IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

IMMUNO-PROTECTIVE IMPACTS OF NEEM OIL ON CATFISH *CLARIAS BATRACHUS* (LINN.) TO SUSTAIN ENVIRONMENT AND AVOID THE RISK OF SYNTHETIC PESTICIDES

Aysha Parvin (research scholar) and Dr. Umesh Shukla (Assoc. Prof.)

Department of Zoology, Agra College, Agra,

Dr. Bhimrao Ambedkar University, Agra. Uttar Pradesh 282004, India

Abstracts-

Pesticides are synthetic substances generally used to control pests, insects, aquatic weeds and plant diseases. Currently, up to 35 % losses of farming yield are due to pests, so defeating this problem, the agricultural and aqua cultural practitioners used pesticides. Besides that pesticides can contaminate water, soil, turf, and other vegetation for consumption of all livings. Additionally killing insects and weeds, pesticides can be deleterious to a host of other livings including beneficiary insects, fish, bird, and non-target plants and animals. Synthetic pesticide nuvan exposures have been linked to the onset of poisonous pathways and adverse outcomes in aquatic species. Discharge of these toxic pesticides may result in accumulation of pollutants in aquatic livings which may occur after a long period has passed pollutants and include immune suppression, low metabolism, and damage to the gills and epithelia. Hence biopesticides are encouraged over synthetic chemical and pesticides. Another promising approach of biopesticides products are lower toxicity that easily decompose into the environment. The present investigation also highlights the utilization of biopesticides for the development of new environmentally safe antioxidant agents of plant origin. Anti-oxidant effects of neem oil (biopesticide) play an important role in protective mechanisms against metabolic disorder and its complications.

Some alterations in albumin and globulin in blood serum of treated catfish *Clarias batrachus* with 1/20 and 1/10 LC_{50} of nuvan and neem oil concentrations separately compared with non-treated one after 7, 15, 30 and 60 days of exposure were recorded and discussed. Observation for most biomarkers revealed patterns of decreasing values with increasing toxicant concentration and exposure duration. Also, depicts behavior and immune system of freshwater catfish *Clarias batrachus* in the presence of nuvan and neem oil. Obtained data could be acquired useful in environmental risk assessment of aquatic organisms. Pollution of surface water and aquatic life can be avoided by using judiciously and environmentally safe pesticides like biopesticides and replacing toxic synthetic pesticides, promoting prudent and proper pest management tools.

Keywords: Nuvan, catfish *Clarias batrachus*, nuvan, neem oil, serum albumin and globulin

Introduction –

Pesticides, otherwise mentioned to as plant protection products, by intentional use and applications in pest management and crop protection have reported increased occurrences in the environment (Chukwuka and Ogbeide, 2021; Saha and Saha, 2021). The eventual contamination of aquatic ecosystems with toxic pesticides either due to spray-drift, leaching, runoff, and/or accidental spills or from aqua-cultural applications (Saha *et al.,* 2021) could culminate into risks of enervating effects and mass mortalities of non-target livings (Sharma *et al.,* 2021). Another factor affecting water quality is rainfall, as high levels of rainfall increase the risk of pesticide contamination increases and adversely affected the aquatic livings and other animals including humans directly or indirectly. In addition, risk of prolonged toxicity to local biota because of their persistence and excessive possession within environmental matrices is also a concern (Stara *et al.,* 2020). Organophosphorus pesticides have moderate to high toxicity to freshwater fish, estuarine, and marine fish and probable high toxicity to birds (Karanth, 2014). Synthetic pesticides are composition of deleterious compounds which damage crops, and reduce the nutritional value of crops. The contamination of surface waters by organophosphorus insecticides is known to have ill effects on the survival, growth and reproduction or death of aquatic animals. Nuvan (dichlorvos) is an organophosphate derivative highly used as an agricultural and household pesticide. These pesticides degrade the soil and loss of soil fertility. Its documented high toxicity to beneficial insects vigorous risks of ecological effects, including loss of invertebrate taxa and trophic cascades (Rogers *et al.,* 2016), which could also impact the survival of fish populations.

