10.5281/zenodo.7566560

Insecticidal Potentials of Black Seed, (*Nigella Sativa)* Powder as an Eco-Friendly Bio-Pesticide in the Management of *Dermestes Maculatus* in Codfish *Gadus Morhua* (Gadidae: Gadiformes)

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Abstract

Background: Insecticidal potentials *Nigella sativa* powder as an eco-friendly bio-pesticide in the management of *Dermestes maculatus* in codfish was evaluated in a laboratory. **Results:** Adults of hide and skin beetle were obtained from infested smoked fish and cultured in laboratory. The culture was kept at room temperature and RH in order to obtain F1 generation of the insect for the experiment to develop. Uniform age and unmated adults *D. maculatus* were obtained for the experiment. *Nigella sativa* seeds were purchased from a vendor of foreign spices and ground into fine powder. Five levels (0.4 g, 0.8 g, 1. 2 g, 1.6 g and 2.0 g.) were weighed using a sensitive balance and added into 40 g codfish kept in a Kilner jar. Two pairs of *D. maculatus* was introduced into the different concentrations and left on work bench for observations. Phytochemical analysis of *N. sativa* was conducted. Data such as mortality were corrected using Abbot Formulae while % weight loss was assessed by direct weighing method and effectiveness of different treatments in protecting the codfish was also calculated. The results indicated that codfish treated with different doses of powders of *N. sativa* had similar number of larvae development after 35 days. Mean of number of adult *D. maculatus* emergence on codfish treated with *N. sativa* powder at different doses was significant. Percent protection conferred by the botanical in the management of *D. maculatus* showed that all the doses applied were effective. Characterizations and quantification of phytochemical properties of N. *sativa* indicated eleven active chemical compounds. **Conclusion:** The finding posited therefore that *N. sativa* seed powder possesses secondary metabolites with high bio-pesticide potentials and may considered being use in IPM programme in stored codfish especially in the tropics.

Keywords: black seeds, *Nigella sativa, dermestes maculatus,* stockfish, progeny emergence

1. Introduction

Fish being one of the cheapest source of protein across the globe especially in the developing world is consumed both fresh and processed (Osarenren & Ojor, 2014). Over 3 million people especially in Africa depend on fish wholly or partially either as sources of protein or other means of livelihood (Adewuyi *et al.,* 2013). The industry in Africa is put at $3 billion worth (El Shaika et al., 2014). *Gadus morhua* is an unsalted cured fish usually dried (Thorarinsdottir *et al.,* 2004) and relished widely across African continent in many dishes and therefore sold at a high cost (Frederick 1969; Dale & Uwonkunda, 2017). It is a good source of high quality protein which aid in building healthy immune system and other health benefits such as assisting in proper weight management (Tidwell and Allan, 2001), brain and nervous system function, prevention of excess fat and stabilizing blood pressure (Berr et al., 2009).

As soon as fish is caught, deterioration commences immediately, therefore, traders device a means of overcoming it by processing such as salting, smoking or even sun drying though it does not preclude it to heavy insect infestation. Zakka et al. (2009) earlier reported Dermestes sp and ham beetle 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 apply different types of synthetic insecticides as control strategy (Abolagba et al., 2011). This leads to several health challenges to the consumers since most of them end up in the environment (Oguh et al., 2019) as run-off or directly exposing animals, and farmers as well as consumers to health risks. Such risk can be overcome by the use of non-synthetic pesticides (biopesticides) such as pyrethrum (*Tanacetum cinerariifolium*) (Sarwar 2015), neem (*Azadirachta indica)* [Castillo-Sánchez et al., 2015], garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*) and thyme (*Thymus vulgaris*) (Joseph and Sujatha, 2012; Sharafzadeh, 2011).

Mahmood et al. (2016) opined the use of biopesticides as an age long tradition until the recent introduction of synthetic pesticides. However, the threat to both environment and human health attributed to the synthetic pesticide (Nefzi et al., 2016) has led to the advocate of the use of plant derived material in pest management that are safer (Karaca et al., 2017; Mishra, 2018), available and mostly edible (Srijita, 2015), inexpensive (Castillo-Sánchez et al., 2015) and easily integrated into other pest management option. Others qualities include its low toxicity to beneficial organism with none or little allelopathic effect (Gurjar et al., 2012) and none residual effect (Dubey et al., 2008; Dubey, 2010). The plant parts utilized ranges from the bark to floral parts and fruits or seeds to special structures like rhizomes and bulbs which is either dried and ground into fine powder and extracted with organic solvents (Chougule & Andoji 2016) and utilized either as plant extracts, essential oils or both (Mizubuti et al., 2007). But biopesticides shortfalls include ability to break down very quickly in sunlight and degrade fast due to its acidic or alkaline nature (Kole et al., 2002; Oguh et al., 2019).

