Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 571-575

Received: 01-11-2019
Accepted: 05-12-2019

Amusan Babatunde
Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Amusan Babatunde
Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Acute toxicity of paraquat dichloride on the whirligig beetle, *Orectogyrus* Sp.

Amusan Babatunde

Abstract
Paraquat dichloride is an organic salt that is widely used to control weeds in many agricultural and non-agricultural areas. However, this and other pesticides have been identified as critical sources of pollution in aquatic ecosystems. This study investigated the acute toxicological effects of the various concentrations of paraquat on whirligig beetles (*Orectogyrus* sp) with the aim of determining the potential lethal effects of the chemical on the organisms. The test substance (paraquat dichloride) was found to have lethal effects on the test organism (*Orectogyrus* sp) as it altered the behavioural patterns and eventually caused death of the test organisms. The 72hr and 96hr LC50 was estimated to be 42.03mg/L and 51.47mg/L respectively. The mortality trend in the test organisms followed a linear pattern with the highest mortality recorded in the highest concentration. Abnormalities in the behaviour of the organisms was attributed to oxidative stress and disruption of their nervous coordination.

Keywords: Paraquat, toxicity, mortality, oxidative stress, whirligig beetles

Introduction
In developing countries, the amount and heterogeneity of discharges that gets into the aquatic ecosystem has recently become alarming. Consequently, approximately three million people have been reported poisoned and about 200,000 death recorded annually all over the world from pesticide contamination. Unsuprisingly, the majority of these incidences were reported from developing countries [1]. The diminishing world's water resources and continuous pollution of waterbodies each day have been attributed to urbanization and the over-exploitation of the available land resources largely due to the ever-growing human population [2-3]. One of the important sources of pollution that have been identified in the aquatic environment is the residue of pesticides from agricultural farmlands. All over the world, pesticides have been used to control the destructive effects of living forms such as insects, rodents and herbs.

In Nigeria, chemical weed control is playing an increasing role in agriculture due to the increasing cost and widespread unavailability of labour required to carry out traditional farming practices. As a result, there has been rapid rise in the indiscriminate and unregulated use of pesticides on agricultural farmlands in the country. Some of the means by which pesticides get into waterbodies include drift during pesticide spraying, runoff from treated areas and by leaching through soil column [4]. Application of pesticides directly onto water surfaces for the control of insects such as mosquitoes is another way in which water is polluted. Although, the magnitude of water pollution depends mainly on the type of pesticides, soil properties, weather conditions, landscape and the distance of the application site to the waterbody. However, spontaneous movement into the groundwater may be hastened by heavy rainfall shortly after the application of the pesticide to wet soils [5].

The introduction of these toxic substances into waterbodies constitute water pollution which may be potentially harmful to non-target aquatic life forms there-in. Pollution may cause reduction in the dissolved oxygen available in the water which may lead to the suffocation or eventual death of the organisms in the waterbody. Aquatic insects have been known to be basically susceptible to even the least concentrations of pesticides as it affects their neurological and other behavioural activities. Whirligig beetle (*Orectogyrus alluadi* [6]) is an important component of the freshwater ecosystem. Aside their usefulness as bio-indicators of water quality, they also play vital role in keeping the water body clean by clearing off debris from the water surface. Paraquat (1,1-dimethyl-4,4-bipyridinium ion) is a contact and non-selective herbicide

commonly used to exterminate vegetative pests. Its effectiveness in the control of terrestrial weeds and aquatic plants in many water bodies in different countries of the world has been widely reported [7-9]. Although, pesticides have been generally known to be toxic to all life forms in the aquatic environment, however, information on the lethal dose or the level of toxicity of these chemicals to aquatic insects is lacking. The essence of this study is to provide information on the acute toxicity of 'Paraquat' to the beetles as well as their behavioural responses to the toxicological stress. It is believed that information on acute toxicity of a toxic substance is essential in the prediction and prevention of acute damage to aquatic organisms in receiving waters as well as in the regulation of toxic waste discharges into the environment.

Materials and Methods

Test substance

Paraquat dichloride ($C_{12}H_{14}Cl_2N_2$) with a molecular weight of 257.158 g/mol is manufactured by Syngenta. It is commonly referred to as "paraquat" and it is classified as a viologen, a non-selective contact herbicide with high solubility in water. It is one of the commonest and widely used chemicals in the control of cultivated farmlands of rice, cotton, fruit, tea, potatoes, sugarcane and vegetable [10].

