sc.Thesis, Fac. Sci., Minia Univ. Egypt.

El-Feki, M.A. (1987): Studies on the host-parasite interaction between carp and *Saprolegnia*. Ph.D. Thesis, Aston Univ., Birmingham, U.K. England.

El-Feki, M.A.; Tawfek, N.S. and Awad, E.M. (1993): Ulcerative Dermal Necrosis (UDN). A freshwater fish disease. VIII. Haematological studies. J. Egypt. Soc. Toxicol., *10*: 25-28.

Ellsaesser, C.F.; Miller, N.W. and Cuchens, M.A. (1985): Analysis of channel catfish peripheral blood leucocytes by bright-field microscopy and flow cytometry. Trans. Am. Fish. Soc., *114*: 279-285.

El-Shahawy, G.A.; Zahran, W.M.; Gabry, M.S. and Mahmoud, M.B. (1995): Haematological changes in the infected white rat (*Rattus norvegicus*) by the trematode *Cynodiplostomum azimi*. J. Egypt. Ger. Soc. Zool., *18* (d). 1-18.

El-Tahir, K.E.H.; Ashour, M.M.S. and Al-Harbi, M.M. (1993): The respiratory effects of the volatile oil of the black seed (*Nigella sativa*) in Guinea Pigs: Elucidation of the mechanism(s) of action. Gen. Pharmac., *24* (5): 1115-1122.

England, J.M.; Chetty, M.C. and Gravey, B. (1982): Value assignment to quality control materials. In: Advances in Haematological Methods: The blood count. CRC Press, Boca Raton, Florida. 239.

Engstad, R.E. and Robertsen, B. (1993): Recognition of yeast cell wall glucan by Atlantic salmon (*Salmo salar* L.) macrophages. Develop. Comp. Immunol., *17*: 319-330.

Engstad, R.E.; Robertsen, B. and Frivold, E. (1992): Yeast glucan induces increase in activity of lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. Fish & Shellfish Immunol., *2*: 287-297.

Erslev, J.A. (1983): Haemopoietic stem cell disorders-aplastic. In: Haematology. 3rd Ed., ch.20, McGraw-Hillbook Company. New York.

Esteve, G.; Amaro, C.; Garay, E.; Sautos, Y. and Toranzo, A. (1995): Pathogenicity of live bacteria and extracellular products of motile *Aeromonas* isolated from eels. J. Appl. Bacteriol., *78* (5): 555-562.

Fawcett, O.W. (1986): A textbook of Histology. 11th Ed. W.B. Saunders Company. Igaku-shoinl/Saunders.

Finn, J.P. and Nielson, N.O. (1971): Effect of temperature on inflammatory response in rainbow trout. J. Pathol. Bacteriol., *105*: 257-268.

Gilman, A.G.; Rall, T.W.; Nies, A.S. and Taylor, P. (1991): The Pharmacological Basis of Therapeutics. 8th Ed., Pergamon Press, New York.

Gomez, K.A. and Gomez, A.A. (1983): Statistical procedures for Agricultural Research. 2nd Ed. John Wiley and Sons. New York, p 680.

Hanafy, M.S.M. and Hatem, M.E. (1991): Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J. Ethnopharmacology., *34*: 275-278.

Harding, J. and Hogland, L.B. (1984): Seasonal variation in blood components of reared Atlantic salmon (*Salmo salar* L.). J.Fish Biol., *24*: 565-579.

Hasan, C.M.; Ahsan, M. and Islam, S.N. (1989): *In vitro* antibacterial screening of the oils of *Nigella sativa* seeds. Bangladesh J. Bot., *18* (2): 171-74.

Hedaya, S.A. (1995): Effect of *Nigella sativa* seeds (Black seeds) extract on some haematological and biochemical parameters in Rats. Alex. J. Vet. Sci., *11* (2): 95-99.

Hirst, I.D. and Ellis, A.E. (1994): Iron-regulated outer membrane proteins of *Aeromonas salmonicida* are important protective antigens in Atlantic salmon against furunculosis. Fish & Shellfish. Immunol., *4*: 29-45.

Hirst, I.D. and Ellis, A.E. (1996): Utilization of transferrin and salmon serum as sources of iron by typical and atypical strains of *Aeromonas salmonicida*. J. Microbiol., *142*: 1543-1550.

Hirst, I.D.; Hastings, T.S. and Ellis, A.E. (1994): Utilization of haem compounds by *Aeromonas salmonicida*. J. Fish Dis., *17*: 365 - 373.

Jeney, G. and Anderson, D.P. (1993): Glucan injection or bath exposure given alone or in combination with a bacterin enhance the non-specific defence mechanisms in rainbow trout (*Oncorhynchus mykiss*). Aquacult., *116*: 315-329.

Jφrgensen, J.B.; Lunde, H. and Robertsen, B. (1993): Peritoneal and head kidney cell response to intraperitoneally injected yeast glucan in Atlantic salmon, *Salmo salar* L. J. Fish Dis., *16*: 313-325.

Jφrgensen, J.B. and Robertsen, B. (1994): Yeast B- glucan stimulates respiratory activity of Atlantic salmon (*Salmo salar* L.) macrophages. In: Stimulation of antibacterial mechanisms in salmonid macrophages by yeast B-glucan and bacterial lipopolysaccharide. Ph.D.Thesis by Jorgensen, J.B. Norwegian college of Fishery Science, Trosmφ Univ. Trosmφ.

Joy, J.E. and Jones, J.C. (1973): Observations on the inflammatory response within the dermis of white bass, *Morone chrysops*, infected with *Lernea cruicata*. J. Fish Biol., *5*: 21-23.

Kandil, O.; Radwan, N.; Hassan, A.; El-Banna, H. and Amer, A. (1993): Toxicity of some plants. Vet. Med. J. (Giza)., *41* (2): 9-14.

Klontz, G.W. (1972): Haematological techniques and the immune response in Rainbow Trout. Symp. Zool. Soc. Lond., *30*: 89-99.

Kompanets, EV.; Isaeva, NM. and Balakhnin, IA. (1992): Bacteria of the genus *Aeromonas* and their role in aquaculture. Microbiol-Zh., *54* (4): 89-99.

Larsen, H.N. and Snieszko, S.F. (1961): Comparison of various methods of determination of haemoglobin in trout blood. Progve. Fish Cult., *23*: 8-17.

Lee, K.K. and Ellis, A.E. (1989): The quantitative relationship of lethality between extracellular protease and haemolysin of *Aeromonas salmonicida* in Atlantic salmon, *Salmo salar*. FEMS Microbiology Lett., *61*: 127-132.

Lee, K.K. and Ellis, A.E. (1990): Glycerophospholipid: cholesterol acyltransferase complexed with lipopolysaccharide (LPS) is a major lethal exotoxin and cytolysin of *Aeromonas salmonicida*:

LPS stabilises and enhances toxicity of the enzyme. J. Bacteriol., *172*: 5382-5393.

MacGregor, R.G.S. (1940): The differential leucocyte count. J. Pathogen. Bacteriol., *51*: 337.

Natt, M.P. and Herrick, C.A. (1952): A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Sci., *31*: 735-738.

Nieto, T.P.; Santos, Y.; Rodriguez, L.A. and Ellis, A.E. (1991): An extracellular acetylcholinesterase produced by *Aeromonas hydrophila* is a major lethal toxin for fish. Microbial pathogenesis., *11*: 101-110.

Robbins, S.L.; Cortan, R.S. and Kumar, V. (1984): Pathologic basis of disease. 3rd Ed. W.B. Saunders Company, Igaku. Shoin/Sounders.

Robertsen, B.; Rϕrstad, G.; Engstad, R. and Raa, J. (1990): Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. J. Fish Dis., *13*: 391-400.

Roitt, M.I.; Brostoff, J. and Male, K.D. (1982): Complement. In: Essential Immunology. Blackwell Scientific Publication, Oxford, p.137.

Roitt, M.I.; Brostoff, J. and Male, K.D. (1985): "Immunology". Churchill Livingstone, Edinburg, London, Melbourne, New York.