In the upcoming days, biopesticides could replace synthetic pesticides without extremely affecting productivity and yield, if their capabilities are fully maximized (Mishra *et al*., 2020). Neem oil is a plant originated biopesticide and obtained from neem *(Azadirachta indica)* tree which is traditionally called as the ‘‘Village dispensary’’ or “Village pharmacy’’ in India (Biswas *et al*., 2002). There are many observations about the biopesticidal, immunomodulatory, antimicrobial and nematocidal activities of neem. Among 40 different active compounds of neem, more specifically azadirachtin is highly oxygenated triterpenoids and an important bioactive compound, responsible for pesticidal and antimicrobial activity (Govindachari and Gopalkrishnan 1998). Other components present include meliantriol, fatty acids (oleic, stearic, and palmitic), nimbin, nimbidin, nimbinin, nimbolides and salannin. The composition of the neem oil may widely differ depending on growth habitat of the neem tree, and environmental conditions (Aiello *et al.,* 2011). Moreover, neem oil is non-toxic to animals and beneficial insects, biodegradable, hydrophobic, low-cost, that has multiple applications in food, agriculture, and health sectors, as well as its historic significant values in Ayurveda, Unani, and homoeopathic medicines for centuries (Quraishi *et al.,* 2018).

Fisheries and aquatic resources such as ponds, streams, rivers, the seas and oceans are supplying peoples with long term benefits that can be the direct financial ones which deliver employment, profit, and save money as the sea food industry. There are occupational threat and safety concerns in the aquaculture industry. The aquaculture industry regulations and international oversight are extremely complex, with many agencies managing aquaculture practices, including water quality, selection of site, polluted control, feed supply and food safety (David *et al.,* 2009). The most important aquaculture species of catfish is the Asian Catfish, *Clarias batrachus*. It belongs to family-Claridae. *Clarias batrachus* is widely recognized in Indian sub-continent for its nutritional and economic significance. Biochemical parameters have been declared as biomarkers of health status of any animal in various pathological and eco-toxicological assessments. The information given in this investigation facilitates the evaluation of potential toxic hazard resulting from exposure to different doses of nuvan and beneficiary impacts of neem oil on fish health including humans and environments along with current and future prospects.

Materials and methods –

Experimental organism the freshwater, air-breathing catfish *Clarias batrachus* were collected from a local fish farm in Agra district, U.P., and transported to the laboratory for experimentation (weight 60-120 g; length 20-25 cm). Fish were conditioned to laboratory settings for 20 to 25 days in flow-through outdoor tanks with dechlorinated water (pH 7.5–7.8) and atmospheric temperature under a natural photoperiod (12:12 h light-dark. Every two days, the water quality was maintained by replacing the water of tanks. The catfishes *Clarias batrachus* were provided every day with commercial fish food at a rate of 2.5% body weight. Nuvan or dichlorvos 76% EC (CAS- 2, 2-dichloroethenyl dimethyl phosphate) belonging to the organophosphate class of insecticide and pure and natural cold pressed biopesticide neem oil was used as experimental substances.

The laboratory determination of median lethal concentration (LC_{50}) through a static bioassay test was performed. Data were measured statistically using the Probit Analysis Statistical Method of Finney (1971). In a randomized design, acclimatized catfish in experimental tanks with 20 L of water in each were set up. The chronic toxicity experiments were conducted for 60 days with control and four treatments groups. Group I was declared as control or untreated group. Group II and III were exposed to nuvan concentrations (0.137 ml/L and 0.274 ml/L), and other groups IV and V were treated with doses of neem oil (0.424 ml/L and 0.848 ml/L) which corresponded to 1/20th and 1/10th of the 96h LC_{50} respectively obtained for *Clarias batrachus*. To ensure the water quality and doses of the test media, the medium in all the groups was completely replaced every week with freshly made nuvan and neem oil solution. For 60 days, the fish were fed two times a day until they were visually satisfied. Blood was obtained by puncturing the fish's caudal vessels with a 5 mL dispovan syringe after 7, 15, 30 and 60 days, a portion of the blood was taken and centrifuged for 20 min at 2500 rpm in a cooling centrifuge. The serum was taken with a micropipette, transferred to microtubes, and stored at −20 ◦C for further assessment of serum albumin and globulin. Biochemical estimation of serum albumin and globulin was calculated using the technique by Dumas *et al*., (1971) method. Then, One-Way ANOVA is applied to compare the means between exposed and control groups.

Results –

The main objective of this assessment was to determine how the organophosphate pesticide nuvan and biopesticide neem oil affected biochemical (serum albumin and globulin) parameters in *Clarias batrachus* after chronic exposure. The 96 h LC_{50} of nuvan and neem oil was estimated 0.274 ml/L and 0.848 ml/L respectively. Nuvan was administered at predefined exposure sub-lethal doses 0.0137 ml/L (1/20 of LC_{50}) and 0.0274 ml/L (1/10 of LC_{50}) while neem oil at (0.0424 l/L and 0.0848 ml/L) and monitored at 7, 15, 30, and 60 days in the investigation. Fish from nuvan exposure (0.0137 ml/L and 0.0274 ml/L) displayed very highly significant lower serum albumin activity while neem oil treated (0.0424 ml/L and 0.0848 ml/L) catfish *Clarias batrachus* shows non-significantly increase activity of blood serum albumin after 7, 15, 30 and 60 days when compare with control groups (table- 1; graph- 1). In this observation, the specific growth rate of test fish exposed to nuvan remained less than control fish throughout the exposure intervals from 7, 15, 30 and 60 days.

