

**STUDY ON THE EFFECT OF *NIGELLA SATIVA*
SUPPLEMENTATION ON THE GROWTH RATE PERFORMANCE
AND EXPERIMENTAL INFECTION BY *SALMONELLA*
TYPHIMURIUM IN SWISS MICE**

ROFABIL, S. K. AND A. M. DAUD

*Veterinary Serum and Vaccine Research Institute, Agricultural Research Centre,
Ministry of Agriculture, Giza, Egypt.*

(Manuscript received 26 July 2004)

Abstract

Nigella Sativa was used as diet supplementation in group of mice experimentally infected with *Salmonella Typhimurium*. It was found that mice that took diet with *Nigella Sativa* and experimentally infected with *Salmonella Typhimurium* showed improving in body weight and survival ratio was 96% compared with the group that had diet without *Nigella Sativa* and was experimentally infected with *Salmonella Typhimurium*. Bacteriological examination revealed that mice of treated group showed no histopathological changes and regained their normal appearance, while those of non-treated group showed infiltration of liver with mature and premature leucocytic cells in the hepatic tissues, the heart showed leucocytic inflammatory cells infiltration in between the vascular degenerated myocardial bundles. Finally, *Nigella Sativa* can be used in enhancing the immune system as well as it improved the growth rate performance.

INTRODUCTION

Nigella Sativa is a herb native to the Mediterranean, that belongs to the family Ranunculaceae. The active principle of *Nigella Sativa* was found to affect growth rate performance (Abaza *et al.*, 2003). Moreover, it inhibits the growth of several species of pathogenic bacteria and also has a beneficial effect on the immune system (Khaled *et al.*, 1998).

Nutrition has a profound effect on growth, immunity and health of animals, the nutrition deficiencies impair the immune responsiveness and thereby, increase mortality, subsequently the production capacity (James *et al.*, 2002).

Supplementation of natural growth promoters to mice feed in order to improve the utilization of nutrient and improvement in their body weight gain and mortality rate was discussed by Abaza *et al.* (2003).

Salmonella Typhimurium is one of the most important bacteria causing disease condition and high mortalities, in which the immune status deficiency plays an

important role in the progress of the bacteria (Rofaail, 1997).

Furthermore, the possibility of preventing the disease using dietary supplementation and/or herbal medicine has attracted considerable attention.

In Egypt, a little effort was done for investigation of the role played by *Nigella Sativa* in growth rate performance and enhancing the therapeutic effect for microbial agents.

Thus, the aim of the present study was to throw light on the role of *Nigella Sativa* supplementation on growth rate performance and their protecting effect in mice artificially infected with *Salmonella Typhimurium*.

MATERIALS AND METHODS

***Nigella Sativa* seeds** they were obtained from commercial sources, crushed and thoroughly mixed with a basal ration.

Experimental Mice one hundred and fifty mice were kept in separated cages under strict hygienic conditions, during the experiment.

***Salmonella Typhimurium* strain** local isolate of *Salmonella Typhimurium* was confirmed through Gram staining, colonial morphology, biochemical and serological reactions as described by Forbes *et al.* (1998).

Experimental designs mice were divided into three groups, 50 mice each and were treated as follows:

Group (1): Mice were fed on commercial ration supplemented with *Nigella Sativa* crushed seeds in concentration of 2.5%.

Group (2): Mice were fed on commercial ration without *Nigella Sativa* supplementation. The body weight of the two groups was recorded before feeding and then weekly after feeding for 10 weeks.

The mice of groups 1 and 2 were experimentally injected intraperitoneally with 0.5 ml twenty four hours broth culture of *Salmonella Typhimurium* containing 1.5×10^8 CFU/ml such mice were observed daily after experimental infection, any cases of mortality was recorded. Smears from internal organs of freshly dead mice were subjected to bacteriological examination. Reisolation of the organism from internal organs and confirmatory diagnosis was done as described by Forbes *et al.* (1998).

For histopathological examination tissue samples from the liver and heart were collected and fixed in 10% neutral formalin, washed by tap water, dehydrated in graded ethanol, embedded in paraffin sections at 4 - 5 microns and stained by

Hematoxylin and Eosin following the standard technique of Bancroft *et al.* (1994).

The mice of group (3) were left as control group had a normal ratio without *Nigella Sativa* and not experimentally infected with *Salmonella Typhimurium*.

RESULTS AND DISCUSSION

This work has been designed to investigate the possible effects of *Nigella Sativa* supplementation on the growth rate performance in mice experimentally infected with *Salmonella Typhimurium*.

Nigella Sativa is a herb that was reported to have a growth and immune stimulant, the extract of such plant contained several ingredients with potential value. It is composed of more than 100 components that worked synergistically, it is a rich source of poly-unsaturated fatty acids, which are the building blocks of cells, protein which sustained body health as well as thymohydroquinone which was proved to have a high anti-microbial effect (Khaled *et al.*, 1998).

