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Therapeutic impact of biopesticide neem oil on health status of walking catfish *Clarias batrachus* for replacing synthetic organophosphorus pesticides

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Abstract

Pesticides are widely used in agricultural purposes and indiscriminate use of pesticides resulted in unintentional harming of the environment. Lack of consciousness and education coupled with indifferent attitude also contributed to indiscriminate use of pesticides. Enormous amount of these toxic pesticides and other chemicals reaches in the aquatic environment by the runoff and other sources and polluted the water, air and soil for any living organisms. One of the fastest growing food producing sectors is aquaculture, supplying approximately 40% of the world's fish food. Pesticides may be used in aquatic ecosystem to control the fish parasites, diseases, and other pests that have also adversely affected the aquatic ecosystem. Nuvan is an organophosphorus pesticide is widely used due to low persistence in environment but also affects the non-targeted organisms including fish. Synthetic chemicals generate environmental problems; researchers have been leaning towards pest control with natural or plant based substances. Protection of water quality is possible when rationalize the use of natural pesticides and promote biopesticides. Neem oil is a natural or mixtures of chemicals obtained from neem plant (*Azadirachta indica*) which has many significant benefits to overcome from the chemicals or synthetic pesticides. By making the health beneficial uses of neem oil accessible to the farmers in the society, the use of chemicals in agriculture can be reduced.

The aim of the current study is to observe the impacts of nuvan and neem oil on the survival and health status of fishes which also affected the other animals by the food chain. Changes in blood parameters of fish are related to the response of the whole animal, which affect on survival, reproduction and growth of fish. Some alterations in cholesterol and triglycerides in serum of treated fish *Clarias batrachus* with 1/20 and 1/10 LC_{50} concentrations of nuvan and neem oil separately compared with non-treated one after 7, 15, 30 and 60 days of exposure were recorded and discussed. Also, this assessment may be essential for encourage suitable methods of application in aquatic organism production facilities to be fully explored in future.

Keywords: Neem Oil; Nuvan; *Clarias batrachus*; Serum Cholesterol; Serum Triglycerides; Aquatic Environment

1. Introduction

Pesticides are extensively used in the agriculture purposes which are the large occupation in developing countries [1]. They play an essential role in enhancing agricultural productivity but also have accompanied by a variety of undesirable environmental effects. Farmers often use pesticides unusually manners at higher concentrations than recommended. Despite the usage of such a large quantity of pesticides, there is an assessed 10-30% loss due to pests alone [2]. Over-spray and/or runoff of toxic pesticides from agricultural areas may easily pollute water bodies. The principal hazards of the excess amount of pesticides in water are twofold as great number of non target animals may be killed or gets accumulated in the tissues of animals. Bioaccumulation of pesticides and other hazardous chemicals in tissues of aquatic

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animals [3] can be more dangerous for human consumption. High levels of these toxic pesticides accumulation in the body of the animal and later interfere with the maintenance of their homeostasis and influence their performance [4]. Among the various harmful pesticides organophosphorus pesticide comprises about 80% of the agricultural pesticides. These are irreversible inhibitors of cholinesterase resulting in detrimental effects on metabolism of fats, protein and carbohydrates [5]. They depict cholinergic quality; hence act as neurotoxins to a broad spectrum of pests.

The most essential alternative of toxic chemicals or pesticides is botanical pesticides or biopesticides like plant extracts and essential oils, which can be helpful in pest management and control other disease vectors without any adverse effects on our environment. Neem is known as a medicinal plant of containing diverse chemical active substances of many biological qualities. The neem tree *Azadirachta indica* is a source of neem oil belonging to the family of Meliaceae. With the large utilization of biopesticides or neem oil can help in the sustainable agriculture. Neem oil can manage soil fertility and its nutrients and also stop the contamination of water for animals and other livings consumption. Neem oil contains the active ingredient azadirachtin, a triterpenoid active compound and its antiviral, antibacterial and antifungal qualities have been known for hundred years which has proven insecticidal activity [6]. It breaks down quickly within 4 to 5 days in water or light and would not cause long-term effects on the environment. Main component of neem oil, Azadirachtin is commercially exploitable [7] and is being used traditionally to treat various diseases from past decades. It has also been used in aquaculture systems to manage fish predators [8]. The neem oil is identified by a variety of compositions and modes of action, which have an impact on insects by repelling or exhibiting insecticidal activities. They can be manifest physiological functions with hormonal action, keep coenzymes in a low form, and represent a source of energy. Hence, the use of neem-based products may offer a selective, harmonious and eco-friendly approach to remove toxic pesticides in water quality management [9].