The alterations in serum globulins activities was noted (table-2; graph-2) after exposure of nuvan and neem oil in blood of experimental fish *Clarias batrachus* at 7, 15, 30 and 60 days. The reduction in serum globulin activities was noted significantly after treated with 1/20 of LC_{50} of nuvan (0.0137 ml/L) and significantly elevation noted in treated fish after exposure of neem oil (0.0848 ml/L) in test fishes after 7, 15, 30 and 60 days . When specimen exposed with 1/10 of LC_{50} of nuvan concentration (0.0274 ml/L) than very-highly significant reduction in serum globulin while treated with 1/10 of LC_{50} of neem oil concentration (0.0848 ml/L), very-highly significant elevation was noted in blood serum globulin activities of experimental fish *Clarias batrachus* throughout the exposure periods (7, 15, 30 and 60 days).

Table 1: **Albumin (gm/dl)** in blood serum of *Clarias batrachus* after Nuvan and neem oil toxicity

Concentration (ml/L)	Albumin (gm/dl)	Exposure time				Significance level ANOVA
		7 day	15 day	30 day	60 day	
Control (Group- I)	Mean	2.63	2.63	2.63	2.63	Normal
	± S. Em.	0.05	0.05	0.05	0.05	
Nuvan– 0.0137 ml/L (1/20 of LC_{50}) (Group- II)	Mean	2.405	2.295	1.97	1.89	(P<0.001) Decrease
	± S. Em.	0.11	0.13	0.04	0.02	
Nuvan – 0.0274 ml/L (1/10 of LC_{50}) (Group- III)	Mean	2.12	1.98	1.63	1.32	(P<0.001) Decrease
	± S. Em.	0.06	0.02	0.04	0.01	
Neem oil – 0.0424 ml/L (1/20 of LC_{50}) (Group- IV)	Mean	2.63	2.63	2.63	2.75	(P>0.05) Increase
	± S. Em.	0.05	0.05	0.05	0.04	
Neem oil –0.0848 ml/L (1/10 of LC_{50}) (Group- V)	Mean	2.65	2.84	2.91	2.91	(P>0.05) Increase
	± S. Em.	0.03	0.04	0.02	0.02	

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

Graph- 2. Impact of nuvan and neem oil on serum albumin levels (gm/dl) in *Clarias batrachus*

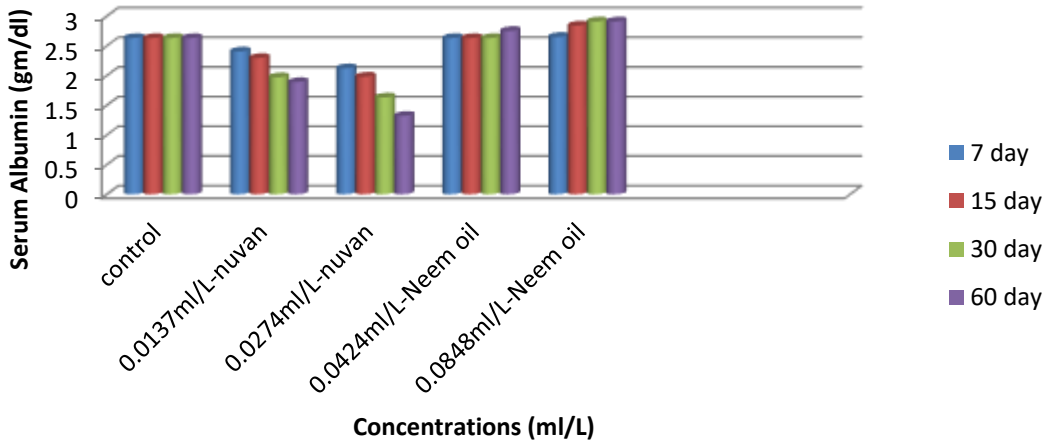
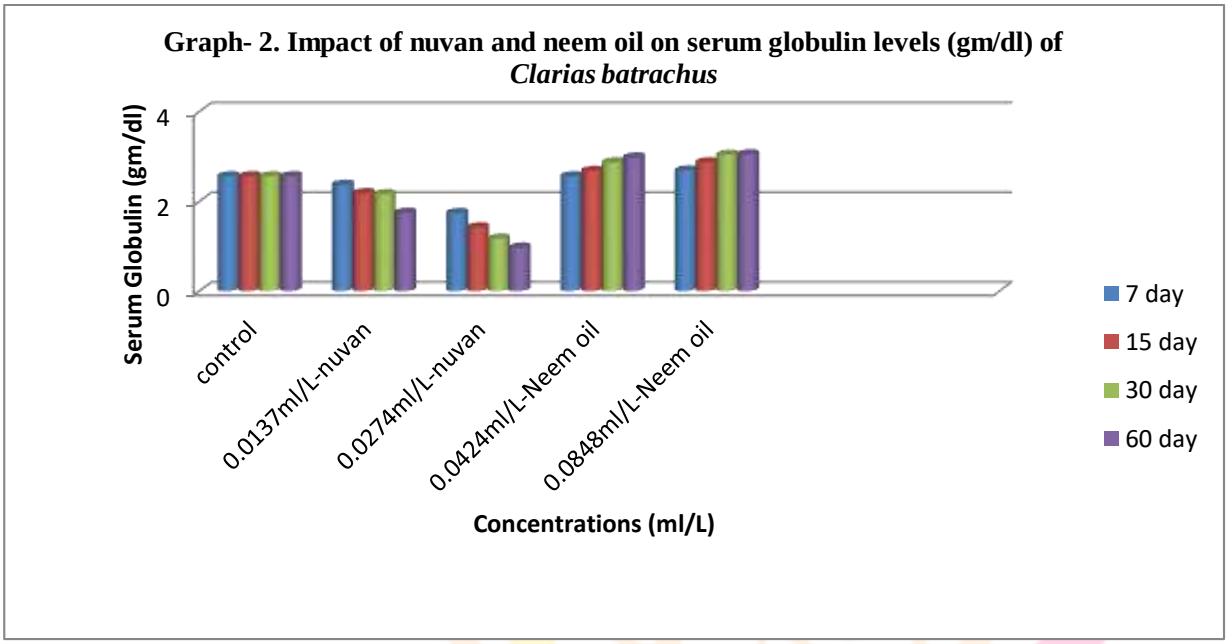