Rosjo, C.; Thomassen, M. and Eggset, G. (1993): Glycerophospholipid-cholesterol acyltransferase complexed with lipopolysaccharide of *Aeromonas salmonicida* produces lipophospholipids in salmonid red cell membranes: a probable haemolytic mechanism. J. Fish Dis., *16*: 87-99.

Said, H. (1990): Toxicological studies with anticoagulants, brodifacoum and flucoumafen on rats. M.Sc.Thesis, Fac. Agr. Cairo Univ., Egypt.

Sallal, AKJ. and Alkofahi, A. (1996): Inhibition of the haemolytic activities of snake and scorpion venoms *in vitro* with plant extracts. Biomedical, Lett., *53* (212): 211-215.

Slicher, A.M. (1961): Endocrinological and haematological studies in fundulus heterocltius (Linn.). Bull. Birmingham Oceanographic Coll., *17*: 3-55.

Smith, G.C.; Lewis, W.M. and Kaplan, H.M. (1952): A comparative morphologic and physiologic study of fish blood. Progve Fish Cult., *14*: 169-172.

Solanki, D.L. and Blackburn, B.C. (1985): Spurious red blood cell parameters due to serum cold agglutinins: observation on Ortho ELT-8 cell counter. Am. J. Clin. Pathol., *83*: 218.

Sövényi, J.F.; Yamamoto, H.; Fujimoto, S. and Kusuda, R. (1990): Lymphomyeloid cells, susceptibility to erythrodermatitis of carp and bacterial antigens. Develop. Comp. Immunol., *14*: 185-200.

Stoebner, J.A. and Payne, S.M. (1988): Iron -regulated haemolysin production and utilization of haem and haemoglobin by *Vibrio cholerae*. Inf.& Immun., *56*: 2891-2895.

Stoskopf, M.K. (1993): Clinical Pathology. In: Fish medicine. Stoskopf, M.K. (ed.): ch.9, I: 113-131.

Sveinbjφrnsson, B. and Seljelid, R. (1994): Aminated B-1,3-D-polyglucose activates salmon pronephros macrophages *in vivo*. Vet. Immunol. Immunopathol., *41*: 113-123.

Tennekoon, K.II.; Jeevathayaparan, S.; Kurukulasooriya, A.P. and Karunanayake, E.II. (1991): Possible hepatotoxicity of *Nigella*

sativa seeds and *Dregea volubilis* leaves. J. Ethnopharmacol., *31* (3): 283-89.

Thrope, J.E. and Robert, R.J. (1972): An aeromonad epidemic in the brown trout (*Salmo trutta* L.). J. Fish Biol., *4*: 441-452.

Watson, L.J.; Shehmeister, I.L. and Jackson, L.L. (1963): The haematology of goldfish (*Carassius auratus*). Cytologia, *28*: 118-130.

Wedemeyer, G.A. and Mcleay, D.J. (1981): Methods for determining the tolerance of fishes to environmental stressors. In: Stress and Fish. Pickering, A.D. (ed.): London, Academic Press. 247-275.

Williams, D.L.; Pretus, H.A.; McNamee, R.B.; Jones, E.L.; Ensley, H.E.; Browder, I.W. and Di Luzio, N.R. (1991): Development, physicochemical characterization and preclinical efficacy evaluation of a water soluble glucan sulfate derived from *Saccharomyces Cerevisiae*. Immunopharmacology., *22*: 139-156.

Wong, E.L. and Davidson, R.J. (1983): Raised coulter mean corpuscular volume in diabetic ketoacidosis, and its underlying association with marked plasma hyperosmolarity. J. Clin. Pathol., *36*: 334.

Wood, C.M.; Turner, J.D. and Graham, S. (1983): Why do fish die after severe exercise ?. J. Fish Biol.,*22*: 188-201.

Yamamoto, K. and Itazawa, Y. (1988): Erthrocyte supply from the spleen of exercised carp. Comp. Biochem. Physiol., *92* (A): 139-144.

Yano, T.; Mangindaan, R.E.P. and Matsuyama, H. (1989): Enhancement of the resistance of carp *Cyprinus carpio* to experimental *Edwardsiella tarda* infection, by some β-1,3-glucans. Nippon Suisan Gakkaishi., *55* (10): 1815-1819.

Yoshida, T.; Hatanaka, K.; Uryu T.; Kancko, Y.; Suzuki, E.; Miyano, H.; Mimura, H.; Yoshida, O. and Yamamoto, N. (1990): Synthesis

and structural analysis of curdlan sulfate with a potent inhibitory effect *in vitro* of AIDS virus infection. Macromolecules, *23*: 3717-3721.

Zahran, W.M.; El-Feki, M.A.; Gabry, M.S. and Abd-Rabou, H.E. (1996): The anticarcinogenic effect of the black seed (*Nigella sativa*) on leukemia. El-Minia Sci. Bull., *9* (2): 1-19.

Zahran, W.M.; El-Feki, M.A.; Gabry, M.S. and El-Bakry, H.A. (1992): Effects of brodifacoum rodenticide on the gerbil, *Gerbillus gerbillus*. I-Symptomological and Haematological studies. J. Egypt. Soc. Toxicol., *8*: 129-132.

Zarka, R. (1993): Investigation of the biological effects of *Nigella sativa* L. seeds. M. Sc. Thesis, London Univ.

Table 1. Multi-Factor Analysis of Variance (MANOVA) for the effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the total red blood cell counts (RBCs) and haemoglobin concentration (Hb Conc.) of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

	Group	S.V	d.f	RBCs			Hb Conc.		
				SS	MS	F Cal.	SS	MS	F Cal.
Laminarin	Control Treat. with lamin. Protect. by lamin.	General effect Time Lamin. Time-lamin. Error Total	 4 2 8 75 89	 0.998 0.020 1.580 0.151 2.749	 0.249 0.010 0.198 0.002	 123.600*** 4.995** 97.851***	 20.374 0.877 43.269 1.727 66.247	 5.094 0.439 5.409 0.023	 221.167*** 19.043*** 234.845***
N. sativa	Control Treat. with *N. sativa* Protect. by *N. sativa*	General effect Time N. sativa Time-*N. sativa* Error Total	 4 2 8 75 89	 1.011 0.672 2.250 0.208 4.141	 0253 0.336 0.281 0.003	 91.111*** 121.199*** 101.373***	 0.786 13.910 71.524 2.889 89.108	 0.196 6.955 8.940 0.039	 5.099*** 180.567*** 232.115***
Treatment	Control Treat. with lamin. Treat. with *N. sativa*	General effect Time Treat. Time-Treat. Error Total	 4 2 8 75 89	 2.750 0.264 0.890 0.198 4.102	 0.688 0.132 0.111 0.003	 260.570*** 50.023*** 42.158***	 45.160 1.027 37.953 2.611 86.751	 11.290 0.514 4.744 0.035	 324.251*** 14.750*** 136.252***
	Control Treat. with lamin.	Between group Within group Total	9 50 59	1.526 0.095 1.621	0.170 0.002	89.702***	50.626 0.912 51.539	5.625 0.018	308.244***
	Control Treat. with *N. sativa*	Between group Within group Total	9 50 59	2.473 0.127 2.600	0.275 0.003	108.191***	35.942 1.930 37.873	3.994 0.039	103.454***
Protection	Control Protect. by lamin. Protect. by *N. sativa*	General effect Time Protect. Time-Protect. Error Total	 4 2 8 75 89	 1.144 0.763 1.054 0.162 3.123	 0.286 0.382 0.132 0.002	 132.817*** 177.137*** 61.181***	 22.518 7.905 30.322 2.005 62.750	 5.630 3.953 3.790 0.027	 210.614*** 147.878*** 141.800***
	Control Protect. by lamin.	Between group Within group Total	9 50 59	1.190 0.080 1.271	0.132 0.002	82.155***	15.593 1.046 16.639	1.733 0.021	82.817***
	Control Protect. by *N. sativa*	Between group Within group Total	9 50 59	1.799 0.105 1.904	0.200 0.002	95.468***	46.293 1.190 47.483	5.144 0.024	216.146***