Among the inland fisheries the walking catfishes and other air breathing fishes are second most important group with commercial purpose [10] because they can survive even in adverse environmental conditions and can grow in swamps and marshes. The freshwater catfish *Clarias btrachus* is high in protein and iron, and low in fat content, nutritious and delicious so have great valuable position in protein rich food demand. Blood is an easily available fluid used as an important diagnostic tool to assess toxicity of pesticides instead of tissue analyses. Almost all living tissues are exposed to this fluid for exchange of substances. Blood analysis is crucial in various areas of ichthyologic research and aqua farming and in the area of toxicology and environmental assessment as possible marker of physiological or pathological changes in aquaculture management and diseases assessment [11]. The purpose of this investigation is to assess and contributes to knowledge on the biochemical alterations in blood serum of freshwater catfish *Clarias batrachus* following the exposure of the fish to organophosphorus pesticide nuvan and biopesticide neem oil. In order to make the aquaculture and agricultural system safer and more sustainable plans to reduce the use and risk of chemical and more hazardous pesticides and multidisciplinary research that will provide good, safe, effective, and inexpensive plant protection products like biopesticides should be encouraged [9].

2. Materials and methods

The freshwater catfish, *Clarias batrachus* (Linn.) of size 20 to 25 cm in length and weight 50 to 120 g were brought from local fish market from Agra, UP. These fishes were acclimated to laboratory environment for 15 days in well aerated dechlorinated tap water at 20 ±2^{o}C prior to experimentation. Commercial preparations of nuvan (DDVP) and neem oil are only use in agricultural fields so commercial grade formulations of materials were used in this work. All the precautions are followed to conduct the acute and chronic fish bioassay experiments. The LC_{50} value was determined following the procedure of Finney's Probit Analysis [12].

Based on the results of acute toxicity study, doses of 1/20th and 1/10th of LC_{50} values of nuvan and neem oil were selected for sub lethal studies. *Clarias batrachus* was placed in five different tanks containing 0.0137 ml/L (group II) and 0.0274 ml/L (group III) of nuvan while 0.0424 ml/L (group IV) and 0.0848 ml/L (group V) of neem oil concentrations with 20 L of water and the last tank as control (group I) without any pesticide. The experimental fish *Clarias batrachus* was fed with formulated feed and for biochemical analyses catfish from each tank were taken and sacrificed at 7, 15, 30 and 60 days. The blood was collected from the caudal vain of walking catfish with the help of sterilized disposable syringe, scissor and forceps. The blood was allowed to clot in the serum separator tubes for 30 to 40 minutes and now centrifuged at 2500 rpm for 25 minutes. After centrifugation the supernatant or serum was used for evaluate different biochemical parameters (serum cholesterol and triglycerides). The biochemical estimation of serum cholesterol was analyzed by Roeschlau *et al.,* [13] and serum triglyceride by Schettler and Nussel [14]. The obtained data were statistical analyzed by one way analysis of variance (ANOVA).

3. Results

The aim of this study was to analyze the effect induced by nuvan an organophosphorus pesticide and neem oil a biopesticide on biochemical indices (serum cholesterol and triglyceride) of *Clarias batrachus*. The acute poisoning symptoms due to the exposure of nuvan were characterized by change in behavior such as hyperactivity, restlessness, loss of balance, and low feeding followed by abnormal posture ultimately leading to death. On the other hand catfish shows comparatively low reactivity in behavioral patterns with the neem oil than the nuvan. LC_{50} (50% mortality at 96 hours) of nuvan and neem oil for *Clarias batrachus* after 96hr exposure was found to be 0.274 ml/L and 0.848 ml/L consequently. A large variation in the values of median lethal concentrations (LC_{50}) of nuvan and neem oil was obtained which indicates that nuvan is about four times more toxic than neem oil for *Clarias batrachus* noted on the present study. After calculating the LC_{50} values of nuvan and neem oil biochemical analysis was conducted for calculating the activity of serum cholesterol and serum triglyceride in blood of specimen.