Table 2: **Globulin (gm/dl)** in blood serum of *Clarias batrachus* after Nuvan and neem oil toxicity

Concentration (ml/L)	Globulins (gm/dl)	Exposure time				Significance level ANOVA
		7 days	15 days	30 days	60 days	
Control (Group- I)	Mean	2.56	2.56	2.56	2.56	Normal
	± S. Em	0.207	0.207	0.207	0.207	
Nuvan – 0.0137 ml/L (1/20 of LC_{50}) (Group- II)	Mean	2.37	2.185	2.16	1.745	(P<0.05) Decrease
	± S. Em	0.127	0.095	0.138	0.187	
Nuvan – 0.0274 ml/L (1/10 of LC_{50}) (Group- III)	Mean	1.74	1.42	1.17	0.97	(P<0.001) Decrease
	± S. Em	0.187	0.138	0.057	0.08	
Neem oil – 0.0424 ml/L (1/20 of LC_{50}) (Group- IV)	Mean	2.56	2.68	2.87	2.98	(P<0.05) Increase
	± S. Em	0.207	0.017	0.034	0.051	
Neem oil–0.0848 ml/L (1/10 of LC_{50}) (Group- V)	Mean	2.69	2.87	3.04	3.05	(P<0.001) Increase
	± S. Em	0.034	0.04	0.04	0.04	

± S.Em.– Standard error of mean; (P<0.05) – Significant; (P<0.001) – Very highly Significant



Discussion –

Aquatic organisms are affected by toxic pesticides that contaminate water through agricultural runoff and are considered an essential bio-indicator of aquatic pollution (Bhat *et al.,* 2012). There has been a significant elevation in the number of publications over the past decade, concerning the use of neem oil to control agricultural pests (Bakry *et al.,* 2016). Various scientific researchers have shown that neem oil is also safe for farmed workers, with no handling risks, and can be applied throughout the entire crop production cycle (Boeke *et al.,* 2004). Behavioural alterations are one of the most sensitive signs of potential harmful effect in fishes (Banaee *et al*., 2011). The assessment of biochemical parameters in fish has become an essential application to understand normal and pathogenic action of aquatic livings as well as toxicological impacts (Sudova *et al*., 2009). In this study, assessment of biochemical parameters such as serum albumin and globulin revealed significant difference in walking catfish *Clarias batrachus* treated with nuvan and neem oil as compare with control. Various investigators examined biochemical parameters had variations due to the exposure of nuvan in various fish species. But very few observations have been done so far regarding blood sensitivity of the fish to the neem oil biopesticides used in agricultural fields.