From the obtained data in Table 1 it is clear that the average live body weight of mice fed on ration supplemented with *Nigella Sativa* crushed seeds is better than that of mice fed on ration without *Nigella Sativa*.

These results are in agreement with the results of Osman and El-Barady (1999) who found that the addition of *Nigella Sativa* in broiler diet had significant effect on average body weight and improved feed conversion. In addition, Harkness and Wagner (1995) recorded that improvement of growth performance as a result of *Nigella Sativa* supplementation of growing mice, can be attributed mainly to the increased animals resistance during physiological and/or environmental stress, through the noticeable improvement in animal health, and increase of the responsiveness of immune system besides the other functions being affected by growth performance.

This finding was already discussed by Abdel Aziz *et al.* (1995) who attributed the improvement of the live body weight as well as feed conversion to the choleric action of *Nigella Sativa* through increasing the bile flow as well as improving the exocrine function of the pancreas and accordingly the digestive performance. It may also be related to the stimulating effect of *Nigella Sativa* on the thyroid gland directly or through the pituitary level, hence anabolic effect is expected.

Data recorded in Table 2 showed that protective efficacy of *Nigella Sativa* supplementation with ration on the mice experimentally infected with *Salmonella Typhimurium*, increased the survival percent among the treated group with *Nigella*

Sativa and reached 96%, with a mean death time of 7 days, with a mean lesion score (+), while in non-treated group with *Nigella Sativa*, the survival percent was 20% with a mean death time of 3 days and a mean lesion score (+++). It could be said that the action of *Nigella Sativa* in reducing the mortality rate of mice infected with *Salmonella Typhimurium* is due to its properties as antimicrobial agent (Hanafy and Hatem, 1991).

From the obtained results the anti-microbial response produced by feeding mice on ration supplemented with *Nigella Sativa* is similar to that reported by Benirsckle *et al.* (1978) who explained the improvement of the immune status of mice, the active principle of *Nigella Sativa* enters the blood stream and soon reaching all parts of body. It was referred to that the regaining of normal immune status is due to the enhancement of the natural killer cells to restore their activity.

The results also are similar to those obtained by Wahba (2002) who studied the prophylactic and therapeutic effect of *Nigella Sativa* on rats infected with the fatal protozoan *Pneumocystis carinii*. He found that rats treated with the *Nigella Sativa* could tolerate the infection, while those non-treated did not survive. El-Refaii (2003) reported that *Nigella Sativa* had a therapeutic effect on *Cryptosporidium parvum* in experimentally infected mice and found that treated group with *Nigella Sativa* showed a reduction in oocysts count, when compared to those non-treated group.

From the pathological point of view, the group of mice supplemented with *Nigella Sativa* in ration resisted the intraperitoneal inoculation with *Salmonella Typhimurium*. They appeared normal and microscopically, the internal organs specially liver and heart did not show any pathological changes (Figures 1 and 2). On the other hand, the group of mice, which was not supplemented with *Nigella Sativa* ration showed friable liver with necrotic foci and congestion of heart as shown in Figures 3 and 4. The liver showed mature and primitive leucocytic cells infiltration in the hepatic tissue, the heart showed leucocytic inflammatory cells infiltration in between the vascular degenerated myocardial bundles.

The control group did not show any pathological changes in liver or heart. So, it was concluded that supplementation of *Nigella Sativa* which is a cheap local plant can be used to increase growth and proved to be beneficial in protecting mice against *Salmonella Typhimurium* and can be used as feed additive.

Table 1. Effect of dietary *Nigella Sativa* supplementation on mice live body weight .

Time of testing	Average live body weight (in gm) in group of mice fed on ration supplemented with <i>Nigella Sativa</i>	Average live body weight (in gm) in group of mice fed on ration not supplemented with <i>Nigella Sativa</i>
Zero time	15.20	15.30
After 1 week	17.30	16.10
After 2 weeks	19.70	17.00
After 3 weeks	22.00	19.40
After 4 weeks	25.60	21.30
After 5 weeks	28.80	23.20
After 6 weeks	33.20	24.80
After 7 weeks	38.60	26.50
After 8 weeks	42.40	30.20
After 9 weeks	48.60	32.60
After 10 weeks	51.20	34.80

N.B. No. of mice were 50 in each group.

Table 2. Efficacy of *Nigella Sativa* supplementation in ration of mice experimentally infected with *Salmonella Typhimurium* .

Groups of mice	No. of dead mice/Total No. of mice	Mean death time (Days)	Mortality rate	No. of survived mice/Total No. of mice	Protection percent	Lesion score
Group (1)	2/50	7	4 %	48/50	96 %	+
Group (2)	40/50	3	80 %	10/50	20 %	+++
Group (3)	0/50	-	0 %	50/50	100 %	-

Group 1: Supplemented with *Nigella Sativa* in ration and infected with *Salmonella Typhimurium*.