* (significant), ** (highly significant) and *** (very highly significant)

Table 2. Multi-Factor Analysis of Variance (MANOVA) for the effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the packed cell volume (PCV) and mean cell volume (MCV) values of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

	Group	S.V	d.f	PCV			MCV		
				SS	MS	F Cal.	SS	MS	F Cal.
Laminarin		General effect							
	Control	Time	4	152.428	38.107	68.937***	9724.401	2431.100	434.788***
	Treat. with lamin.	Lamin.	2	31.572	15.786	28.558***	131.218	56.609	11.734***
	Protect. by lamin.	Time-lamin.	8	245.956	30.744	55.618***	8295.464	1036.933	185.449***
		Error	75	41.458	0.553		419.360	5.591	
		Total	89	471.414			18570.443		
N. sativa		General effect							
	Control	Time	4	109.419	27.355	37.869***	20219.486	5054.871	263.582***
	Treat. with *N. sativa*	N. sativa	2	147.013	73.506	101.758***	2939.436	1469.718	76.637***
	Protect. by *N. sativa*	Time-*N. sativa*	8	505.647	63.206	87.499***	13456.419	1682.052	87.709***
		Error	75	54.177	0.722		1438.320	19.178	
		Total	89	816.256			38053.661		
Treatment		General effect							
	Control	Time	4	471.892	117.973	136.275***	20350.756	5087.689	409.531***
	Treat. with lamin.	Treat.	2	11.151	5.576	6.441***	4154.596	2077.298	167.211***
	Treat. with *N. sativa*	Time-Treat.	8	395.092	49.386	57.048***	14123.259	1765.407	142.106***
		Error	75	64.927	0.866		931.740	12.423	
		Total	89	943.062			39560.350		
	Control	Between group	9	373.000	41.444	87.868***	7120.914	791.213	152.924***
	Treat. with lamin.	Within group	50	23.583	0.472		258.694	5.174	
		Total	59	396.583			7379.608		
	Control	Between group	9	533.484	59.276	62.602***	31960.200	3551.133	230.181***
	Treat. with *N. sativa*	Within group	50	47.344	0.947		771.379	15.428	
		Total	59	580.828			32731.580		
Protection		General effect							
	Control	Time	4	27.739	6.935	16.937***	11112.932	2778.233	225.034***
	Protect. by lamin.	Protect.	2	135.050	67.525	164.919***	1879.903	939.951	76.135***
	Protect. by *N. sativa*	Time-Protect.	8	118.728	14.841	36.247***	6108.824	763.603	61.851***
		Error	75	30.708	0.409		925.940	12.346	
		Total	89	312.225			20027.598		
	Control	Between group	9	88.537	9.837	20.602***	12709.356	1412.151	272.617***
	Protect. by lamin.	Within group	50	23.875	0.478		258.999	5.180	
		Total	59	112.412			12968.356		
	Control	Between group	9	247.267	27.474	107.042***	7253.614	805.957	52.658***
	Protect. by *N. sativa*	Within group	50	12.833	0.257		765.274	15.305	
		Total	59	260.100			8018.888		

* (significant), ** (highly significant) and *** (very highly significant)

Table 3. Multi-Factor Analysis of Variance (MANOVA) for the effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) values of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

	Group	S.V	d.f	MCH			MCHC		
				SS	MS	F Cal.	SS	MS	F Cal.
Laminarin	Control Treat. with lamin. Protect. by lamin.	General effect Time Lamin. Time-lamin. Error Total	 4 2 8 75 89	 154.579 32.393 385.092 22.201 594.265	 38.645 16.196 48.137 0.296	 130.548*** 54.714*** 162.613***	 114.077 16.280 196.397 5.265 332.019	 28.519 8.140 24.550 0.070	 406.244*** 115.951*** 349.699***
N. sativa	Control Treat. with *N. sativa* Protect. by *N. sativa*	General effect Time N. sativa Time-*N. sativa* Error Total	 4 2 8 75 89	 784.332 674.218 2314.474 64.224 3837.248	 196.083 337.109 289.309 0.856	 228.985*** 393.675*** 337.854***	 70.511 42.898 297.245 8.881 419.536	 17.628 21.449 37.156 0.118	 148.859*** 181.128*** 313.762***
Treatment	Control Treat. with lamin. Treat. with *N. sativa*	General effect Time Treat. Time-Treat. Error Total	 4 2 8 75 89	 1032.732 675.888 2054.312 55.965 3818.896	 258.183 337.944 256.789 0.746	 345.998*** 452.887*** 344.130***	 172.561 32.340 177.463 7.375 389.738	 43.140 16.170 22.183 0.098	 438.744*** 164.453*** 225.603***
	Control Treat. with lamin.	Between group Within group Total	9 50 59	193.656 15.108 208.764	21.517 0.302	71.212***	231.130 2.889 234.019	25.681 0.058	444.440***
	Control Treat. with *N. sativa*	Between group Within group Total	9 50 59	3555.456 45.888 3601.344	395.051 0.918	430.450***	205.034 5.047 210.081	22.782 0.101	225.700***
Protection	Control Protect. by lamin. Protect. by *N. sativa*	General effect Time Protect. Time-Protect. Error Total	 4 2 8 75 89	 85.673 33.548 465.760 30.460 615.441	 21.418 16.774 58.220 0.406	 52.737*** 41.302*** 143.352***	 163.721 12.643 164.485 6.772 347.621	 40.930 6.321 20.561 0.090	 453.296*** 70.007*** 227.705***
	Control Protect. by lamin.	Between group Within group Total	9 50 59	397.500 12.125 409.625	44.167 0.242	182.136***	143.421 2.938 146.359	15.936 0.059	271.243***
	Control Protect. by *N. sativa*	Between group Within group Total	9 50 59	206.470 23.367 229.837	22.941 0.467	49.090***	234.033 4.396 238.429	26.004 0.088	295.758***

* (significant), ** (highly significant) and *** (very highly significant)

Table 4. Multi-Factor Analysis of Variance (MANOVA) for the effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the total leucocytic (WBCs) and neutrophil (Nt) counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

	Group	S.V	d.f	WBCs			Nt		
				SS	MS	F Cal.	SS	MS	F Cal.
Laminarin	Control Treat. with lamin. Protect. by lamin	General effect							
		Time	4	113.681	28.420	18.884***	4864.956	1216.239	151.651***
		Lamin.	2	2246.031	1123.016	746.177***	2838.467	1419.233	176.962***
		Time-lamin.	8	159.965	19.996	13.286***	15225.978	1903.247	237.313***
		Error	75	112.877	1.505		601.500	8.020	
		Total	89	2632.555			23530.900		
N. sativa	Control Treat. with *N. sativa* Protect. by *N. sativa*	General effect							
		Time	4	681.157	170.289	39.056***	1527.178	381.794	19.343***
		N. sativa	2	3860.605	1930.302	442.716***	13998.067	6999.033	354.601***
		Time-*N. sativa*	8	1093.850	136.731	31.359***	4440.333	555.103	28.124***
		Error	75	327.010	4.360		21446.400	19.738	
		Total	89	5962.622					
Treatment	Control Treat. with lamin. Treat. with *N. sativa*	General effect							
		Time	4	526.855	131.714	65.444***	4934.822	1233.706	140.300***
		Treat.	2	2875.661	1437.831	714.405***	10747.756	5373.878	611.131***
		Time-Treat.	8	653.524	81.690	40.589***	8391.578	1048.947	119.289***
		Error	75	150.947	2.013		659.500	8.793	
		Total	89	4206.987			24733.656		
	Control Treat. with lamin.	Between group	9	1534.630	170.514	234.010***	12920.483	1435.609	356.821***
		Within group	50	36.433	0.729		201.167	4.023	
		Total	59	1571.063			13121.650		
	Control Treat. with *N. sativa*	Between group	9	3763.732	418.192	168.431***	13913.683	1545.965	140.756***
		Within group	50	124.144	2.483		549.167	10.983	
		Total	59	3887.876			14462.850		
Protection	Control Protect. by lamin. Protect. by *N. sativa*	General effect							
		Time	4	233.642	58.411	15.162***	6687.156	1671.789	88.154***
		Protect.	2	3425.230	1712.615	444.453***	10428.289	5214.144	274.943***
		Time-Protect.	8	634.632	79.329	20.591***	6045.378	755.672	39.847***
		Error	75	288.940	3.853		1422.333	18.964	
		Total	89	4582.444			24583.156		
	Control Protect. by lamin.	Between group	9	2079.079	231.009	134.193***	11441.417	1271.269	129.413***
		Within group	50	86.073	1.721		491.167	9.823	
		Total	59	2165.152			11932.583		
	Control Protect. by *N. sativa*	Between group	9	3798.291	422.032	99.304***	13205.933	1467.326	71.787***
		Within group	50	212.496	4.250		1022.000	20.440	
		Total	59	4010.787			14227.930		