Serum Cholesterol content of specimen *Clarias batrachus* was increased highly significant at dose 0.0137 ml/L (1/20 of LC_{50}) and significantly elevated at 0.0274 ml/L (1/10 of LC_{50}) concentration of nuvan exposure after 7, 15, 30 and 60 days while neem oil treated fish showed decrease in serum cholesterol content non-significantly at 0.0424 ml/L (1/20 of LC_{50}) dose and very highly significant decrease at 0.0848 ml/L (1/10 of LC_{50}) concentration of neem oil when compared with control group as shown in Table (1) and Figure (1).

Serum Triglyceride levels of test animal *Clarias batrachus* were very highly significant elevated at both concentrations of nuvan (0.0137 ml/L and 0.0274 ml/L) but when specimens were exposed with 0.0424 ml/L (1/20 of LC_{50}) dose of neem oil the non-significant reduction and with 0.0848 ml/L (1/10 LC_{50}) dose of neem oil highly significant reduction were noted in serum triglyceride activity when compare with non-treated group (control) after 7, 15, 30 and 60 days as shown in Table- (2) and Figure- (2).

Table 1 Cholesterol (mg/dl) levels in blood serum of *Clarias batrachus* after exposure of Nuvan and Neem oil

Concentration (ml/L)	**Cholesterol (mg/dl)**	**Exposure Time**				**Significance level ANOVA**
		7 days	15 days	30 days	60 days	
Control (Group- I)	Mean	178.2	178.2	178.2	178.2	Normal
	± S.Em.	1.73	1.73	1.73	1.73	
Nuvan – 0.0137 ml/L 1/20 of LC_{50})(Group- II)	Mean	181.2	183	184.8	188.4	(P<0.01) Increase
	± S.Em.	2.30	1.73	0.577	1.73	
Nuvan – 0.0274 ml/L (1/10 of LC_{50})(Group-III)	Mean	185.3	187	194	202	(P<0.05) Increase
	± S.Em.	1.15	1.73	2.30	1.15	
Neem oil – 0.0424 ml/L (1/20 of LC_{50})(Group-IV)	Mean	178.2	178.2	172.7	171.3	(P>0.05) Decrease
	± S. Em	3.46	4.04	2.30	3.46	
Neem oil – 0.0848 ml/L (1/10 of LC_{50})(Group- V)	Mean	173.7	168.5	168.4	167	(P<0.001) Decrease
	± S. Em	1.15	4.61	1.73	2.30	

± S.Em – standard error of mean; (P<0.05) – significant; (P<0.001) – Very highly significant; (P<0.01) – highly significant; (P>0.05) – Non-significant.

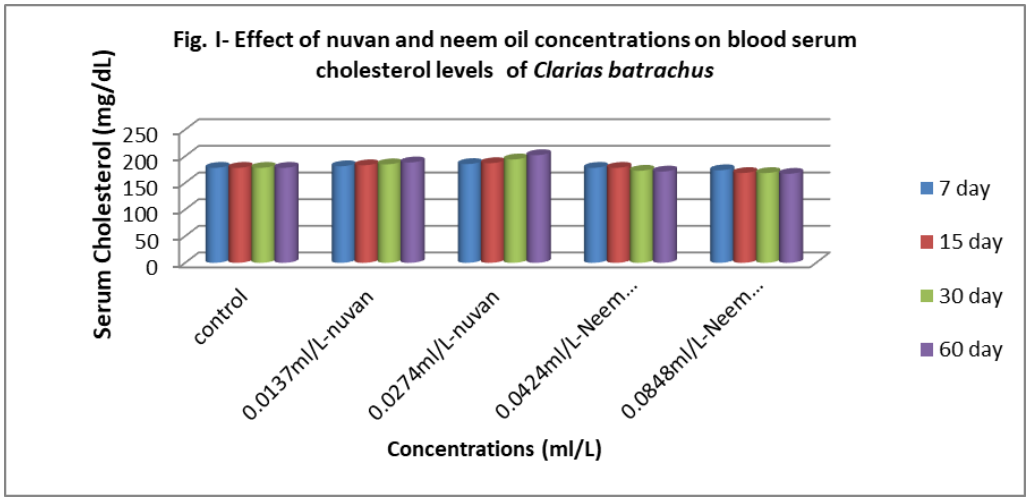

Figure 1 Effect of nuvan and neem oil concentrations on blood serum cholesterol levels of *Clarias batrachus*