In the present study, catfish *Clarias batrachus* exposed to nuvan exhibited extreme behavioral alterations. Fish showed stress throughout the investigation and attempted to escape the aquaria. Abnormal, rapid movement and loss of balance of catfish was noted throughout the experiment. Similar behavioral alterations recorded by Tiwari *et al.,* (2012) in *Labio rohita* exposed with cypermethrin. Catfish *Clarias batrachus* treated with neem oil were less affected from such stressful situation in this experiment. Experimental Fishes moved in groups in aquaria and whenever they reached to water surface to consume air but shows abnormal behavior after increment in dose and exposure duration. The LC_{50} value of neem oil (0.848 ml/L) is higher than the nuvan (0.274 ml/L) after 96 hours on *Clarias batrachus* that indicates more toxicity of nuvan than neem oil. Comparison of toxicity potential of synthetic and biopesticide shows that neem oil can be applied as eco-friendly plant based pesticide to control pests on agricultural fields as an alternative of detrimental and toxic synthetic pesticides.

Albumin is one of the most abundant proteins made by the liver. Serum albumin is referred to generally as blood albumin which is found in vertebrate's blood. It is the most essential proteins in blood because it acts in coagulation, free radical scavenging, transportation of substrate, and maintenance of COP, buffering capacity and wound healing (www.vetfolio.com). The blood albumin is an easily available protein reserve and a protein transporter (Anderson *et al*. 1979). The result indicates a changed value with decrease of albumin; it may be indicate an issue with the liver or kidney. Reduced albumin level in blood may occur when enough nutrients does not get into the body. Nutritional deficiency is also cause of hypoalbuminuria (www.medicalnewstoday.com). Pesticides have strength to accelerate oxidative stress in fish (Monteiro *et al*., 2006). Patnaik (2010) also noticed significant inhibition in albumin levels and protein activities, possibly because of tissue repair, impaired food intake, and increased energy cost of homeostasis, and detoxification mechanisms during stress. Prusty (2011) also recorded the decrease in albumin level of *Labio rohita*, treated with fenvalerate and resulted that under stress situation, various animals utilize protein as a source of energy during the oxidation process of amino acids. Similarly Velisek *et al*. (2012) mentioned significant decrease in albumin due to exposure of simazine in common carp. Same as significant albumin decrease in fish *Labio rohita* treated with acetamiprid reported by Alam *et al.,* (2014), suggests that the pesticide may have cause renal disease and liver insufficiency. This is consistent with preceding research on different fish treated with multiple pesticides (Majumder and Kaviraj 2018).

Globulins are protein, synthesis by liver in the body of animals. Globulins play an essential role in liver function, fighting infection and blood clotting. About 40% of proteins in blood are alpha, beta and gamma globulins (my.clevelandclinic.org, 2022). Serum albumin is the remaining protein part in blood. The gamma globulin fraction is the source of mainly all the serum biochemical active proteins of blood. Globulins like gamma globulins also known as immunoglobulin, is essential for maintaining a healthy immune system. Since, gamma fraction of globulin makes the largest portion of globulin, it can be inferred that subsequent enhanced globulin level in exposed groups may boost the immune response and initially stage for stress mitigation of catfish *Clarias batrachus*. Reduction in albumin and globulin levels in blood serum of specimen signifies that synthetic pesticide nuvan could have caused nutritional insufficiency, kidney disease, and liver inefficiency and may accelerate hazardous impacts on immune system. Antioxidant also protected immune responses in animal from certain environmental sources of free radicals. Free redicals (oxidants) are produced from immune system (Biesalski et al., 1995).

The quality of antioxidant to remove free radicals protects the structural integrity of tissues and cells. The antioxidative system ensures that the emergence of free radicals during various physiological processes should not result in cellular damage. Presence of organophosphorus pesticides like nuvan can be increased energy lost of homeostasis, tissue repair, impaired food consumption and detoxification mechanisms during the period of stress (Patnaik, 2010) damage the antioxidants and immune responses. Narra, (2016) suggested that the growth and survival performance of test fish *Clarias batrachus* affected by the sub-lethal pesticide concentrations hazardously. This might be due to oxidative stress induced by toxic pesticide, resulting in decrement of feeding or energy drain leading to the reduction in liver mass which correlates with the protein degradation (Mohapatra *et al*., 2012 and Narra *et al*., 2015). They also observed decreased globulin and albumin levels indicate that, it may be due to protein amalgamation or may be due to increased adverse effects on the immune system (Kumar *et al*., 2011; Mohapatra *et al*., 2012; and Yonar *et al*., 2014).