Collection and acclimation of test organisms

The *Orectogyrus alluaudi* specimens were collected from Opa reservoir in Obafemi Awolowo University campus, Ile-Ife, Nigeria. The coordinate of the sampling station is Latitude 07º 32.561'N and Longitude 004º 32.550'E. The beetles were collected with the aid of a D-frame net by scooping the water surface. The collected specimens were transported to the Laboratory in a plastic bowl. Thereafter, the beetles were allowed to acclimatize to the Laboratory conditions at 25 ± 2 ºC with 12:12 h light:dark photoperiod. Identification of the beetle specimens was done with the aid of the identification keys provided by [11]. During the test, the organisms were fed with *Drosophila melanogaster* Meigen, 1930 (fruit flies) for 24 h prior to the test. The fruit flies used for feeding the organisms were produced in the laboratory using ripe banana as food source.

Test procedures

Acute toxicity test was conducted in the laboratory in accordance with the standard methods prescribed by [12]. However, prior to the definitive test, a range-finding test was conducted to determine the range of concentrations suitable for the specimens' exposure. The concentrations that were selected based on the range finding test include; 0.02 mg/L, 0.2 mg/L, 2 mg/L, 20 mg/L, 200 mg/L and a control. These concentrations were obtained from the stock solution of the pesticide. Each concentration was replicated three times. The de-chlorinated tap water used had the same physical and chemical properties with the one used in acclimatizing the beetles. The test solution was allowed to stand for 12 hours to bring it to equilibrium before introducing the test organisms. A total of 180 beetles were exposed, giving a loading rate of 10 beetles per test chamber. The survival rate and mortality rate were monitored and recorded 6 hourly for a 96-hour period. A test organism is considered dead when movement ceased and there was no response to gentle probing. Such dead test organisms were removed to prevent the build-up of Ammonia in the test solution. Behavioural and morphological indicators were also observed in this study. Each test chamber was observed for 5-10mins and responses were recorded if they differed from the controls. Some physicochemical parameters of the test solutions such as; Alkalinity, pH, dissolved oxygen, salinity, total dissolved solid and conductivity were determined at the commencement and the end of the exposure period using standard methods for chemical analysis of water [13].

Statistical analysis

Median lethal concentration (LC_{50}) values of the pesticides to *O. alluaudi* were calculated using probit analysis method [14]. Two-way ANOVA was used to compare the results of the physico-chemical parameters of the test solutions for significant differences. Percentage mortality recorded at 24hr, 48hr, 72hr and 96hr for each concentration and control was compared using Fisher's exact binomial test with Bonferroni correction for pair-wise comparison. Test of significance was at 5% level.

Results

Behavioural responses

Avoidance behaviour was observed to be high in the test chambers. This avoidance movement was observed to be very rapid in the higher concentrations as the beetles were seen to be attempting to escape from the test medium. It was also observed that the movement of the beetles was disorientated after 48hrs of exposure period (Table 1). General activity and movement reduced drastically after 48 hrs of exposure.

Table 1: Diagnostic behavioural effects of paraquat on *Orectogyru alluaudi*

S/No	Behavioural and Morphological Changes	Observations
1.	Loss of equilibrium	Yes
2.	Avoidance/ Agitation behaviour	Increases with increasing concentration.
3.	Movement	Disorientation in the movement of the beetles especially at high concentrations.
4.	Deformities	No observable deformities

Toxicity, Survival and Mortality

Beetle mortality occurred in all the test chambers. Mortality of the beetles in the test medium was observed to increase progressively along the concentration gradient within the test period. Mortality in each test concentrations (0.02 mg/l, 0.2 mg/l, 2 mg/l, 20 mg/l and 200 mg/l) was observed to be significantly higher than the control. It was further observed that mortality in the beetles was time-dependent as it increased with increased duration of exposure (Fig 1). There was about 75% mortality in the highest concentration (200 mg/L) after 24hrs of exposure and 100% mortality was recorded within 72 hrs of exposure. In the 20 mg/L concentration, 20% mortality was recorded after 96 hrs (at the end of the test period). The mortality in 2 mg/L, 0.2 mg/L and 0.02 mg/L were 35%, 15% and 10% respectively after 96 hrs of exposure.

