



## **Insecticidal Property of Black Seed (*Nigella sativa*) Powder as an Eco-friendly Management of Skin Beetle *Dermestes maculatus* (Coleoptera: Dermestidae) in Atlantic Codfish *Gadus morhua* (Gadiformes: Gadidae)**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors compiled the literature search, assembled, proofread and approved the work. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

The bio-pesticidal potential of *Nigella sativa* seed powder in the management of *Dermestes maculatus* in codfish (*Gadus morhua*) was evaluated in the laboratory. *D. maculatus* beetles were obtained from naturally infested smoked fish, cultured at ambient temperature for the establishment of new stock and same age adults. Purchased *N. sativa* seeds were ground into fine powder, weighed at 0.4 g, 0.8 g, 1.2 g, 1.6 g and 2.0 g for use in the bioassay. The treatments were separately added into 40 g codfish kept in Kilner jar into which two sexed pairs of *D. maculatus* were introduced and observed. From the results, the number of the developmental stages (larvae, pupae and adults) of *D. maculatus* in codfish treated with *N. sativa* seed powder

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was inversely proportional to the concentration of the seed powder. Thus, an increase in the concentration of *N. sativa* powder generated reduction in the mean number of *D. maculatus* progeny found in the codfish after 35 days as follows: at 0.4 g, progeny development was (103.50, 7.75, 2.50) and 77.00, 8.25 and 1.00 at 2.0 g respectively for larva, pupa and adult stages. Percentage protection conferred by the botanical on *D. maculatus* showed that all the doses applied were effective. Corrected mortality of *D. maculatus* adults after 45 days of exposure to the different doses of *N. sativa* treatments also increased with an increase in concentration of *N. sativa* from 0.4 g to 2.0 g. Frass weight generated by *D. maculatus* activity in codfish treated with *N. sativa* powder was high in codfish treated at 1.6 g. Finally, *N. sativa* characterized both qualitatively and quantitatively for phytochemicals, proved the secondary metabolites with bio-pesticide potentials against *D. maculatus* such as phenols, Alkaloid, flavonoid, terpenoid, saponin, tannin, Cardiac glycoside, anthraquinone, steroid and oxalate in different proportion, therefore, should be utilized in Integrated Pest Management program of stored codfish.

**Keywords:** *Nigella sativa*; *Dermestes maculatus*; codfish; progeny emergence; phytochemicals.

## 1. INTRODUCTION

Fish being one of the cheapest sources of protein across the globe especially in the developing world is consumed both fresh and processed [1]. About 3.5 million people in Africa depend on fish wholly or partially either as sources of protein or other means of livelihood [2]. The industry in Africa is put at three billion dollar worth [3]. *Gadus morhua* commonly called stockfish or codfish is an unsalted cured fish usually dried by cold air and wind on wooden racks [4], a process known as freeze drying. Codfish is relished widely across African continent especially in Nigeria where [5,6] long ago reported that Nigeria is one of the highest consumers of stock fish in Africa. It is used in many West African dishes and soups. Cod/Stockfish are saltwater whitefish that turns creamy after they are dried and sold at a very high cost [5]. Sea foods such as codfish, crabs, periwinkles, shrimps, oyster, crayfish, shark, mackerel, haddock, salmon and tuna are good sources of high quality protein that help in building healthy immune system, support blood circulation, assist in proper weight management, and provide other nutritional functions needed by the body [7]. Codfish is high in omega-3 fatty acids required for proper brain and nervous system function and reduce blood pressure and prevents excess fatty substances in the arteries and in turn lower cholesterol level in the blood. Codfish has no fibre and carbohydrate content and the fresh codfish has about 72 kilo calorie of energy per 100 g [8].

As soon as fish is caught, deterioration commences immediately, therefore, traders device a means of overcoming it by processing using either salt, smoke or even sun drying.