Black seed plant is found in southwestern Asia and parts of the Mediterranean and African continents. It has a long history of use in diverse culinary and traditional medicine (Hassanien *et al*., 2014) and is widely grown for its pungent seeds and used as a spice, skin cream for treating eczema and to stimulate lactation. Other uses include treatment of intestinal worms, digestive disorder, asthma, bronchitis and rheumatoid arthritis (Babayan et al., 1978). The seeds have an aroma similar to fennel and have a pungent flavour somewhat similar to nutmeg (Bharat, 2009) and usually roasted and ground as a spice in some parts of India, the Middle East, and North Africa (Karapinar & Aktug, 1987).

The seed was earlier reported to have pharmacological potentials (Colovic et al., 2013) and contains a variety of chemicals such quinine compounds with thymoquinone as the most abundant (Kahsai, 2002). There is also clinical evidence of antimicrobial, antiparasitic, and antifungal properties and ability to suppress tumour (Shanmugam et al., 2017). *Dermestes maculatus* like any other major pest of stored product has developed some resistance to certain pesticides thereby leading to significant economic losses. Therefore, there is need to study and ascertain, certain natural protectants of codfish against *D. maculatus* infestation. This study examined the insecticidal potentials of *N. sativa* on the development of hide beetle on codfish in the laboratory.

Materials and Method

Dermestes maculatus culture

Adults of skin beetle were obtained from naturally infested smoked fish from open market and cultured in laboratory on codfish purchased from Rumuokoro Market in Obiokpor Local Government Area in Port Harcourt, Rivers State and placed in plastic containers. The top of the lids were cut open but covered with netting material to allow for sufficient aeration and to avoid escape of the *D. maculatus* culture and entrance of unwanted insect pest. The culture was kept at ambient temperature and relative humidity for new generations of *D. maculatus* for the experiment to develop.

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 a well labeled test tubes containing pieces of codfish and allowed to complete their life cycle and left unmated until needed.

Preparation of Black seed (*Nigella sativa*) powder

Nigella sativa seeds were purchased from a vendor of foreign spices who imports them from India with the 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 250µm standard test sieve and stored in a specimen bottle for 24 hrs prior to the start of the experiment to avoid possible depreciation of the active ingredient.

Experimental set-up

40 g fleshy parts of the codfish were weighed and placed in plastic containers. The plant materials at 5 levels (0.4 g, 0.8 g, 1. 2 g, 1.6 g and 2.0 g.) were weighed using a sensitive balance and added into each substrate. Two pairs of *D. maculatus* was introduced into the different concentrations and left on work bench for observations. Each treatment was replicated four times.

Sexing of *Dermestes maculatus*, DeGeer, 1776.

Dermestes maculatus adults were sexed using the features described by Imai *et al*. (1990) that the males are distinguished from the females by their possession of a deep depression and brush of hairs on the 4th abdominal sternite.

Phytochemical Analysis of Black seed, *Nigella sativa* powder

Quantitative Phytochemical analysis of *N. sativa* was conducted in Autino Research laboratory using the method described by Harborne (1973) and Obadoni and Ochuko (2002).

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

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 percent weight loss calculated.

Percent Protection of Treatment

The effectiveness of different treatments in protecting the codfish was calculated according to (El-Ghar *et al*., 1987).

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

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 (ANOVA) and significant means were separated using LSD at 5% level of probability.

Results and Discussion

Table 1 shows the result of *D. maculatus* progeny developed on cod fish *Gadus morhua* in a laboratory treated with *Nigella sativa* powder as an eco friendly bio-protectant. Cod fish treated with different doses of the plant bio-pesticide responded differently at each development stages. From the result higher mean number of larvae was recorded in a control experiment while codfish treated with different doses of powders of *N. sativa* had similar number of larvae developing on them after 35 days while codfish treated with pestox had the least number of larvae. From the same table the result shows that 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 0.4 g dose where least mean number of pupae was recorded. Mean of 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 *Dermestes maculatus* on codfish treated with *Nigella sativa* powder at different doses as eco-friendly bio-control strategy.

Treatment	Larvae	Pupae	Adult emergence
0.4	103.50[b]	7.75[b]	2.50[bc]
0.8	83.00[b]	13.50[ab]	4.25[b]
1.2	82.75[b]	12.50[ab]	2.75[c]
1.6	103.50[b]	11.00[ab]	1.25[c]
2.0	77.00[b]	8.25[ab]	1.00[c]
Pestox	25.75[c]	9.75a[b]	1.25[c]
Control	131.75[a]	14.00[a]	9.50[a]
LSD	25.26	5.77	1.89

The result of bio-efficacy of *N. sativa* powder as protectant against *D. maculatus* infestation in *G. morhua* is shown in table 2. Percent 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 and control experiment had the least percent 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, 0.8 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* was higher in codfish treated with pestox followed by *G. morhua* treated with 2.0 g and the least was recorded in codfish treated with 0.4 g of *N. sativa* though was not significantly different from those treated with 0.8 g *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 1.6 g though not significantly different from those treated at 0.4 and 1.2 g while the least mean frass weight was generated in a cod treated with pestox.