Table 2 Triglycerides (mg/dl) in blood serum of *Clarias batrachus* after exposure of Nuvan and Neem oil

Concentration (ml/L)	**Triglycerides (mg/dl)**	**Exposure time**				**Significance level ANOVA**
		7 days	15 days	30 days	60 days	
Control (Group- I)	Mean	161	161	161	161	Normal
	± S.Em	2.30	2.30	2.30	2.30	
Nuvan – 0.0137 ml/L (1/20 of LC_{50})(Group II)	Mean	166	175	179	192	(P<0.001) Increase
	± S.Em	1.73	1.73	2.30	2.30	
Nuvan – 0.0274 ml/L (1/10 of LC_{50})(Group III)	Mean	179	185	195	210	(P<0.001) Increase
	± S.Em	2.30	2.30	1.73	2.30	
Neem oil –0.0424 ml/L (1/20 of LC_{50})(Group IV)	Mean	161	161	161	159	(P>0.05) Decrease
	± S. Em	2.30	2.30	2.30	2.30	
Neem oil– 0.0848 ml/L (1/10 of LC_{50}) (Group- V)	Mean	151	150	142	140	(P<0.01) Decrease
	± S. Em	2.30	3.46	1.73	1.15	

± S.Em – standard error of mean; (P<0.001) – Very highly significant; (P<0.01) – highly significant; (P>0.05) – Non-significant;

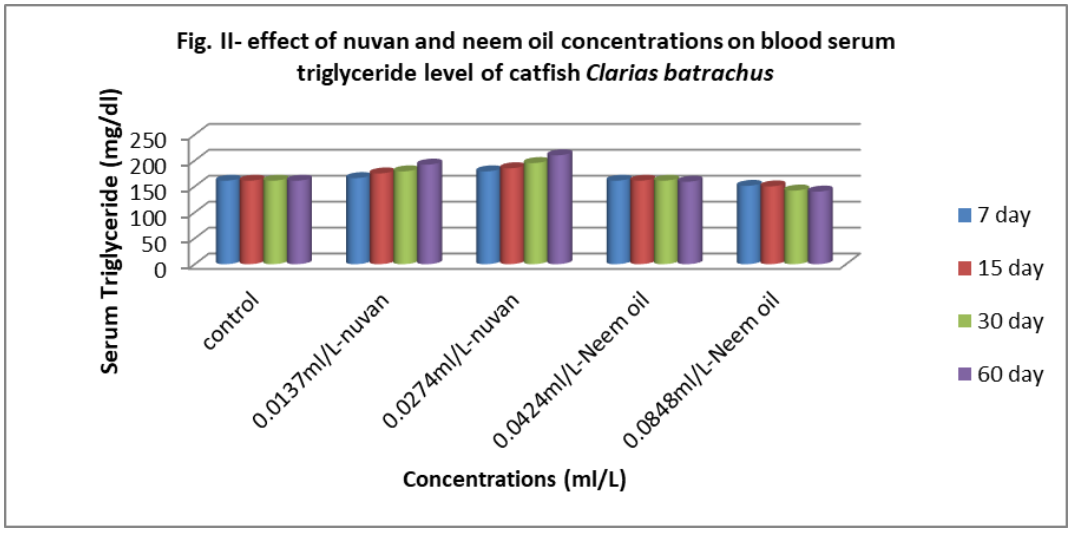

Figure 2 Effect of nuvan and neem oil concentrations on blood serum triglyceride level of catfish *Clarias batrachus*

4. Discussion

Various studies have shown that when the water quality is affected by toxic substances, many physiological changes will be reflected in the values of one or more of biochemical parameters. Pesticides may also enter in the aquatic ecosystem from nearby agricultural areas [15] where they prove hazardous to both invertebrates and vertebrates [16]. Thus quality of water is one of the major factors, responsible for individual variations in fish biochemically, since they are sensitive to slight fluctuation that may occur within their internal environment. Ample numbers of researchers have investigated the effect of different pesticides on behavior and biochemical responses of many species of fish [17, 18, and 19] and have found varying responses after exposing the fish to different sub-lethal concentrations using the acute toxicity tests. The estimation of various biochemical parameters in fish is essential to understand normal and pathological activities of aquatic livings as well as toxicological impacts [20]. In this study, the protective effects of neem oil against the complications of nuvan are reported. Increase activity of blood serum cholesterol and triglyceride exposed to nuvan while reduction in the serum cholesterol and triglyceride levels after treated with neem oil were noted in the experimental freshwater catfish *Clarias batrachus* after 7, 15, 30 and 60 days.