* (significant), ** (highly significant) and *** (very highly significant)

Table 5. Multi-Factor Analysis of Variance (MANOVA) for the effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the lymphocyte (L) and monocyte (Mn) counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

	Group	S.V	d.f	L			Mn		
				SS	MS	F Cal.	SS	MS	F Cal.
Laminarin	Control Treat. with lamin. Protect. by lamin.	General effect Time Lamin. Time-lamin. Error Total	 4 2 8 75 89	 5898.156 5164.689 17816.97 242.500 29122.32	 1474.539 2582.344 2227.122 3.233	 456.043*** 798.663*** 688.801***	 178.844 1003.889 451.556 266.833 1901.122	 44.711 501.944 56.444 3.558	 12.567*** 141.084** 15.865***
N. sativa	Control Treat. with *N. sativa* Protect. by *N. sativa*	General effect Time N. sativa Time-*N. sativa* Error Total	 4 2 8 75 89	 1782.156 20384.02 5784.978 239.167 28190.32	 445.539 10192.01 723.122 3.189	 139.716*** 3196.10*** 226.763***	 324.889 820.067 154.378 275.167 1574.500	 81.222 410.033 19.297 3.669	 22.138*** 111.760** 5.260***
Treatment	Control Treat. with lamin. Treat. with *N. sativa*	General effect Time Treat. Time-Treat. Error Total	 4 2 8 75 89	 4778.600 13863.80 10682.86 118.833 29444.10	 1194.650 6931.900 1335.358 1.584	 753.987*** 4374.97*** 842.793***	 106.267 344.867 230.133 128.833 810.100	 26.567 172.433 28.767 1.718	 15.466*** 100.382** 16.746***
	Control Treat. with lamin.	Between group Within group Total	9 50 59	14089.66 74.667 14164.33	1565.519 1.493	1048.34***	41.017 39.167 80.183	4.557 0.783	5.818***
	Control Treat. with *N. sativa*	Between group Within group Total	9 50 59	17887.15 63.833 17950.98	1987.461 1.277	1556.76***	574.750 113.833 688.583	63.861 2.277	28.050***
Protection	Control Protect. by lamin. Protect. by *N. sativa*	General effect Time Protect. Time-Protect. Error Total	 4 2 8 75 89	 8391.933 16700.46 7428.867 362.833 32884.10	 2097.983 8350.233 928.608 4.838	 433.667*** 1726.05*** 191.949***	 242.622 1056.689 530.644 413.167 2243.122	 60.656 528.344 66.331 5.509	 11.010*** 95.908*** 12.041***
	Control Protect. by lamin.	Between group Within group Total	9 50 59	17211.48 187.500 17398.98	1912.387 3.750	509.970***	1370.350 251.833 1622.183	152.261 5.037	30.231***
	Control Protect. by *N. sativa*	Between group Within group Total	9 50 59	20327.00 195.000 20522.00	2258.556 3.900	579.117***	997.483 185.500 1182.983	110.831 3.710	29.874***

* (significant), ** (highly significant) and *** (very highly significant)

Table 6. Multi-Factor Analysis of Variance (MANOVA) for the effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the eosinophil (Es) and basophil (Ba) counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

	Group	S.V	d.f	Es			Ba		
				SS	MS	F Cal.	SS	MS	F Cal.
Laminarin	Control	General effect							
	Treat. with lamin.	Time	4	50.489	12.622	19.125***	4.600	1.150	9.409***
		Lamin.	2	12.156	6.078	9.209***	1.067	0.533	4.364*
	Protect. by lamin.	Time-lamin.	8	96.178	12.022	18.215***	5.267	0.658	5.386***
		Error	75	49.500	0.660		9.167	0.122	
		Total	89	208.322			20.100		
N. sativa	Control	General effect							
	Treat. with *N. sativa*	Time	4	25.156	6.289	8.708***	5.511	1.378	11.698***
		N. sativa	2	8.622	4.311	5.969***	2.022	1.011	8.585***
	Protect. by *N. sativa*	Time-*N. sativa*	8	41.044	5.131	7.104***	5.422	0.678	5.755***
		Error	75	54.167	0.722		8.833	0.118	
		Total	89	128.989			21.789		
Treatment	Control	General effect							
	Treat. with lamin.	Time	4	8.933	2.233	4.295**	10.600	2.650	17.036***
		Treat.	2	14.067	7.033	13.526***	0.467	0.233	1.500
	Treat. with *N. sativa*	Time-Treat.	8	27.600	3.450	6.635***	2.867	0.358	2.304*
		Error	75	39.000	0.520		11.667	0.156	
		Total	89	89.600			25.600		
	Control	Between group	9	22.817	2.535	4.844***	8.933	0.993	6.473***
	Treat. with lamin.	Within group	50	26.167	0.523		7.667	0.153	
		Total	59	48.983			16.600		
	Control	Between group	9	31.333	3.481	6.447***	11.350	1.261	7.138***
	Treat. with *N. sativa*	Within group	50	27.000	0.540		8.833	0.177	
		Total	59	58.333			20.183		
Protection	Control	General effect							
	Protect. by lamin.	Time	4	102.956	25.739	29.852***	1.711	0.428	5.066***
		Protect.	2	13.400	6.700	7.771***	2.156	1.078	12.763***
	Protect. by *N. sativa*	Time-Protect.	8	73.378	9.172	10.638***	5.622	0.703	8.322***
		Error	75	64.667	0.862		6.333	0.084	
		Total	89	254.400			15.822		
	Control	Between group	9	149.350	16.594	22.126***	8.400	0.933	7.368***
	Protect. by lamin.	Within group	50	37.500	0.750		6.333	0.127	
		Total	59	186.850			14.733		
	Control	Between group	9	50.267	5.585	6.756***	8.150	0.906	9.368***
	Protect. by *N. sativa*	Within group	50	41.333	0.827		4.833	0.097	
		Total	59	91.600			12.983		

* (significant), ** (highly significant) and *** (very highly significant)

Table 7. Multi-Factor Analysis of Variance (MANOVA) showing the least significant difference (LSD) values for the studied haematological parameters.