Group 2: Not supplemented with *Nigella Sativa* in ration and infected with *Salmonella Typhimurium*.

Group 3: Control not supplemented with *Nigella Sativa* in ration and not infected with *Salmonella Typhimurium*

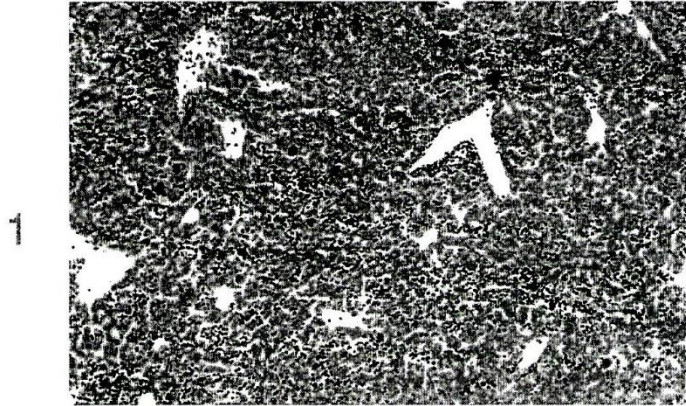


Figure 1. Liver of mice had *Nigella Sativa* in ration and inoculated (I/P) by *Salmonella Typhimurium* showing recovery of pathological lesions to its normal histological structure (H & E x40)

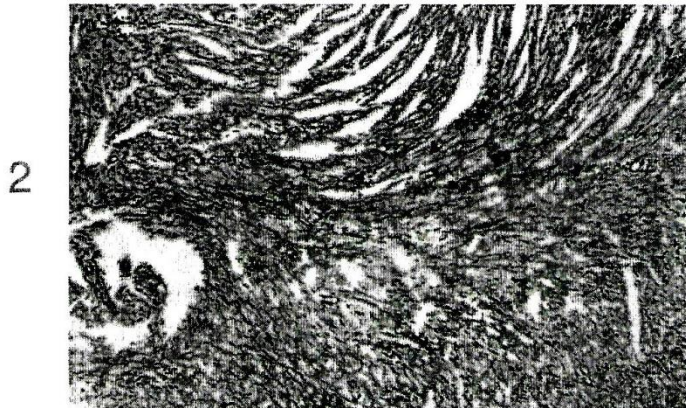
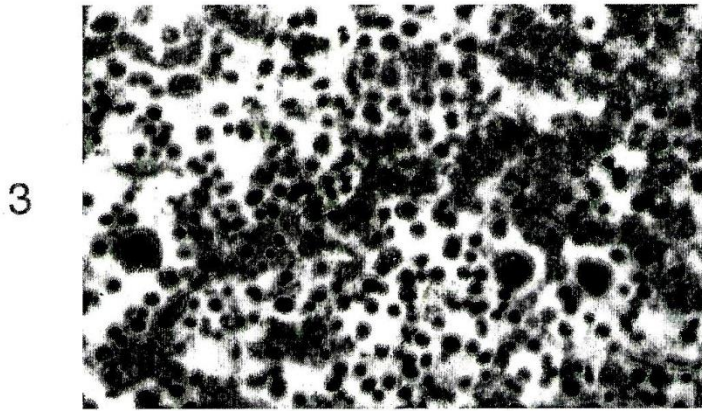
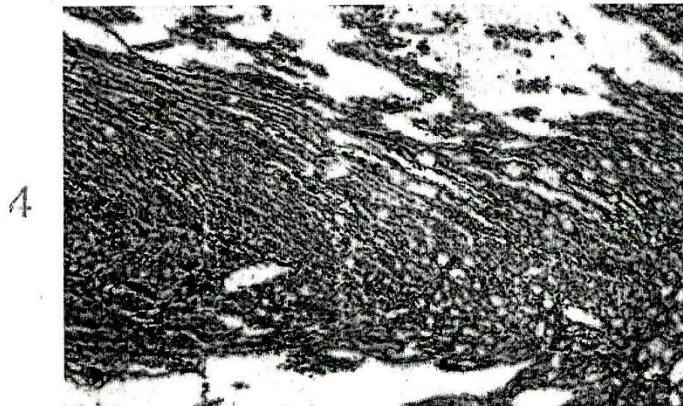


Figure 2. Heart of mice had *Nigella Sativa* in ration and inoculated (I/P) by *Salmonella Typhimurium* showing recovery of pathological lesions to its normal histological structure (H & E x40)



3
Figure 3. Liver of mice not supplemented *Nigella Sativa* in ration and inoculated (I/P) by *Salmonella Typhimurium* showing the mature and premature leucocytic cells infiltration in the hepatic tissues (H & E x160)