Despite these processing techniques, smoked/dried fish is prone to heavy insect infestation [9]. Haines and Rees [9], Zakka et al. [10] earlier reported *Dermestes* species and *Necrobia rufipes* as the major pests on cured fish especially when stored over a long period of time. To avert such loss especially in qualitative and quantitative, traders and even consumers that buy fish in bulk use diverse classes of synthetic insecticides as control strategy. Currently, the insect pest control practices rely heavily on application of pesticides which has led to several problems such as health hazards especially to the consumers. About 80% of pesticides applied as control measures against either field or store crops and against public and animal health vectors end up in the environment as run-off or directly exposing animals, and farmers as well as consumers of such agricultural produce and animals to severe health problem [11]. Such risk can be overcome by the use of non-synthetic pesticides otherwise biopesticides that are regarded as natural eco-friendly [12] pesticides with adequate natural compounds that can control insect pests and plants with such bioactive compounds have had great success stories [13,14,15,16]. Such plants include pyrethrum (*Tanacetum cinerariifolium*) [13,17,18], neem (*Azadirachta indica*) [19,20], garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*) and thyme (*Thymus vulgaris*) [21,22]. Mahmood et al., [23] stated that pest management using biopesticides is an age long tradition especially among rural dwellers [24], until the recent introduction of synthetic pesticides where the former was considered ineffective especially in the management of major pest and disease of pandemic magnitude such as rusts and blights [12] which required quick action and wide

coverage area. However, the threat to both environment and human health attributed to the synthetic pesticide has called for man to advocate the use of natural plant material in pest management to overcome the dangers of hazardous chemical pesticide residues in foods and feed stuffs which are usually above the minimum residual or permissible levels in food and feed stuffs [25-27]. Biopesticides are capable of repelling, inhibiting growth or killing pests [28]. The plant parts utilized include the bark, leaves, roots, flowers, fruits, seeds, cloves, rhizomes and stems depending on the active ingredient of interest and their abundance in certain plant parts, which is either dried and ground into fine powder and extracted with organic solvents [29]. These are then utilized as plant extracts, essential oils or both [30]. The advantage of using biopesticides especially plant derived pesticides include its availability in every locality, multiple uses such as medicinal, spices, ornamentals and even food and or as feed [31]. Castillo-Sanchez et al. [19] stated that they are inexpensive and are easily integrated into other pest management option. Other advantages include least toxicity to beneficial organism since they are target specific with none or little allelopathic effect [32]. Dubey et al., [33,34] viewed other complimentary attributes of biopesticides such as residual effect in treated crop products and environment, reliable and acceptable in sustainable agriculture. Although Kole et al., [35] stated some of the shortfalls of biopesticides such as their ability to degenerate or break down very quickly in sunlight. Also both highly alkaline and acid conditions accelerate their degradation thus they require repeated applications for optimal effectiveness.

Black seed (*Nigella sativa*) plant is found in southwestern Asia and parts of the Mediterranean and Africa with a long history of use in diverse culinary and traditional medicine [36]. It is also grown for its pungent seeds used as spice, skin cream for treating eczema and stimulate lactation [37]. It has been used for menstrual and postpartum problems in some cases. Other uses include treatment of intestinal worms, digestive disorder, asthma, bronchitis and rheumatoid arthritis [38]. The black cumin plant is an annual hardy plant that grows in a variety of soils. The plant readily reseeds, becoming weedy in some areas as it grows up to 60 cm [39]. The seeds have an aroma similar to fennel with pungent flavour somewhat similar to nutmeg [40] and commonly roasted and ground as a spice in India, the Middle East, and parts of

North Africa where it is used to season curries, rice, breads, and sweet confections [41].

*Dermestes maculatus* DeGeer, 1776 is a black, hairy coleopteran beetle commonly known as hide or skin or leather beetle. The beetle has a holometabolous life cycle transcending from eggs, larvae, pupae and adults. It feeds on dried fish, carrion and other dry animal products and are considered the predominant insect pest of codfish [42-44]. They are known to eat up completely flesh from bones exposing the skeleton of fishes [45]. *Dermestes maculatus* has been reported to have developed some resistance to certain pesticides, which has led to several economic losses. Therefore, there is need to study and ascertain, certain natural protectant of codfish against *D. maculatus* infestation hence the relevance of this study in examining the insecticidal potentials of *N. sativa* on the development of the insect pest *D. maculatus* beetle on codfish in the laboratory.

## 2. MATERIALS AND METHODS

### 2.1 *Dermestes maculatus* Culture

Several unsexed adults of skin beetles obtained from naturally infested smoked fish materials from open market were placed in plastic containers and then cultured in the laboratory on codfish purchased from Rumuokoro Market in Obio Akpor Local Government Area of Rivers State. The top of the lids were cut open but netted to allow for sufficient aeration, avoid escape of the *D. maculatus* culture and the entrance of unwanted insect pest. Adopting the methodology of [46], the culture was kept at ambient temperature and relative humidity for development of new generation and same age stock of *D. maculatus* for use in the bioassay.