The LC_{50} values obtained at 48, 72 and 96hrs of exposure of *Orectogyrus. alluaudi to* Paraquat were 6.96 (28.74-206.07) mg/l (Fig 2), 42.03 (8.60-205.39) mg/l (Fig 3) and 51.47 (10.26-258.19) mg/l (Fig 4) respectively. The low observed

effect concentration (LOEC) and No observed effect concentration (NOEC) values estimated were 0.2 and 0.02 mg/l at 48 hrs, 72 hrs and 96 hrs respectively (Table 2).

Most of the physico-chemical parameters of the test solution that were determined at the commencement (0 hr) and at completion (96 hrs) showed variations at different concentrations (Table 3). The most observable differences were observed in the concentration of dissolved oxygen and Total dissolved solids (TDS).

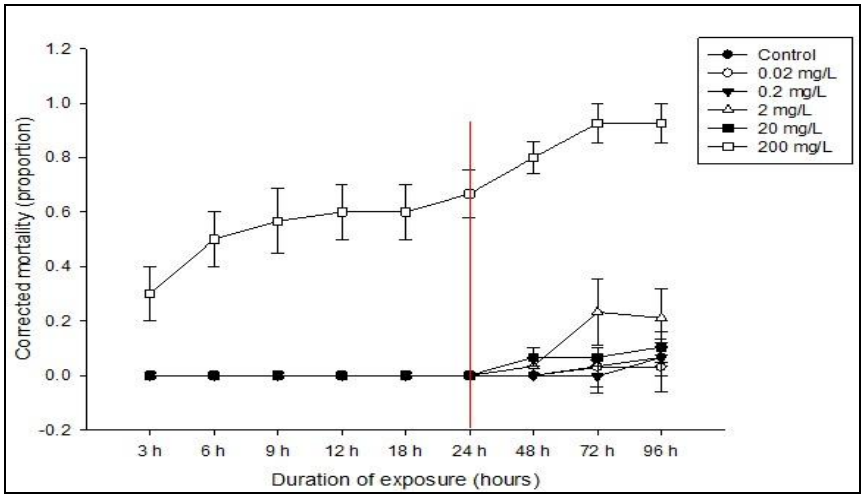

Fig 1: Relative mortality of *Orectogyrus sp* in test concentrations at different test periods

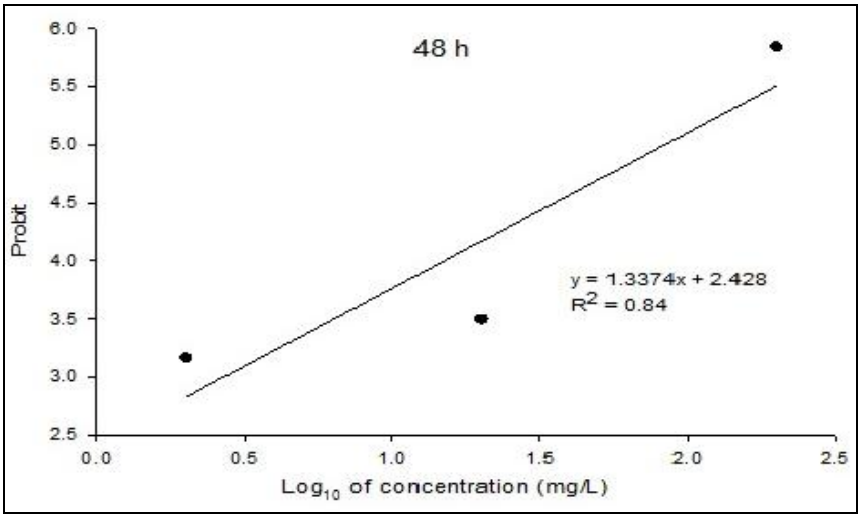

Fig 2: Concentration with 50% mortality at 48 hours of the test period.