The precursor molecule of all steroid hormones and one of the essential substitutes of cell membranes is cholesterol [21]. Liver regulates cholesterol in body, by generating cholesterol to be delivered to the whole body cells, and by transferring it to bile salts so body can get rid of it in bile and faeces. If liver functioning is poor or damaged, the production of bile may be inhibit, which can result in increased level of cholesterol in the blood of animals [22]. Higher level of blood cholesterol might have been due to the release of extra reserve energy [23]. It could be a result of the hepatic impairment along with the reduction of enzymes which change cholesterol into bile acids [24] and causing release of excess cholesterol into the blood [25]. Similarly Rani and Gautam [26] were also observed same findings on *Channa punctatus* due to the exposure of nuvan and suggested elevation in serum cholesterol. They were also said that increased activity of serum cholesterol initiate the lipogenesis including sterols tissue steroidogensis was activated that resulting evolution of corticosteriods therefore stress condition elevate the corticosteroids in the blood of animal.The enhanced level of cholesterol in African catfish, *Clarias gariepinus* after exposure of lambda cyclothrin were observed by Ogueji, and Auta, [27].

Triglycerides are another form of body fat that can raise risk for heart disease. Triglycerides store unused calories and provide energy to the body of animals. There are many investigations which suggested that elevated levels of persistent pollutants such as pesticides are associated with high levels of serum lipids which are a huge risk factor for cardiovascular disease [1]. If this association seems to be causal, it may have essential effects on human health. Therefore any type of destruction either intra and extra hepatic, will cause various alterations of total cholesterol and triglyceride levels in the blood. As a consequence action of negative environmental factors such as stress and contamination due to pesticide toxicity has increased blood serum cholesterol and triglyceride levels in experimental fishes. High level of triglycerides is a general sign of various conditions that raise the risk of heart disease and stroke, including obesity and metabolic syndrome. Higher level of triglycerides in the blood may cause to hardening of arteries or thickening of the artery walls (arteriosclerosis) which elevates the risk of heart attack, stroke and heart disease (www.mayclinic.org).

Packard *et al.,* [28] suggested that elevation in triglyceride is the result of overproduction and impaired clearance of triglyceride-rich lipoproteins- very low density lipoproteins and chylomicrons. The metabolism of cholesterol and triglycerides is intertwined because of shared physicochemical qualities of these molecules. Both need to be transported by the aqueous medium of blood from sites of production or depot to tissues that require them for cell functions or energy production. In the present study it is suggested that liver necrosis affecting the rate of triglyceride synthesis. The high synthesis rate of hepatic triglyceride increases the secretion of triglyceride in blood serum of exposed fish *Clarias batrachus* after exposure of synthetic pesticide nuvan, may cause hypertriglyceridemia. The estimation of serum cholesterol and triglyceride in blood of test catfish plays an important role in the diagnosis of risk of coronary heart diseases in organophosphorus poisoning. An increase in cholesterol and triglyceride (TG) levels in the blood are important components of the metabolic syndrome and one of the great risk factors for cardio vascular diseases (CVD) investigated by Saeed *et al.,* [29].

In the present study, the reduction in serum cholesterol and triglycerides in the blood of test animal *Clarias batrachus* shows hepato-protective and health promoting effect of neem oil. Health-promoting effect of neem oil is attributed because it is a great source of antioxidant hence prevention and treatment of diseases via the regulation of different biological and physiological pathways [30]. The neem seed oil contained a variety of phytochemicals with biological significance. The neem oil could be exploited for chemicals that find application as antioxidant agents for the treatment of various diseases caused by pesticides and hazardous chemicals. Source plant (neem) of neem oil and their ingredients play an essential role as hepatoprotective without any adverse complications studied by Alzohairy, [30]. A dose of neem oil dependently decreased hepatocellular necrosis [31]. The hot neem extract contained previously known antioxidants [32]. Hyperlipidemia contributes to damage endothelial function, expansion of atherosclerosis, and coronary heart disease (CHD) through the enhancement of oxidative stress [33]. Antioxidant defense system (SOD and GPx) protects plasma lipoproteins against oxidative stress [34]. Biswas *et al.,* [35] and Gupta *et al*., [36] showed anti-inflammatory and immunomodulator effect of leave extracts and bark of neem and antipyretic and anti-inflammatory activities of oil seeds of neem. They were also confirmed that neem and its constituents play an essential role in the scavenging of free radical generation and hindrance of disease pathogenesis. Neem oil has quercetin and nimbolide which can help protect from free radical damage, thus keeping complications like heart deseases, diabetes and cancer at bay [37].