In this study, elevations in albumin and globulin levels in blood serum of specimen *Clarias batrachus* signifies that biopesticide neem oil could have potency to handle the demand of nutritional substitutes by improving energy utilization or nutritional sufficiency, protect kidney and liver functioning and may improve immune system. The immune system functioning depends on intake of micronutrients, which can behave as antioxidants (Bendich, 1993). In this work increase level of albumin and globulin shows antioxidant property

of neem oil in serum of catfish. Increase in albumin and globulin levels in the blood serum is considered a strong innate response in fish declared by Wiegertjes *et al.*, (1996). Although body possesses an effective defense system to stop radical damage, this innate system can be affected and lead to a state of immunosuppression or oxidative stress and can ever trigger carcinogenesis. Topical supplementation of antioxidants like neem oil can provide additional protection to neutralize reactive oxygen species from both exogenous and endogenous sources (Chen *et al*., 2012). Sarkar and Parames Sil, (2022) investigated a proper antioxidant mechanism and suggested that appropriate amount of neutralizing antioxidants must be existing in the body which will remove the excess ROS/RNS generated during an immune response. Apart from mechanism of intracellular antioxidant, dietary antioxidants like micronutrients or neem oil treatment also contribute to combat the oxidative stress. Ansari and Ahmad, (2010) suggests that biopesticides are fewer hazardous and more eco-friendly as compared to toxic synthetic pesticides. When fish *Labeo rohita* treated with *Azadirachta indica* leaves extract then non-significant decrease level of albumin were noted by Alam *et al.*, (2014) which proves that biopesticide is safe for non-target organisms. On the other hand, the results of Winkaler (2007) suggest that although neem extract is slightly toxic to *P. lineatus* than other synthetic pesticides used in aquaculture it does cause morphological and functional alterations in fish species. Levels that are too high of serum globulin in any animals indicate autoimmune disease, infections or cancer. Many other researchers have proved that neem oil is used to get rid of any disease or to protect crops and their products or to save aquaculture in an eco-friendly manner.

Future prospective –

Aquaculture has important possibility to provide a healthy and sustainable protein source for future populations comparable to plant sources. However, to reach this possibility, a substantial increase in production is required to ensure future demands for protein. This enhanced production must be matched by essential reductions in environment impact and improvements in efficiency of resource (WWF and GSI report, 2018). Biopesticides are also an essential part of sustainable aquaculture and agriculture, which fulfills the requirements of agricultural and aqua-cultural support workers without any deleterious health effects on plant and other animals including fish. Several studies have been reported the different pharmacologic effects of neem, including hepatoprotective (Igwenyi *et al*., 2017), antimicrobial, anticancer, and anti-diabetes (Moga *et al*., 2018) properties. More than 300 compounds are derived from different parts of neem, such as leaves, flower, seed, fruit, bark, and root which can improve the major source of protein like fish immunity and organs functioning for a better prospective. Along with this, the wearing effects on the environment and the health of its organisms can be prevented by using less pesticides and more use of biopesticides. It offers an unlimited capability of biodegradable, economical, and renewable alternative pest control measures. Hence, in order to balancing in demands and availability, it is essential to improve efficacy and stability through new active substances like biopesticides and advanced formulations for sustain the next generation.

Conclusion –

The enormous use of synthetic pesticides can cause health hazards by disturbing the composition of water, soil and air which is not suitable for consumption and affected different levels of consumers by food chain. The present study concluded that synthetic pesticide nuvan should be noted in list of toxic pesticide as it brings alterations in biochemical parameters such as albumin and globulin of aquatic ecosystem inhabitants such as fish. So it can disrupt the functioning of main organs of fish and other livings. The growing harmful effects on the health risks associated with pesticides can be inhibited, by making farmers aware of their harm potential, and by providing information about the benefits of biopesticides studied in nature. By increasing the use of biopesticides, the increasing negative health effects on aquatic animals can also be reduced, so that safe and sustained protein resources can be passed on the next generations and for the upcoming times.

References –

Aiello, C., Berardi V., Ricci F., Risuleo G, 2011. Biological properties of a Methanolic extract of neem oil, A natural oil from the seeds of the neem tree (*Azadirachta indica* var. A. Juss), in: V.R. Preedy, R.R. Watson, V.B. Patel (Eds.), Nuts and Seeds in Health and Disease Prevention. Academic Press, San Diego, Chapter 96: 813–821.

Alam A., Amtul Bari Tabinda, Mahmood-ul-Hassan, and Abdullah Yasar, 2014. Comparative Toxicity of Acetamiprid and *Azadirachta indica* Leave Extract on Biochemical Components of Blood of *Labeo rohita* Pakistan J. Zool., 46 (6): 1515-1520.