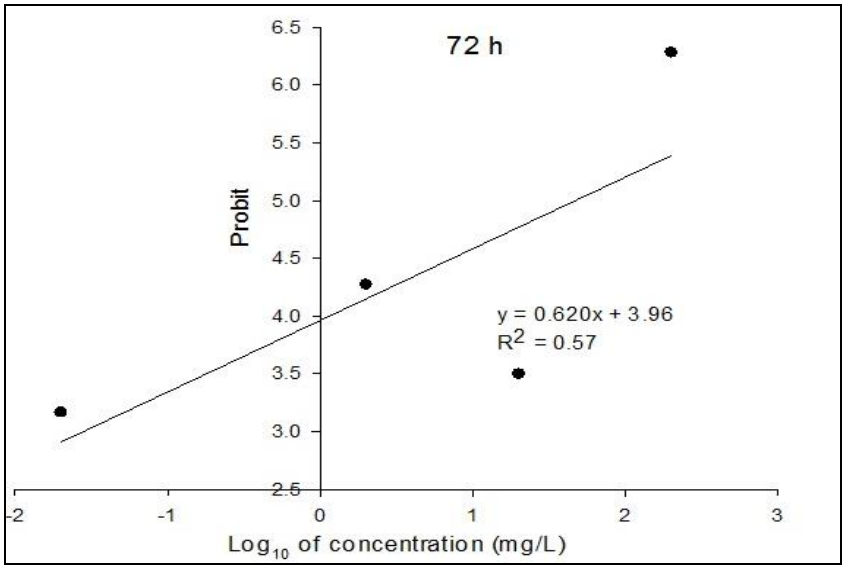

Fig 3: Concentration with 50% mortality at 72 hours of the test period

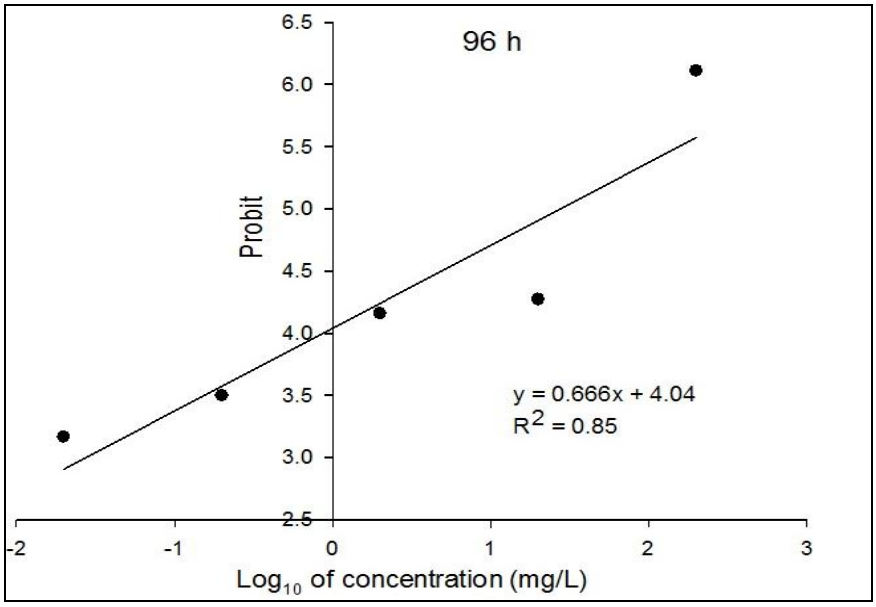

Fig 4: Concentration with 50% mortality at 96 hours of the test period

Table 2: Median lethal concentration (LC_{50}) of Paraquat on *Orectogyrus alluaudi* at 48, 72 and 96hrs of exposure

	48hrs	**72hrs**	**96hrs**
LC_{50} (mg/L)	76.96 (28.74-206.07)	42.03 (8.60 – 205.39)	51.47 (10.26 – 258.19)
LOEC (mg/L)	0.2	0.2	0.2
NOEC(mg/L)	0.02	0.02	0.02

Table 3: Physico-chemical parameters of the test solutions (at 0hr and 96hrs of exposure)

Conc.	**Dissolved oxygen (mg/L)**		**Alkalinity (mEq/L)**		**Conductivity (S/m)**		**pH**		**Total dissolved solid (ppm)**		**Salinity (g/kg)**	
200 mg/L	0 hr	96 hrs	0 hr	96 hrs	0 hr	96 hrs	0 hr	96 hrs	0 hr	96 hrs	0 hr	96 hrs
	8.60	7.62	20.01	21.02	332	440.5	5.38	7.02	221.30	29.32	0.02	0.02
20 mg/L	10.61	11.72	11.20	25.02	1.40	45.95	5.81	7.35	1.01	30.65	0.00	0.05
2 mg/L	8.80	10.23	12.10	26.20	1.71	44.55	5.65	7.28	1.21	32.05	0.00	0.10
0.2 mg/L	10.43	11.21	9.01	22.01	2.50	41.43	5.72	7.27	1.70	27.21	0.00	0.13
0.02 mg/L	9.02	12.02	10.03	26.25	1.61	42.95	5.81	7.23	1.10	28.33	0.00	0.09
control	9.41	8.24	10.02	20.01	59.00	28.65	7.01	7.07	39.70	18.64	0.00	0.00