It has been noted by Yarmohammadi *et al.,* [38] that neem oil can control hyperglycemia, hyperlipidemia and hypertension by over-expression of transcription nuclear factor erythroid 2–related factor 2 (Nrf2) and anti-oxidant effects. It is suggested that given the importance of neem oil and its worldwide use for combating numerous pests in different crops and in aqua farms.Jothigayathri *et al*., [39] analyzed the influence of neem oil on malathion in fish *Oreochromis mossambicus* and suggested that neem oil interacts with melathion and has a protective effect upon the adverse effects of toxicants. Many medicinal plants showed positive effects on hyperlipidemia such as neem and its products (neem oil) were studied by many researchers Kataria *et al*.,[40]; Zuraini *et al.,* [41]; Bisht and Sisodia [42]; Mgbeje *et al.,*[43]. They were also suggested that various doses of neem and its derivatives decreased serum total cholesterol and triglyceride levels and normalized lipid profile of animals. Oniovosa *et al*., [44] suggested that cholesterol levels were not significantly affected when treated with neem leaves aqueous extract but triglyceride values were significantly lower in rainbow trout, *Oncorhynchus mykiss* compare with the control group. Ajitha and Jayaprakas, [45] revealed that exposure of Menma (biopesticide) to *Oreochromis niloticus* causes no significant alterations in its biochemical and hematological parameters. This was an opposite outcome with previous study with biopesticide, Manisha and Anjana, [46] reported increase of cholesterol in fresh water fish *Channa punctatus*.

The fatty acid profile of the neem seed oil were report by many observers who identified fourteen fatty acids as energy sources. The fatty acid profile of the neem seed oil is characterized by a ratio of saturated/unsaturated fatty acids ranging from 0.53 to 0.54 [47].Although fish contain small amount of cholesterol, they are low in saturated fats, and experts recommend eating fish regularly for healthy heart. The beneficial omega- 3 fatty acids in oily fish may also help prevent cardiovascular diseases associated with high cholesterol [48]. For this reason, it becomes very important to protect the health of fish because their deteriorating health will directly affect the organisms that will use them as food. Therefore, these health risks can be reduced by the use of neem oil as shown by this experiment. Thus, it can be resulted that exposure of organophosphorus pesticides leads to severe hindrance in vital metabolic processes and induce hazardous changes in lipid metabolism in fishes while hepato-protective effect of neem oil can be beneficial for the health status of fish. Large-scale field investigation for suitability is mandatory, to screen biologically different neem formulations [49]. Regular monitoring of biochemical parameters in addition to effective interventions in regards to decreasing pesticide exposure to prevent health effects should be provided to farmers and our society [50]. So that can be made aware of the toxicants effects on aquatic environment which is essential for fish health directly and other animals indirectly.

5. Conclusion

Unusual amount of chemical pollutants and toxic pesticides from industry, urbanization and from agricultural practices are entering aquatic ecosystem and posing great harm to fish and other aquatic species which is a cause of adverse effects on human health through food chain. Therefore, it is recommended that in order to preserve the health of various organisms associated with food chain, it is necessary to use natural biopesticides instead of synthetic pesticides for sustain life of organisms and the environment. It could be concluded that the application of neem oil can be used in agriculture and aquaculture purposes as protective supplement that also cure the health of aquatic organism and environment friendly material instead of deleterious nuvan (organophosphorus pesticides). It can be helpful to conserve the natural resources for future generations. Although neem oil has been made safe for agricultural purposes, internal use cannot be recommended without carrying out the bioassays and toxicological analyses. It is suggested that give the importance of neem oil and its worldwide use for combating numerous pests in different crops and in aqua farms.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of informed consent

No additional information is available for this paper.

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