Groups	LSD values	Parameters											
		RBCs	Hb	PCV	MCV	MCH	MCHC	WBCs	Nt	L	Mo	Eo	Bo
Control Treat. with lamin. Protect. by lamin.	Time	0.030	0.101	0.493	1.569	0.361	0.176	0.814	1.879	1.193	1.251	0.539	0.232
	Lamin.	0.023	0.078	0.382	1.215	0.280	0.136	0.630	1.455	0.924	0.969	0.417	0.180
	Time x Lamin.	0.052	0.174	0.854	2.717	0.625	0.304	1.409	3.254	2.066	2.167	0.933	0.402
Control Treat. with *N. sativa* Protect. by *N. sativa*	Time	0.035	0.130	0.564	2.905	0.614	0.228	1.385	2.947	1.185	1.271	0.564	0.228
	N. sativa	0.027	0.101	0.437	2.250	0.475	0.177	1.073	2.283	0.918	0.984	0.437	0.176
	Time x *N. sativa*	0.061	0.225	0.976	5.031	1.063	0.395	2.399	5.104	2.052	2.201	0.976	0.394
Control Treat. with lamin. Treat. with *N. sativa*	Time	0.034	0.124	0.617	2.338	0.573	0.208	0.941	1.967	0.835	0.869	0.478	0.262
	Treat.	0.026	0.096	0.478	1.811	0.444	0.161	0.729	1.524	0.647	0.673	0.371	0.203
	Time x Treat.	0.059	0.214	1.069	4.050	0.992	0.360	1.630	3.407	1.446	1.506	0.829	0.453
Control Treat. with lamin.	Treat. Lamin.	0.050	0.157	0.797	2.641	0.638	0.279	0.991	2.329	1.419	1.028	0.840	0.455
Control Treat. with *N. sativa*	Treat. *N. sativa*	0.059	0.228	1.130	4.560	1.112	0.369	1.829	3.848	1.312	1.752	0.853	0.488
Control Protect. by lamin. Protect by *N. sativa*	Time	0.031	0.108	0.424	2.331	0.423	0.199	1.302	2.889	1.459	1.557	0.616	0.193
	Protect.	0.024	0.084	0.329	1.805	0.327	0.154	1.009	2.238	1.130	1.206	0.477	0.149
	Time x Protect.	0.053	0.188	0.735	4.037	0.732	0.345	2.255	5.003	2.527	2.697	1.067	0.334
Control Protect. by lamin.	Protect. Lamin.	0.047	0.168	0.802	2.643	0.572	0.281	1.523	3.639	2.248	2.606	1.006	0.413
Control Protect. by *N. sativa*	Protect. *N. sativa*	0.053	0.179	0.588	4.542	0.794	0.344	2.394	5.249	2.293	2.236	1.056	0.361

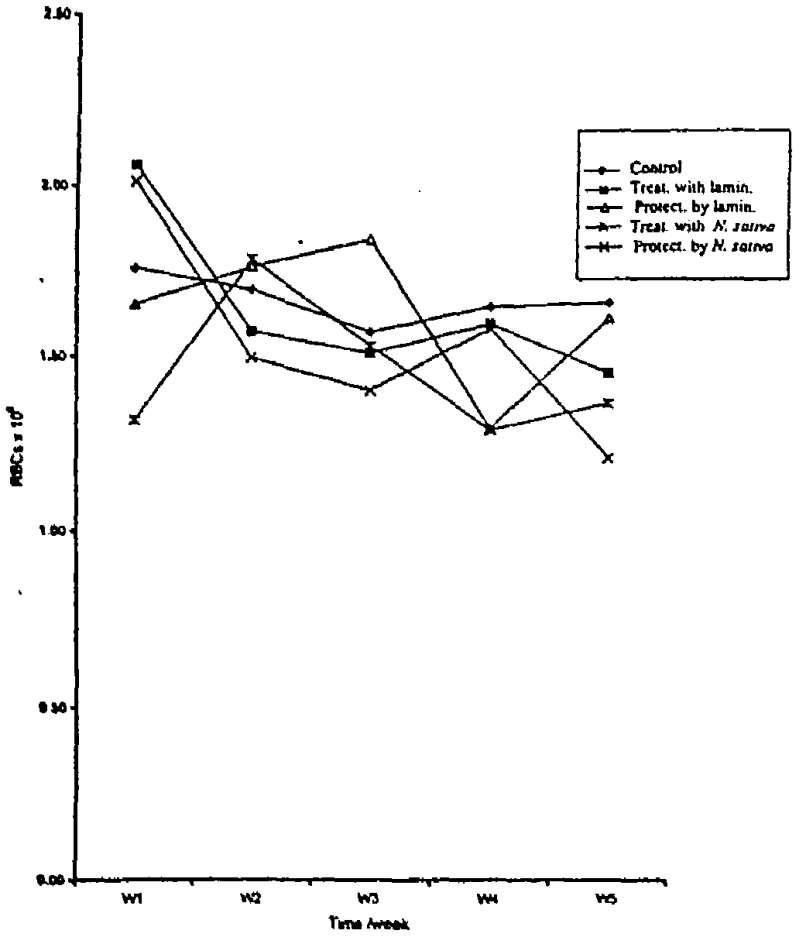

Fig. (1) The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the total red blood cell counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

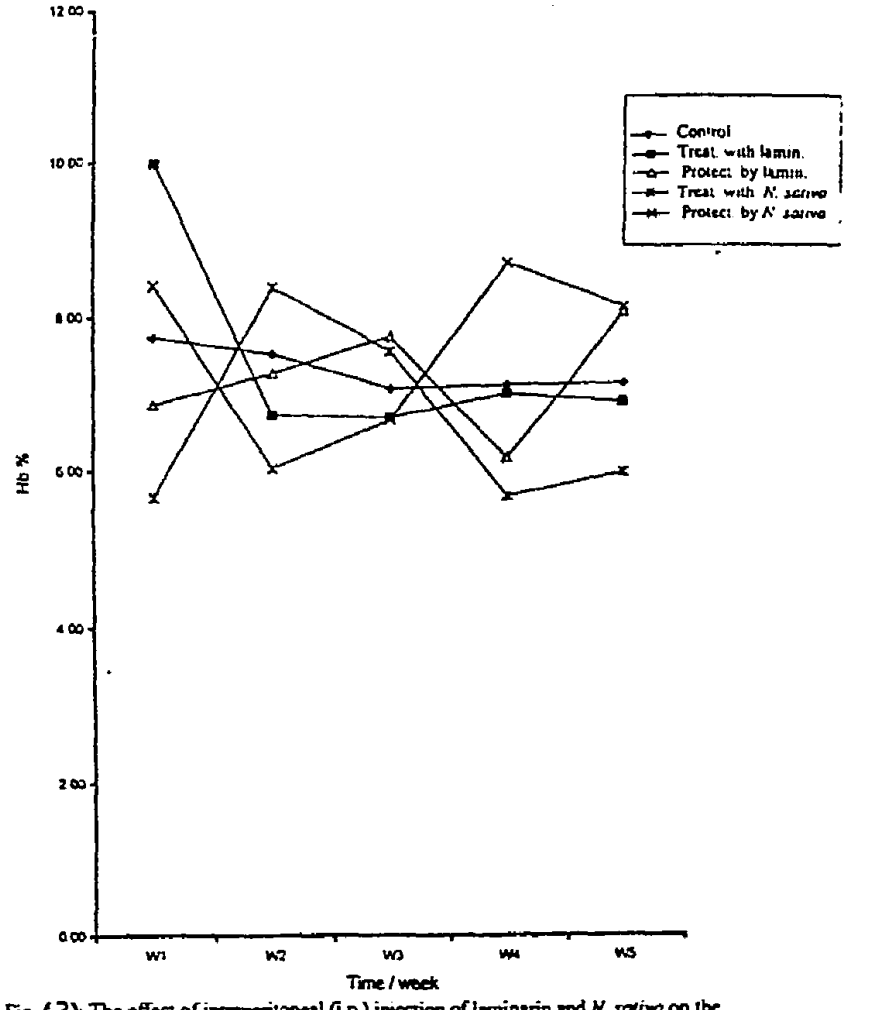

Fig (2): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the haemoglobin concentration of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