Discussion

There were significant differences ($p > 0.05$) in the values obtained for the physico-chemical parameters of the test solutions. This finding contradicts the observations of [15, 16] in wich no marked differences were observed in the physico-chemical parameters of the test soulutions. However, the range of values obatained for the physico-chemical parameters of the test solutions were found to be within the tolearance threshold of aquatic insects. This suggested that the variations in the values may not have caused abnormal behavioural changes in the beetles in this study

The percentage mortality trend in the beetles followed a linear pattern with the highest mortality recorded at the highest concentration. This is similar to the observations of [17-19] in which linear patterns was observed in the mortality trend with respect to concentration on exposure of test organisms to paraquat. In this study also, there was 10% mortality in the least concentration and 100% mortality in the highest concentration thus showing a similar linear pattern. This pattern also conformed with the report of [19] which stated that there should be less than 35% mortality in the lowest concentration and at least more than 65% mortality in the highest concentration. However, the 96hr LC50 values (51.47) obtained in this study indicated less sensitivity of O. alluaudi to paraquat compare to other invertebrates [20-22]. Variations in the sensitivity of these aquatic invertebrates could be attributed to differences in the route of exposure, physiological sensitivity, and biochemical responses [23]. According to [24] differences in sensitivity may also be attributed to the selectivity of the insecticidal action in relation to the species and this has been responsible for the unpredictability of uptake of the insecticide.

Behavioural abnormalities such as disorientation in movement and avoidance mechanism were induced in the whirligig beetles as a result of the exposure to acute concentration of paraquat. The abnormalities in their behavior may be attributed to disruption of the nervous system cordination or biochemical body derangement [25]. This finding agrees with the report of [26, 27] in which similar disruption of activities of test organisms were reported. In some cases, these behavioural changes may be accompanied by physiological changes in the test organisms. For instance [28] reported a 45% shift in the emergence time of the 4th instar larvae of *Chironomus riparius* on a short-term exposure to Lindane. However, the effect of the exposure on the physiological state of the beetles was not ascertained in this study. This may suggest biochemical tests which will reflect the physiological changes in the test organisms. Further studies in this direction are thus required.

Conclusion

This study has shown that paraquat dichloride can be toxic to insects and other life forms in the aquatic ecosystems. This was reflected in the mortality pattern exhibited in the test organisms as mortality was observed to increase with increased concentration of the test substance. As such, controlled use of this pesticides and other organic salts in the environment is strongly advised.