#### 2.1.1 *Dermestes maculatus* sub-culture

In order to obtain adult *D. maculatus* with uniform age and unmated adults, the last larval instars were carefully handpicked using soft brush and placed in well labeled test tubes containing pieces of codfish where they were allowed to complete their life cycle and left unmated until needed [46].

#### 2.1.2 Mating of *Dermestes maculatus*

*Dermestes maculatus* adults were sexed using the features described by [47] that the males are distinguished from the females by their

possession of a deep depression and brush of hairs on the 4<sup>th</sup> abdominal sternite.

## 2.2 Preparation of Black Seed (*N. sativa*) Powder

*Nigella sativa* seeds purchased from an importer vendor of foreign spices from India with due approval by National Agency for Food and Drug Administration and Control (NAFDAC) was taken to the laboratory. The black seeds were ground into fine powder using laboratory pestle and mortar and sieved using 250 µm standard test sieve and stored in a specimen bottle for 24 hours prior to the start of the experiment to avoid possible depreciation of the active ingredient.

## 2.3 Experimental Set-up

40 g fleshy parts of the codfish were weighed and placed in plastic containers. The plant materials were respectively weighed at five treatment levels (0.4 g, 0.8 g, 1.2 g, 1.6 g and 2.0 g) using a sensitive balance and added into each substrate. Two pairs of *D. maculatus* were introduced into the different concentrations and left on work bench for observations. Each treatment was replicated four times.

## 2.4 Phytochemical Analysis of Black Seed (*N. sativa*) Powder

Qualitative phytochemical screening of *N. sativa* was conducted in Autino research laboratory to determine the presence of secondary metabolites such as alkaloids, flavonoids, tannins, cardiac glycosides, saponins, terpenoids, phenols, oxalate and others using standard laboratory procedures described by [48-50]. Furthermore, quantitative phytochemical analysis to determine the percentage composition of phytoconstituents screened were carried out using standard gravimetric and spectrophotometric procedures spelt out by [48-50]. All experiments were done in three replicates.

## 2.5 Corrected Mortality

Data on percentage adult *D. maculatus* was corrected using Abbott's, 1925 formula

$$\% \text{ Corrected mortality} = \frac{(Po - Pc) \%}{(100 - Pc) \%} \times 100$$

Where: Po = Observed mortality  
Pc = Control mortality

## 2.5.1 Weight loss assessment

Percentage weight loss was determined by direct weighing method in which the initial and final weights of each fish substrate were recorded and the percentage weight loss calculated [51].

## 2.5.2 Percentage protection of treatment

The effectiveness of different treatments in protecting the codfish was calculated using the formula employed by [51].

$$\text{Percentage protection} =$$

$$\frac{\text{Total F1 progeny in control} - \text{Total F1 progeny in treatment}}{\text{Total F1 progeny in control}} \times 100$$

## 2.6 Data Collection and Analysis

Data collected were progeny development such as number of larvae, pupa and adults, frass weight, adult mortality, percentage weight loss and protection. All data were subjected to analysis of variance and significant means were separated using LSD at 5% level of probability.

## 3. RESULTS AND DISCUSSION

The result of *D. maculatus* progeny developed on codfish *G. morhua* in a laboratory treated with *Nigella sativa* powder as an eco-friendly bio-protectant (Table 1). Codfish treated with different doses of the plant bio-pesticide responded differently at each development stages. The result showed that the higher the concentrations/ doses of the seed extract treatments, the lower the mean number of the various developmental stages of *D. maculatus* found in the codfish. Higher mean number of larvae was recorded in the control experiment while codfish treated with different doses of powders of *N. sativa* had similar number of larvae developing on them after 35 days. Codfish treated with the conventional insecticide pestox had the least number of larvae. Also the controlled experiment had higher mean number of pupae though not significant from codfish treated with pestox and *N. sativa* powder at different doses except at 0.4g dose where least mean number of pupae was recorded. Mean number of adult *D. maculatus* emergence on codfish treated with *N. sativa* powder at different doses was higher in control experiment followed by cod fish treated with 0.8 and 0.4 g *N. sativa* and the least was recorded in codfish treated with 2.0 g *N. sativa*.