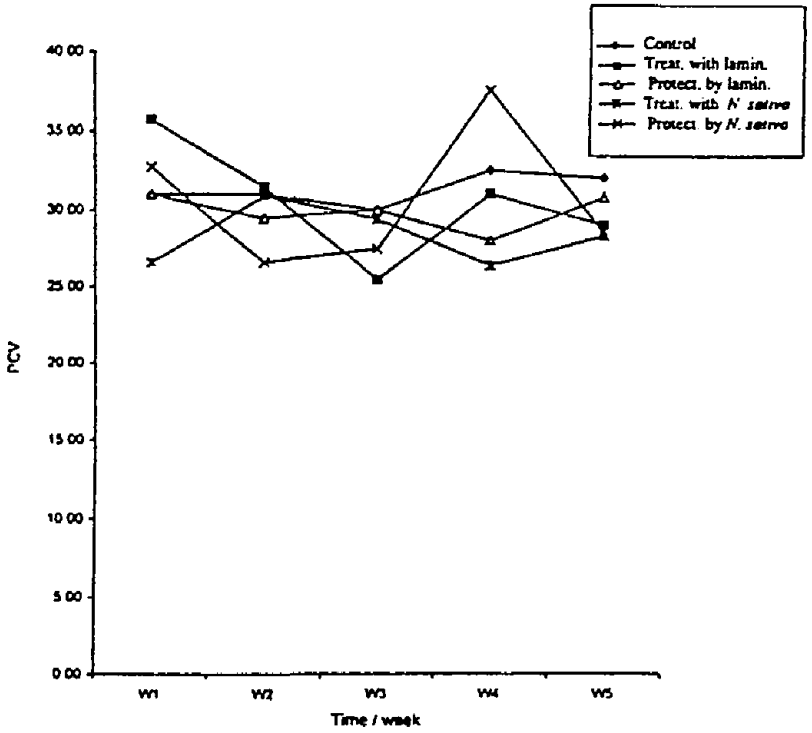

Fig. (3): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the packed cell volume (PCV) of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

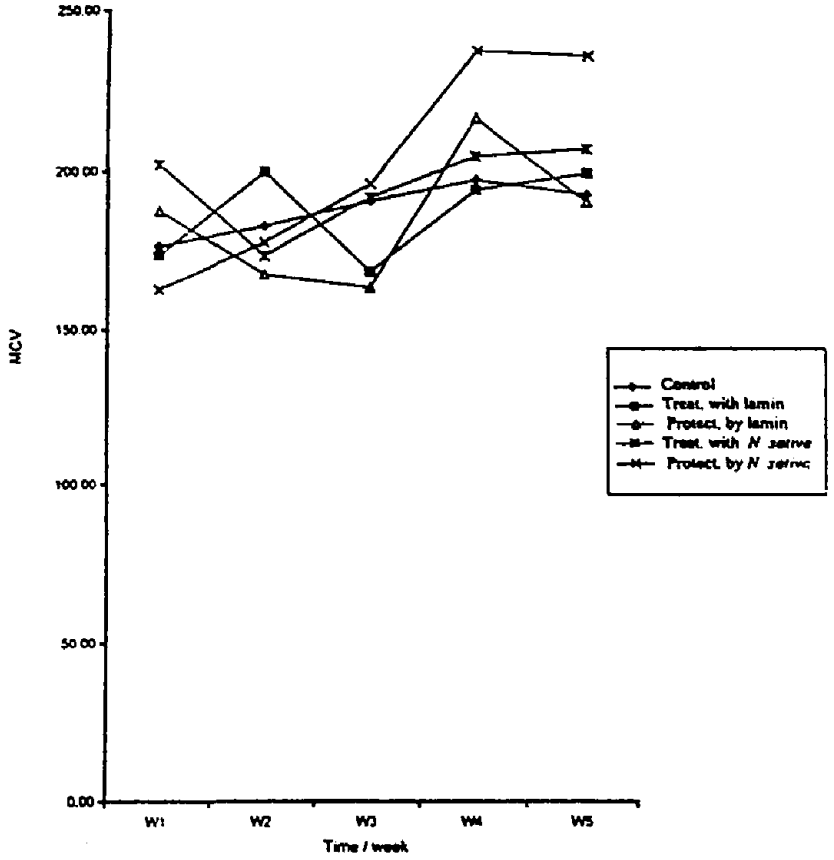

Fig. (4): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the mean cell volume (MCV) values of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

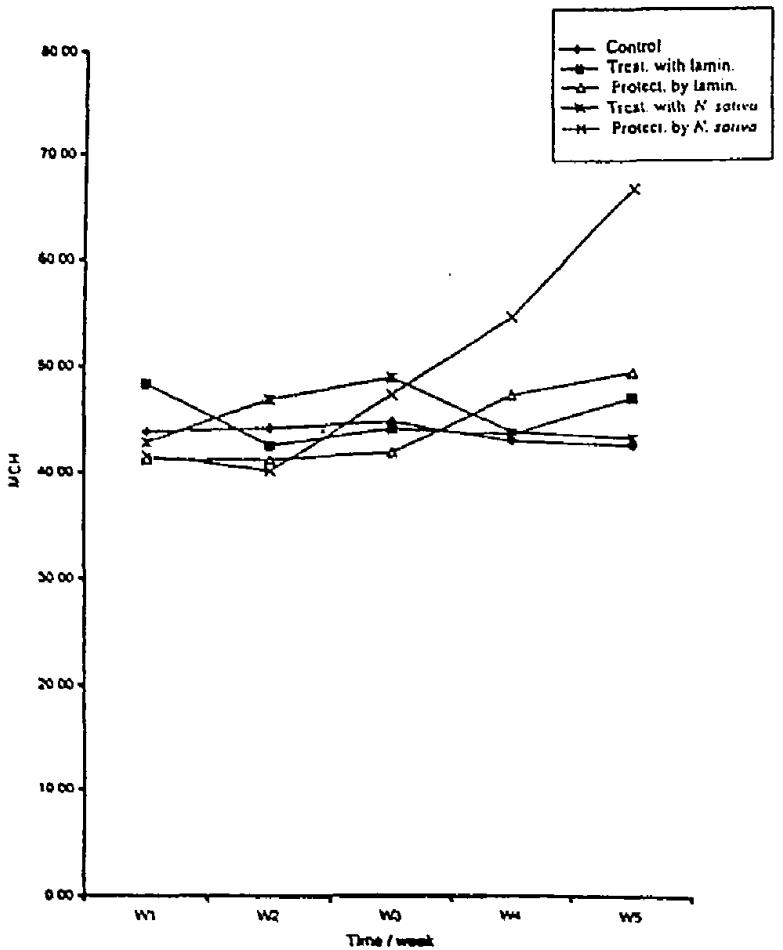

Fig. (5): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the mean cell haemoglobin (MCH) values of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

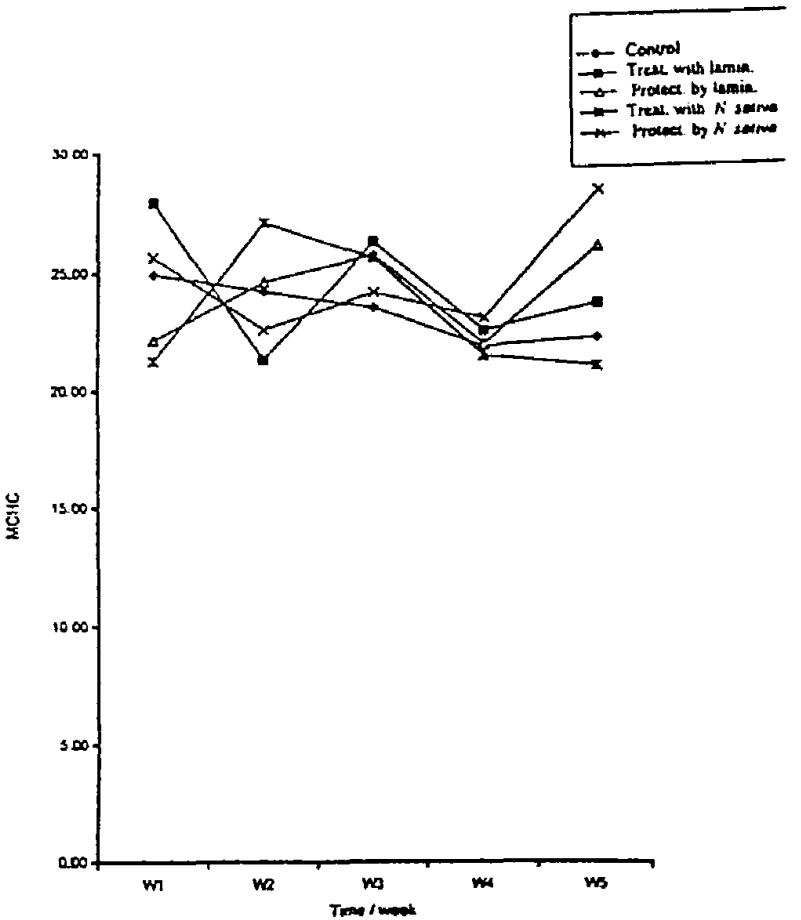

Fig. (6): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the mean cell haemoglobin concentration (MCHC) values of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