References

1. FAO (Food and Agricultural Organisation). The state of world fisheries and aquaculture. FAO/WHO. Residues in food. Report of Joint FAO/WHO food standards programme. Rome: FAO. 2000; 2B:61-81.
2. Ogueji EO, Ibrahim BU, Auta J. Investigation of acute toxicity of chlorpyrifos-ethyl on Clarias gariepinus - (Burchell, 1822) using some behavioural indices. International Journal of Basic and Applied Sciences. 2013; 2(2):176-183.
3. Figen EK, Gullu K, Nazan DY. Insecticide groups and their effects in aquatic environments. Fen Bilimleri Dergisi. 2013; 25(4):167-183.
4. Hossain K, Pant G, Yadav S, Maruthi YA, Quaik S *et al*, Arsenic contamination of groundwater and its effect on soil-crop systems. Research Journal of Environmental Toxicology. 2015; 9:231-240.
5. Morrissey CA, Mineau P, Devries JH, Sánchez-Bayo F, Liess M, Cavallaro MC *et al*. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environment International Int. 2015; 74:291-303. doi: 10.1016/j.envint.2014.10.024.
6. Regimbart M. 'New and Rare Dytiscidae and Gyrinidae from the Musee Collection, Royal of Leiden', Notes from the Leyden Museum. 1889; (11):51-63.
7. Filizadeh Y. An ecological investigation into the excessive growth of *Azolla* in the Anzali Lagoon and its control. Iranian Journal of Natural Resources. 2002; 55(1):65-82.
8. Gao R, Choi N, Chang SI, Kang SH, Song JM, Cho SI, *et al*. Highly sensitive trace analysis of paraquat using a surface-enhanced Raman scattering microdroplet sensor. Analytical Chimica Acta. 2010; 681:87-91.
9. Ismail BS, Sameni M, Halimah M. Evaluation of herbicide pollution in the Kerian Ricefields of Perak Malaysia. World Applied Science Journal. 2011; 15(1):5-13.
10. Eaton AD, Clesceri LS, Rice EW, Greenberg AE, Franson MAH. (editors). Standard methods for the examination of water and wastewater. 21st Edition. Washington, DC: American Public Health Association. 2005, 1368.
11. Brinck P. A revision of the Gyrinidae (Coleoptera) of the Ethiopian region II. 1956; 14:62.
12. ASTM (American Society for Testing and Materials) 'Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians', in 2000 Annual Book of ASTM Standards West Conshohocken, PA: Author, 2000; 11(5):213-233.
13. APHA (American Public Health Association) (1995), Standard Methods for the Examination of Water and Wastewater, Washington, DC: Author.
14. Finney DJ. Probit Analysis (3rd ed.), London: Cambridge University Press. 1971, 187.
15. Seiyaboh EI, Inyang IR, Gijo AH, Adobeni GD. Acute Toxicity of Paraquat Dichloride on Blood Plasma Indices of Clarias gariepinus. IOSR Journal Of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT) e- ISSN: 2319- 2402, p- ISSN: 2319-2399. 2015; (7):15-17.
16. Fajana HO, Amusan BO, Koleosho A, Owojori O. Acute toxicity of a household bleach, sodium hypochlorite, to a whirligig beetle Orectogyrus alluaudi Régimbart, 1889 (Coleoptera: Gyrinidae), *Aquatic Insects*, DOI:2017; 10.1080/01650424. 2017.1326614.
17. Lalruatfela PL, Saminathan M, Ingole RS, Dhama K, Joshi MV. Toxicopathology of Paraquat Herbicide in Female Wistar Rats. Asian Journal of Animal and Veterinary Advances. 2014; 9:523-542.
18. Ayanda OI, Oniye SJ, Auta J, Ajibola VO. Acute toxicity of glyphosate and paraquat to the African catfish (*Clarias gariepinus*, Teugels 1986) using some biochemical indicators, Tropical Zoology. 2015; 28(4):152-162.
19. Rand GM, Petrocelli SR. Eds. Fundamentals of Aquatic Toxicology: Methods and Applications. Hemispheres Publishing, New York, 1985, 374-415.
20. Furlan L, Kreutzweiser D. Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. Environmental Science and Pollution Research. 2015; (22):135-147. doi: 10.1007/s11356-014-3628-7.
21. Woodcock BA, Isaac NJB, Bullock JM, Roy DB, Garthwaite DG, Crowe A *et al*. Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nature Communications. 2016; 7:12459. doi: 10.1038/ncomms12459.
22. Antwi FB, Reddy GVP. Toxicological effects of pyrethroids on non-target aquatic insects. Environmental Toxicology and Pharmacology. 2015; 40(3):915-923.
23. Pickford KA, Thomas-Jones RE, Wheals B, Tyler CR, Sumpter JP. 'Route of Exposure Affects the Oestrogenic Response of Fish to 4-Tert- Nonylphenol', Aquatic Toxicology. 2003; 65(3):267-279.
24. Tang JX, Siegfried BD. Bioconcentration and uptake of a pyrethroid and organophosphate insecticide by selected aquatic insects. The Bulletin of Environmental Contamination and Toxicology. 1996; 57:993-998.
25. Fafioye OO, Fagade SO, Adebisi AA. Toxicity of Raphia vinifera, P. beauv fruit extracts on biochemical composition of Nile Tilapia (Oreochromis niloticus, Trewavas). Biokemistri. 2005; 17:137-142.
26. Ayoola SO. Histopathological Effects of Glyphosate on Juvenile African Catfish (*Clarias gariepinus*). American-Eurasian Journal of Agricultural & Environmental Sciences. 2008; 4:362 367.
27. Hayasaka D, Suzuki K, Nomura T, Nishiyama M, Nagai T, Sánchez-Bayo F *et al*. Comparison of acute toxicity of two neonicotinoid insecticides, imidacloprid and clothianidin, to five cladoceran species. 2013; 38:44-47. doi: 0.1584
28. Hirthe G, Fisher TC, Crane M, Callaghan A. Short-term exposure to sub-lethal doses of lindane affects developmental parameters in *Chironomus riparius* Meigen, but has no effect on larval glutathione-S-transferase activity. Chemosphere. 2001; (44):583-589.