Anderson DP, Robertson BS, Dixon OW., 1979. Plaque-forming cells and humoral antibody in rainbow trout (*Salmo gairdneri*) induced by immersion in a Yersinia ruckeri O-antigen preparation. J Fish Res Board Can, 36 (6): 636– 639.

Ansari, B.A. and Ahmad, M.K., 2010. Toxicity of synthetic pyrethroid lambda-cyhalothrin and neem based pesticide neem gold on zebra fish *Danio rerio* (Cyprinidae). Global. J. environ. Res., 4: 151-154.

Bakry, A. M., Abbas, S., Ali, B., Majeed, H., Abouelwafa, M. Y., Mousa, A., *et al.*, 2016. Microencapsulation of oils: a comprehensive review of benefits, techniques, and applications: encapsulation of marine, vegetable, essential oils. Compr. Rev. Food Sci. Food Saf. 15, 143–182.

Banaee MS, Mirvaghefi AR, Ahmadi K, 2011. Effects of Diazinon on biochemical parameters of blood in rainbow trout (*Onchorhyncus mykiss*). Pesticide Biochemistry and Physiology, 99: 1-6, 2011.

Bendich, A 1993. Physiological role of antioxidants in the immune system. J. Dairy Sci. PubMed

Bhat, I.A, Bhat B.A, Vishwakarma S, Verma A, Saxena G, 2012. Acute toxicity and behavioral responses of *Labeo rohita* (hamilton) to a biopesticide Neem-On. Current World Environment, 7: 175-178.

Biesalski, H.K., Frank *et al*., 1995. Antioxidants in nutrition and their omportance in the anti-/oxidative balance in the immune system. PubMed. 23(5): 166-173.

Biswas, K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U, 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*). Curr Sci 82:1336–1345.

Boeke, S. J., Boersma, M. G., Alink, G. M., van Loon, J. J., van Huis, A., Dicke, M., *et al*., 2004. Safety evaluation of neem (*Azadirachta indica*) derived pesticides. J. Ethnopharmacol. 94, 25–41.

Chen Lucy, Hu JY, Wang SQ, 2012. The role of antioxidants in photoprotection: a critical review. J Am Acad Dermatol. PubMed, 67(5): 1013-1024.

Chukwuka, A.V.; Ogbeide, O. 2021. Riparian-Buffer Loss and Pesticide Incidence in Freshwater Matrices of Ikpoba River (Nigeria): Policy Recommendations for the Protection of Tropical River Basins. River Basin Manag. Sustain. Issues Plan. Strateg. 113.

David WC; Richard C; Steven J Gaydos JG and William WC, 2009. Aquaculture Environmental Toxicological, and Health tissue. Int. J. Hyg. Environ Health, 212, 369-377.

Dumas, B.T. *et al*., 1971. Determination of total protein and albumin in serum. Clin. Chem. Acta., 31: 87-96.

Finney, DT. 1971. Probit analysis. Cambridge University Press: Cambridge; 333.

Govindachari, TR. and Gopalkrishnan GJ, 1998. Insect antifeedant and growth regulating activities of neem seed oil. Indian Chem Soc 75: 655.

Igwenyi I, Eze A, Aja P, Elom S, Uraku A, Awoke J, *et al.* 2017. Cholesterol-lowering and hepatoprotective effect of fruit juice extract of *Azadirachta indica* on Plasmodium berghei infected mice. Int J Curr Microbiol App Sci. 6: 3367-3375.

Karanth, S., 2014. Diazinon. In Encyclopedia of Toxicology; Elsevier BV: Amsterdam, The Netherlands, 55–56.

Kumar Abhishek, Prasad Maniram, Mishra D, K. Srivastav Sunil K., Srivastav Ajai K. 2011. Botanical pesticide, azadirachtin attenuates blood electrolytes of a freshwater catfish *Heteropneustes fossilis* Pesticide Biochemistry and Physiology, 99: 170–173

Majumder, R.; Kaviraj, A 2018. Acute and sublethal effects of organophosphate insecticide chlorpyrifos on freshwater fish *Oreochromis niloticus*. Drug Chem. Toxicol., 42: 487–495.

Mishra, J., Dutta, V., and Arora, N. K. 2020. Biopesticides in India: technology and sustainability linkages. 3 Biotech 10: 1–12.

Moga MA, Bălan A, Anastasiu CV, Dimienescu OG, Neculoiu CD, Gavriș C, 2018. An overview on the anticancer activity of *Azadirachta indica* (Neem) in gynecological cancers. Int J Mol Sci., 19: 3898-3924.