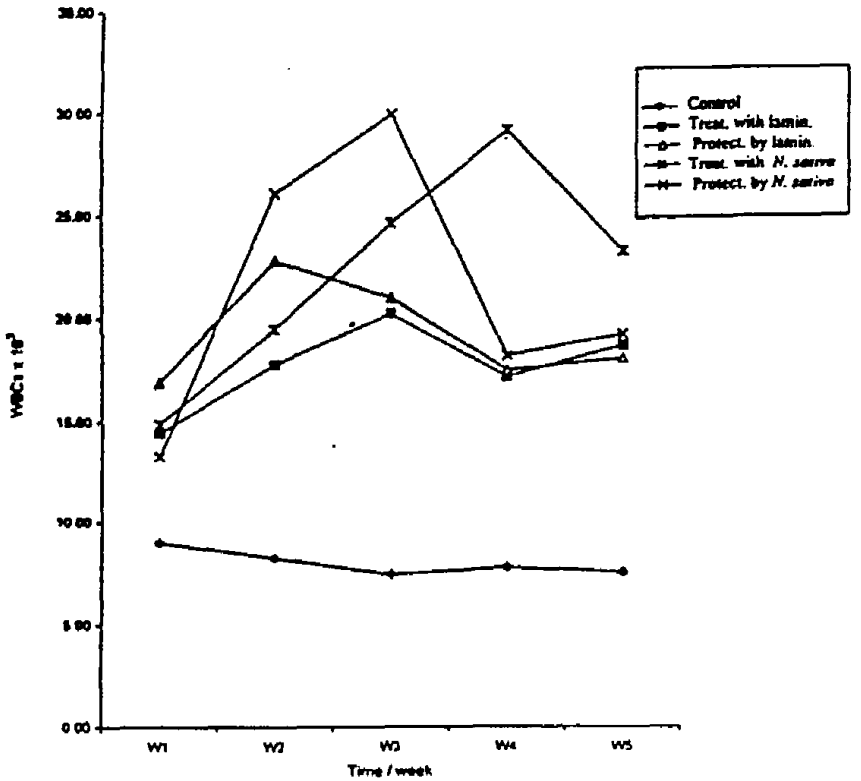

Fig. (7): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the total leucocytic counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

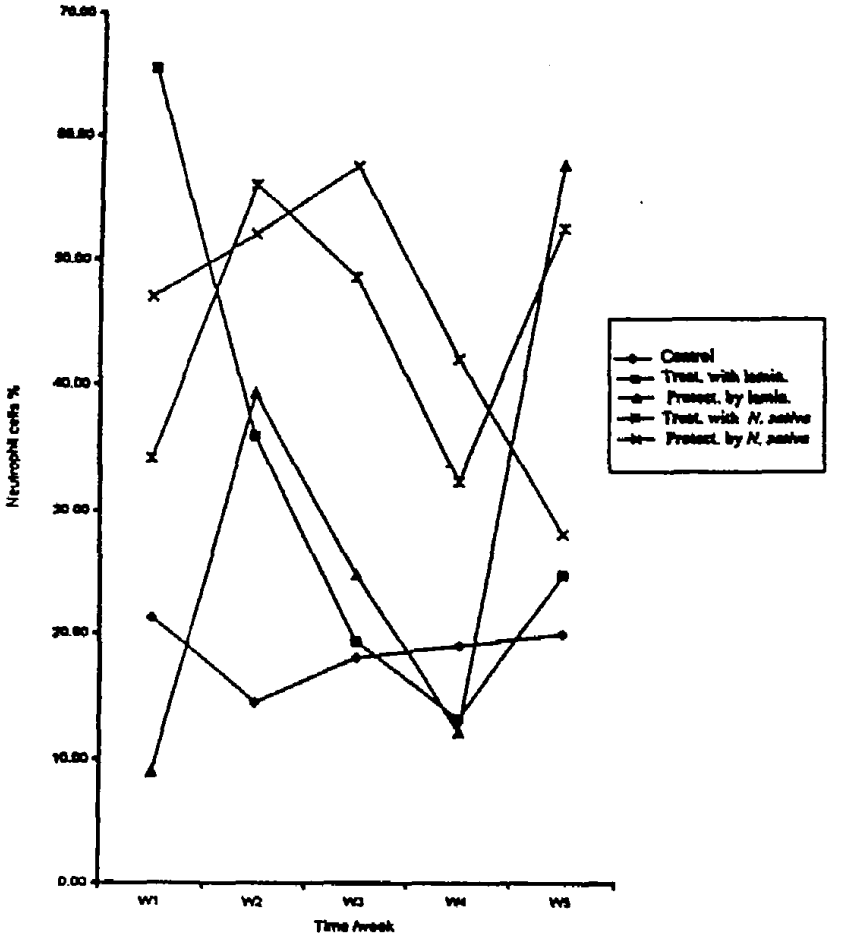

Fig. (8): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the neutrophil counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

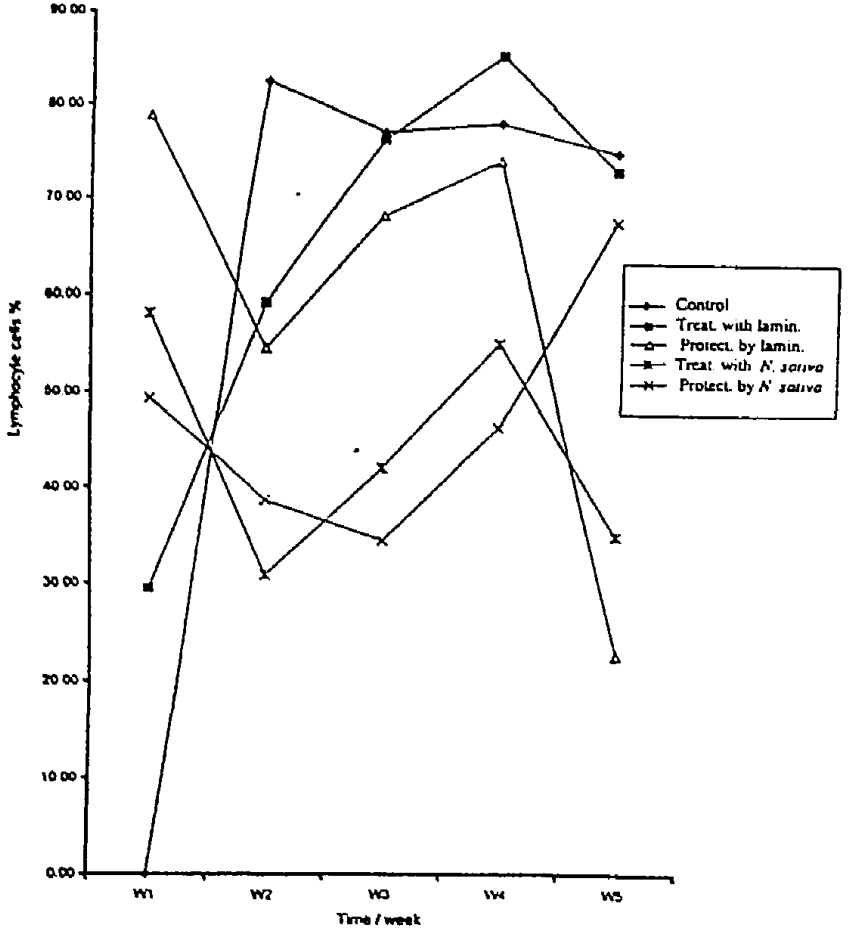

Fig. (9): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the lymphocyte counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