Table 3 shows the characterizations and quantification of phytochemical properties of N. *sativa* carried out showed eleven active chemical compounds with Phenol compounds being the highest which was followed by Alkaloid in terms of quantification while Oxalate was the least compound isolated.

Table 2. Bio-efficacy of *Nigella sativa* powder as protectant against *Dermestes maculatus* infestation in *Gadus morhua* in a laboratory at different rates.

Treatment/Dose	% protection	% Weight loss	% Corrected mortality	Frass weight
0.4	70.50[a]	89.17[a]	2.82[d]	3.09[b]
0.8	75.06[a]	85.83[a]	7.36[cd]	2.81[b]
1.2	73.28[a]	66.50[b]	9.39[c]	3.05[b]
1.6	85.82[a]	89.00[a]	13.20[c]	3.39[ab]
2.0	88.95[a]	69.05[b]	19.25[b]	2.78[b]
Pestox	85.38[a]	26.09[c]	36.20[a]	1.70[c]
Control	46.35[b]	91.11[a]	9.21[c]	3.93[a]
LSD	23.59	12.99	5.80	0.75

Table 3 Chemical compounds and percent compositions of *Nigella sativa*.

S/No	Chemical compound in *N. sativa*	% composition
1	Phenol	9.105
2	Alkaloid	5.040
3	Phylate	2.970
4	Flavonoid	1.700
5	Cardiac Glycoside	1.601
6	Terpenoid	1.078
7	Saponin	0.836
8	Tannin	0.629
9	Athraquinone	0.101
10	Steroid	0.052
11	Oxalate	0.036

Discussion

The presence of high phenol and alkaloid chemical compounds isolated from *N. sativa* agrees with earlier findings which suggest its pharmacological potentials (Colovic et al., 2013) though the presence of thymoquinone (Kahsai, 2002) and athraquinone were not the high as reported by (Kahsai, 2002). However, secondary metabolites such as steroids, alkaloids, tannins, terpenes, phenols, flavonoids and resins as common bioactive compounds identified may suggest its potential candidature as a botanical pesticide such insecticidal, antifungal, antibacterial and antioxidant (Ahmad *et al*., 2017). But further research on its mode of action as biopesticide especially in *D. maculatus* needs to be established which was beyond the scope of this research.

The use of biopesticide in the management of insects infestation in store products has been widely reported (Don-Pedro, 1996; Adedire and Ajayi, 1996; Okonkwo and Ewete, 1998; Okonkwo and Ewete, 2000). The marginal protection conferred by *N. sativa* especially at higher doses over the conventional insecticide suggests that it can be adopted in an IPM strategy in stored products especially in tropical stored products. Although the low mortality rate observed in the study was at variance with Okonkwo and Okoye (1996) who worked on powder seed of *Dennettia tripetala* admixture at 1.5g /25g dried *Clarias sp*. and obtained an impressive mortality results in 7 and 14 days in adult *D. maculatus* and *Necrobia rufipes* (Degeer) respectively. This difference therefore may be attributed to the different active ingredients in the plant species 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. Grdiša and Grši (2013) posited such observation in pyrethrum as targeting nerve cells of insects resulting in paralysis and later death while neem-based act as antifeedant, repellence, moulting abnormalities, oviposition deterrence and disruption of endocrine system. 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 bevahiour was observed by Mbaye et al. (2014) who reported a repulsive effect of *Crataeva religiosa* (Forst.) against *Dermestes spp.* where smoked-dried fish treated had larvae and adults of skin beetles aggregating toward the edges of the jar.

The result indicating lower larvae and pupae progeny over the control suggest that *N. sativa* may have an inhibitory effect at those developmental stages and also may have led to the delay in adult emergence. It may also suggest as posited by Okonkwo and Okoye (2001) in a similar study that cured fish treated with *Dennetia 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.

Increased dose led to reduction in progeny fecundity and adult emergence. Similar result by Don-pedro (1985) on toxicity of some citrus peels to *D. maculatus* was observed where high doses suppressed F1 development of *D. maculatus*. But contrary to Jatau et al. (2014) that *Piper guineense* caused 100 % mortality of the larvae at all the concentrations before pupation and Akinwumi *et al*. (2006) that powders of *D. tripetala*, *Eugenia aromatic*a, *P. 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, but not different from Adesina et al. (2016) who worked on powder of *Clorodendrum capitatum* and recorded lower reduction in adult emergence. The variations observed could be due the length of experiment and response of the insect pest to the available active ingredients in the different test plants which has high tendency to interfere with insect behaviour, physiological activities, biochemical processes, morphology and metabolic pathways (Rattan, 2010).

Conclusion

N. sativa seed powder possesses secondary metabolites with high bio-pesticide potentials and may considered being used in IPM programme in stored codfish especially in the tropics which could be a better management option so as to reduce the over reliance on the synthetic chemical compounds.

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