**Table 1. Progeny development of *D. maculatus* on codfish (*G. morhua*) treated with *N. sativa* powder at different doses as eco-friendly bio-control strategy**

Concentration of seed powder (g)	Progeny development of <i>D. maculatus</i>		<i>Maculatus</i> Adult emergence
	Larva	Pupa	
0.4	103.50 <sup>b</sup>	7.75 <sup>b</sup>	2.50 <sup>bc</sup>
0.8	83.00 <sup>b</sup>	13.50 <sup>ab</sup>	4.25 <sup>b</sup>
1.2	82.75 <sup>b</sup>	12.50 <sup>ab</sup>	2.75 <sup>c</sup>
1.6	79.50 <sup>b</sup>	11.00 <sup>ab</sup>	1.25 <sup>c</sup>
2.0	77.00 <sup>b</sup>	8.25 <sup>ab</sup>	1.00 <sup>c</sup>
Petox	25.75 <sup>c</sup>	9.75 <sup>a</sup>	1.25 <sup>c</sup>
Control	131.75 <sup>a</sup>	14.00 <sup>a</sup>	9.50 <sup>a</sup>
LSD	25.26	5.77	1.89

There was significant difference recorded. Different superscripts indicate significant differences along rows or treatment groups at  $p < 0.05$

**Table 2. Bio-efficacy of *N. sativa* seed powder extract as protectant against *D. maculatus* infestation in codfish (*G. morhua*) at different concentrations**

Concentration of seed powder (g)	% Protection	% Weight loss	% Corrected mortality	Frass weight
0.4	70.50 <sup>a</sup>	89.17 <sup>a</sup>	2.82 <sup>d</sup>	3.09 <sup>b</sup>
0.8	75.06 <sup>a</sup>	85.83 <sup>a</sup>	7.36 <sup>cd</sup>	2.81 <sup>b</sup>
1.2	73.28 <sup>a</sup>	66.50 <sup>b</sup>	9.39 <sup>c</sup>	3.05 <sup>b</sup>
1.6	85.82 <sup>a</sup>	89.00 <sup>a</sup>	13.20 <sup>c</sup>	3.39 <sup>ab</sup>
2.0	88.95 <sup>a</sup>	69.05 <sup>b</sup>	19.25 <sup>b</sup>	2.78 <sup>b</sup>
Pestox	85.38 <sup>a</sup>	26.09 <sup>c</sup>	36.20 <sup>a</sup>	1.70 <sup>c</sup>
Control	46.35 <sup>b</sup>	91.11 <sup>a</sup>	9.21 <sup>c</sup>	3.93 <sup>a</sup>
LSD	23.59	12.99	5.80	0.75

There was significant difference recorded. Different superscripts indicate significant differences along rows or treatment groups at  $p < 0.05$

The result of bio-efficacy of *N. sativa* powder as protectant against *D. maculatus* infestation in *G. morhua* is shown on Table 2. Percentage protection conferred by the botanical in the management of *D. maculatus* showed that all the doses applied were effective as the conventional insecticide (pestox) used as a check. The control experiment had the least percentage protection efficiency. Percentage weight loss in codfish after 45 days of infestation by *D. maculatus* was higher in control experiment although not significantly different from codfish treated with *N. sativa* powder at 0.4 g, 0.8 g and 1.2 g while the least weight loss was recorded in cod treated with pestox. Corrected mortality of *D. maculatus* adults after 45 days of exposure to the different doses of *N. sativa* seed powder also increased with an increase in concentration of *N. sativa* from 0.4 g to 2.0 g. Corrected mortality of *D. maculatus* adults was higher in codfish treated with pestox (conventional pesticide) followed by codfish treated with 2.0 g and the least was recorded in codfish treated with 0.4 g of *N. sativa*. Frass weight generated by *D. maculatus* activity in codfish treated with *N. sativa* powder at

different doses was higher in control closely followed by codfish treated at 1.6g while the least mean frass weight was generated in the codfish treated with pestox.

The characterization and quantification of phytochemicals present in *N. sativa* showed eleven active chemical compounds with phenol compounds being the highest, followed by alkaloid and then oxalate with the least compound isolated as presented on Table 3.

### 3.1 Discussion

The use of plant materials in the management of insects infestation in store products has been widely reported [11,52-58]. The marginal protection conferred by *N. sativa* especially at higher doses over the conventional insecticide suggests that it can be adopted in an integrated pest management (IPM) strategy in stored products especially in tropical stored products. Although the low mortality rate observed in the study was at variance with [59] who worked on seed powder of *Dennettia tripetala* (Pepper fruit)

**Table 3. Qualitative and quantitative screening of *N. sativa***

S/No	Chemical compound in <i>N. sativa</i>	% composition
1	Phenol	9.11
2	Alkaloid	5.04
3	Phylate	2.97
4	Flavonoid	1.70
5	Cardiac Glycoside	1.60
6	Terpenoid	1.08
7	Saponin	0.84
8	Tannin	0.63
9	Athraquinone	0.10
10	Steroid	0.05
11	Oxalate	0.04