4
Figure 4. Heart of mice not-supplemented *Nigella Sativa* in ration and inoculated (I/P) by *Salmonella Typhimurium* showing leucocytic inflammatory cells infiltration in between the vascular degenerated myocardial buddles (H & E x40)

REFERENCES

1. Abaza, I. M. K., M. A. Asar, G. E. El-Shaarrawi and M. F. Hassan. 2003. Effect of using *Nigella Sativa* seeds, chamomile flowers, thyme flowers and Harmala seeds as feed additives on performance of broiler. J. Agric. Res., 81 (2): 735-750.
2. Abdel Aziz, M. J., S. El-Sayed, A. Z. Said and N. N. Nader. 1995. The effect of *Nigella Sativa* (Black cummin) on exocrine and endocrine pancreatic cells: an electron microscopic study. Alex. J. Vet. Sci., 11: 333-343.
3. Bancroft, D. J., C. H. Cook, R.W. Storlig, and D. R. Turner. 1994. Manual of histologic techniques and their diagnostic application. Churchill, Livingstone, Edinburgh.
4. Benirschke, K., F. M. Garver and T. C. Jones. 1978. Pathology of laboratory animals. 2 Vol. Springer-Verlag, New York.
5. El-Refaii, Magda A. H. 2003. Trials o therapeutic effect of *Nigella Sativa* oil extract on *Cryptosporidium parvum* in experimentally infected mice. J. Agric. Res., 81 (2): 805-816.
6. Forbes, B. A., D. F. Sahm, and K. S. Weissfield. 1998. Baily and Scotts Diagnostic Microbiology, Mosby, St. Louis.
7. Hanafy, M. S. M. and M. E. Hatem. 1991. Studies on the antimicrobial activity of *Nigella Sativa* seeds. J. Ethropharma, 34: 275-278.
8. Harkness, J. E. and J. E. Wagner. 1995. The biology and medicine of rabbits and rodents. 4th ed. Philadelphia, Lea and Febiger.
9. James, G. F., C. A. Lynn, M. L. Frankline and W. Q. Fred. 2002. Laboratory animal medicine. 2nd ed. Academic Press, London, New York.
10. Khaled, M. L. A., A. El-Kadery, B. El-Gozy and N. Abdel Fattah. 1998. Effect of *Nigella Sativa* seeds (Black cummin) on experimental *Hymenolepis nana* infection. Egypt. J. Med. Sci., 19 (1): 47-56.
11. Osman, A. M. A. and H. A. A. El-Barady. 1999. Growth performance and immune response of broiler chicks as affected by diet density and *Nigella Sativa* seeds supplementation. Egypt. Poult. Sci., 19 (111): 619-634.
12. Rofail, S. K. 1997. Bacteria causing diarrhoea in Swiss mice. Thesis, M.V.Sc., Microbiology, Fac. Vet. Med., Alexandria Univ.
13. Wahba, A. A. 2002. Efficacy of *Nigella Sativa* oil extract on pneumocystis carinii in immunosuppressed rats. J. Agric. Res., 80 (1): 367-376.

دراسة تأثير الحبة السوداء على معدل النمو والعدوى التجريبية بالسالمونيلا
تيفيموريوم في الفئران السويسرية

صفوت كمال روفائيل ، أحمد محمود داود

معهد بحوث الأمصال واللقاحات البيطرية - مركز البحوث الزراعية - وزارة الزراعة -
الجيزة - مصر.

أجريت هذه الدراسة لمعرفة تأثير إضافة الحبة السوداء على معدل أداء النمو وفاعليته
الوقائية على الفئران السويسرية المعدية تجريبياً بالسالمونيلا تيفيموريوم. وأثبتت النتائج أن هناك
زيادة واضحة في أوزان الفئران في المجموعة المضاف إليها الحبة السوداء في علاقتها بالمقارنة
بالمجموعة الثانية الغير معالجة. وأيضاً وجد أن هناك تأثير مناعي واضح في الفئران التي تم عداها
بالسالمونيلا تيفيموريوم والمعالجة بالحبة السوداء حيث كانت نسبة الفئران المستمرة على قيد الحياة
هي 96% بالمقارنة بالمجموعة الثانية الغير معالجة. علاوة على خلوها من الميكروب بالفحص
البكتريولوجي ولم تظهر أى تغيرات هستوباثولوجية. اما المجموعة الغير معالجة فقد ظهرت بها
تغيرات في الكبد عبارة عن إرتشاح متجمع من خلايا الدم البيضاء في النسيج الكبدي وأيضاً فى
القلب بين الأوعية المنحلة لحزمة عضلة القلب. ومن خلال تلك الدراسة يمكن أن يوصى بإمكانية
استخدام الحبة السوداء فى علائق الفئران لرفع كفاءة الجهاز المناعى وكمحفز للنمو.