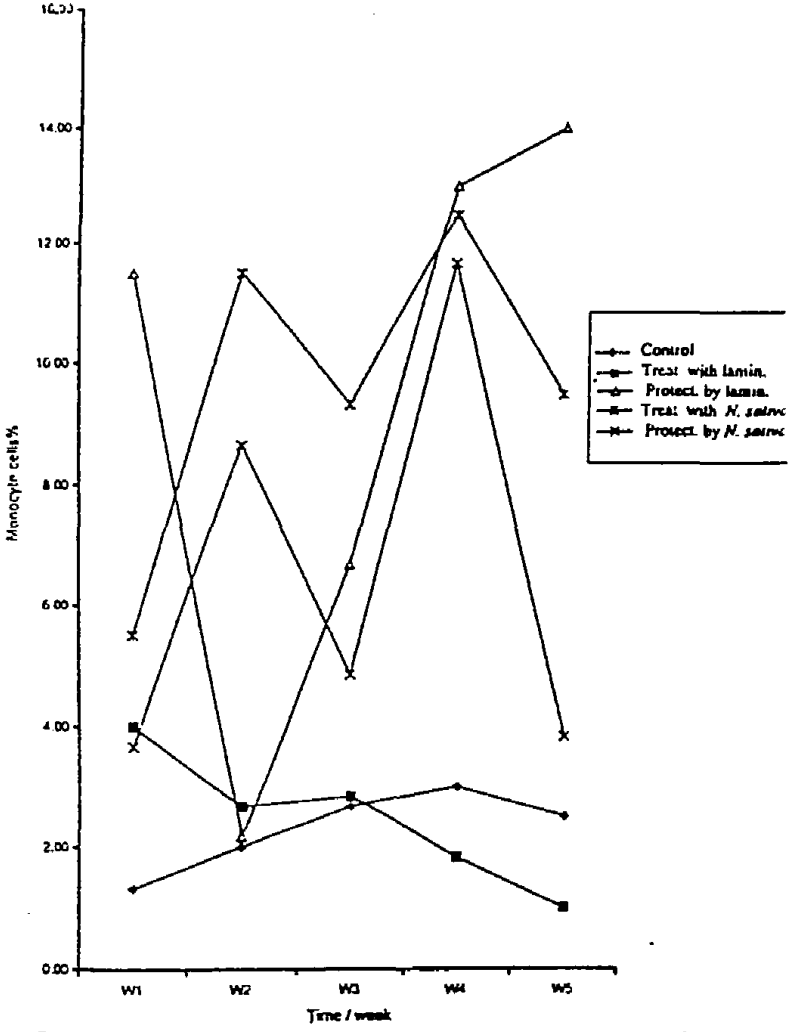

Fig (10). The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the monocyte counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

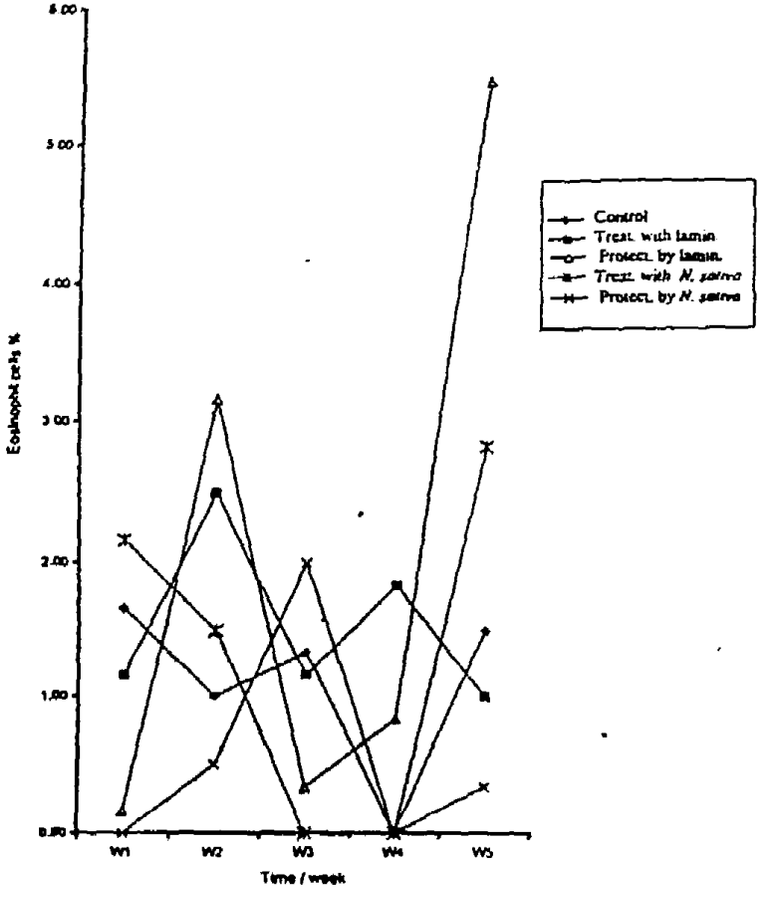

Fig. (11): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the eosinophil counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.

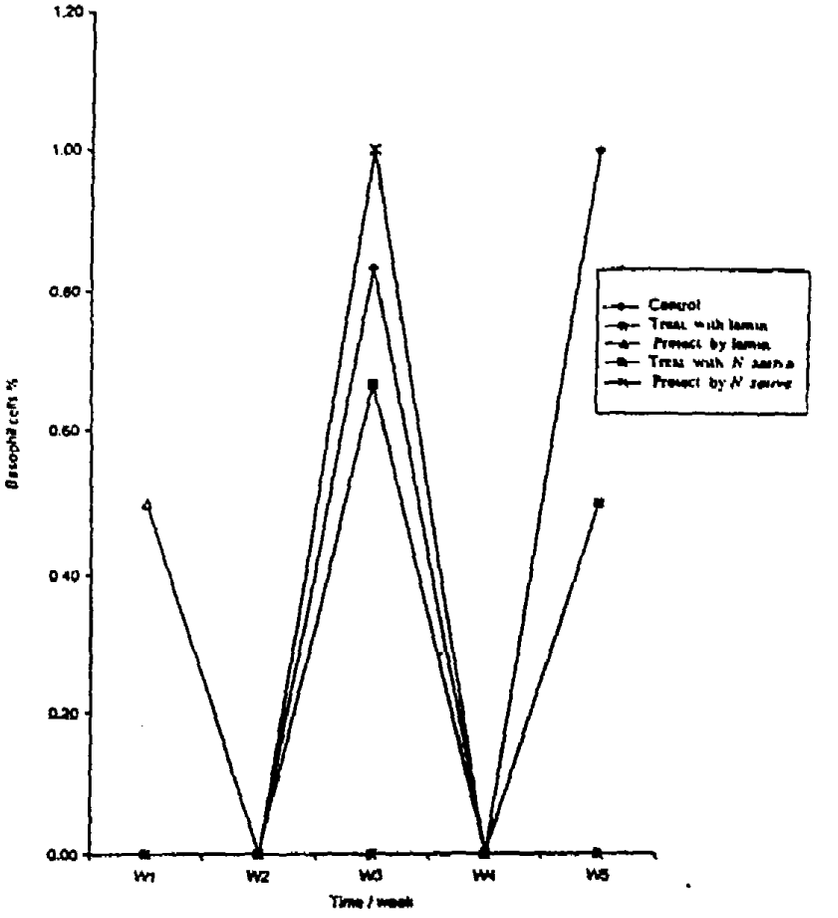

Fig. (12): The effect of intraperitoneal (i.p.) injection of laminarin and *N. sativa* on the basophil counts of common carp (*Cyprinus carpio*) challenged with *A. hydrophila* pathogen at different time periods.