Mohapatra, S., Chakraborty, T., Prusty, A., Kumar, K., Prasad, K., Mohanta, K., 2012. Fenvalerate induced stress mitigation by dietary supplementation of multispecies probiotic mixture in a tropical freshwater fish, *Labeo rohita* (Hamilton). Pest. Biochem. Physiol. 104: 28-37.

my.clevelandclinic.org. 2022. Globulin blood test.

Narra, Madhusudan Reddy 2016. Single and cartel effect of pesticides on biochemical and haematological status of *Clarias batrachus*: A long-term monitoring. Chemosphere 144: 966-974.

Narra, Madhusudan Reddy, Rajender, K., Rudra Reddy, R., Rao, J.V., Begum, G., 2015.The role of vitamin C as antioxidant in protection biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*.Chemospnere 132: 172-178.

Patnaik, L., 2010. Biochemical alterations induced by sevin in *Clarias batrachus*. Asian J. exp. biol. Sci., 1:124-127.

Prusty, A.K., Kohli, M.P.S., Sahu, N.P., Pal, A.K., Saharan, N., Mohapatra, S. And Gupta, S.K., 2011. Effect of short term exposure of fenvalerate on biochemical hematological responses in *Labeo rohita* (Hamilton) fingerlings. Pestic. Biochem. Physiol., 100: 124-129.

Quraishi, H., Islam N, Iqbal A, Bhat S, Jameel A, Ashraf S, Khan Q, 2018. Therapeutical and medicinal properties of Neem (*Azadirachta indica*) in context of Unani system of medicine: a review study, J. Drug Deliv. Therapeut. 8: 394–399.

Rogers, H.; Schmidt, T.S.; Dabney, B.L.; Hladik, M.L.; Mahler, B.J.; Van Metre, P.C, 2016. Bifenthrin Causes Trophic Cascade and Altered Insect Emergence in Mesocosms: Implications for Small Streams. Environ. Sci. Technol. 50: 11974–11983.

Saha, S, Mukherjee D., Dhara K., Saha N.C, 2021. Acute toxicity bioassay of a pyrethroid pesticide bifenthrin to the Asian stinging Catfish, *Heteropneustes Fossilis* (Bloch). Curr. World Environ. 16, 250–258.

Saha, S.; Saha, N.C, 2021. Study on Acute Toxicity of Bifenthrin to (*Clarias batrachus Linn*.). Indian J. Ecol., 48: 545–548.

Sarkar Kasturi and Parames C Sil, 2022. Antioxidants effects in health. Chapter 5.7-Antioxidants and immune functions. Science Direct. 719-740.

Sharma, S.; Dar, O.I.; Singh, K.; Kaur, A.; Faggio, C, 2021. Triclosan elicited biochemical and transcriptomic alterations in *Labeo rohita* larvae. Environ. Toxicol. Pharmacol.

Stara, A.; Pagano, M.; Capillo, G.; Fabrello, J.; Sandova, M.; Albano, M.; Zuskova, E.; Velisek, J.; Matozzo, V.; Faggio, C, 2020. Acute effects of neonicotinoid insecticides on *Mytilus galloprovincialis*: A case study with the active compound thiacloprid and the commercial formulation calypso 480 SC. Ecotoxicol. Environ. Saf. 203: 110980.

Sudova, E. K., Piackova, V., Kroupova, H., Pijacek, M., Svobodov, Z. 2009.The effect of paraziquantel applied on selected hematological and biochemical indices in common carp (*Cyprinus carpio L*.). Fish Physiol. Biochem. 35: 599-605.

Tiwari, S., Tiwari, R. And Singh, R.A., 2012. Impact of cypermethrin on fingerlings of common edible carp (*Labeo rohita*). J. scient. World., 12: 1-7.

Velisek, J., Stara, A., Machova, J. and Svobodova, Z., 2012. Effects of long-term exposure to simazine in real concentrations on common carp (*Cyprinus carpio*). Ecotoxicol. environ. Safe., 76: 79–86.

Wiegertjes, GF., Stet RJM, Parmeatier HK, Van Muiswinkel WB, 1996. Immunogenetics of disease resistance in fish; a comparable approach developmental and comparative Immunology. J Exp Boil 20: 365–381.

Winkaler Elissandra U., Thiago R.M. Santos, Joaquim G. Machado-Neto, Cláudia B., Martinez R, 2007. Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish *Prochilodus lineatus*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 145 (2): 236-244.
WWF and GSI, 2018. The future of aquaculture. Washington DC.

www.medicalnewstoday.com,wikipedia.

www.vetfolio.com.

Yonar, S., Ural, M., Silici, S., Yonar, M., 2014. Malathion-induced changes in the haematological profile, the immune response, and the oxidative/antioxidant status of *Cyprinus carpio carpio*: protective role of propolis. Ecotoxicol. Environ. Saf. 102, 202-209.