*Results are mean percentages of three experimental evaluations*

admixture at 1.5 g/ 25 g dried *Clarias* species and obtained 100% mortality in adult *D. maculatus* in 7 days and *Necrobia rufipes* Degeer in 14 days. This difference therefore may be attributed to the different active ingredients in the plant species, methodology of extraction and dosage of application. It may as well be attributed to the mode of action which may have not led to total kill but reduction in weight loss as observed in the study since bio-pesticides exhibit varying degree of mode of action ranging from repellence, inhibition to denaturation of proteins. Gurjar et al. [60] posited such observation in pyrethrum as targeting nerve cells of insects resulting in paralysis and later death while neem-based biopesticides act as antifeedant or feeding deterrents, repellence, moulting abnormalities, oviposition deterrence and also disrupt the endocrine system of target insects. The response of *D. maculatus* to *N. Sativa* could have resulted to any of those and not necessarily death of the insect pest. One of such behaviour was observed by [61] who reported a repulsive effect of *Crataeva religiosa* Forst against *Dermestes* species with resultant aggregation of the smoked-dried fish treated larvae and adults toward the edges of the treated jar. A substantial number of studies have been conducted that suggest the seeds may indeed have pharmacological potential [29]. The seeds contain a variety of chemicals, but most of the pharmacological properties of black cummin are attributed to the presence of quinine compounds with thymoquinone as the most abundant [62]. There is clinical evidence that the seeds have antimicrobial, antiparasitic and antifungal properties while some studies have demonstrated tumour suppression [63].

The result indicating lower larvae and pupae progeny over the control suggested that *N. sativa* had an inhibitory effect at those developmental stages which led to the delay in adult emergence. This is in conformity with observations posited by [15,64,65] in their various studies that cured fish treated with *Denntia tripetala* and *Piper guineense* powders had fewer or no eggs after 7 days and suggested that the extracts may have affected eggs production and deposition due to the repulsive action of the powders on the insect pest and thereby prevented mating among them and increased doses led to reduction in progeny fecundity and adult emergence. This was further corroborated by the observations by [66] on toxicity of some citrus peels to *D. maculatus*, that high doses suppressed progeny development. According to Jatau et al. [67], *Piper guineense* caused 100% mortality of the larvae at all the concentrations before pupation and [15,17,65] reported that powders of *Denntia tripetala*, *Eugenia aromatica*, *Piper guineense* and *Monodora myristica* effectively prevented adult emergence in fish protected against *D. maculatus* and *N. rufipes* and had suppression rate of adult emergence of both insect pest between 99.60%-100% while [68] who worked with the powder of *Clorodendrum capitatum* recorded reduction in adult emergence between 28.8%- 26.7%. The variations observed could be attributed to the length of experimentation and response of the insect pest to the available active ingredients in the different test plants which had high tendency to interfere with the insect behaviour, physiological activities, biochemical processes, morphology and metabolic pathways [69,70].

The presence of high phenol and alkaloid chemical compounds isolated from *N. sativa* agrees with earlier findings which suggest its pharmacological potentials [29,71,72] and mostly attributed to the presence of quinine compounds with thymoquinone as the most abundant although the presence of athraquinone was established, it was not however, the highest as reported by [62]. However, the presence of major secondary metabolites such as steroids, alkaloids, tannins, terpenes, phenols, flavonoids and resins as common bioactive compounds [73] suggest its potential candidature as a botanical pesticide with insecticidal, antifungal, antibacterial and antioxidant properties [64,74].

#### 4. CONCLUSION

The study indicated that *N. sativa* seed powder possesses secondary metabolites with high bio-pesticide potentials against the skin beetle *D. maculatus*, a predominant insect pest of stored codfish (*G. morhua*) especially in the tropics which could be a better management option so as to reduce the over reliance on the synthetic chemical pesticides which have proven hazardous to animal and human life.

#### 5. RECOMMENDATION

The authors therefore recommend that the plant *Nigella sativa* incorporated in the integrated pest management program (IPM) for the prevention, management and or control of the insect, *D. maculatus* owing to the success recorded by this study. Furthermore, research on the mode of action of the secondary metabolites contained in the plant *N. sativa* be researched upon to ascertain their efficacy as biopesticide especially against the insect *D. maculatus*.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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