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**Effect of sublethal concentrations of Malathion on the
behaviour of *Heteropneustes fossilis***

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Abstract

The present study was performed to investigate the impact of sublethal concentrations of malathion on different behavioural aspects of *Heteropneustes fossilis*. LC₅₀ of malathion for 96 hrs by static bioassay test was found to be 21.34 µl/L. The fishes were exposed to three different sublethal concentrations (2.13 µl/L, 1.07 µl/L and 0.71 µl/L) for 21 days. Malathion exposure caused decrease in resting period, increase in opercular movement and air gulping, increase in S-jerk, threat and burst swimming behaviour. Slow response during feeding, swimming on the upper surface of water hanging vertically, spreading throughout aquarium was also observed in pesticide treated fishes. In the highest concentration i.e. 2.13 µl/L mortality of fishes occurred. Fishes died with bending of the mid section of the body and opening mouth. Fishes lay down motionless at the bottom of the aquarium before death. Fading of body colour was also observed in malathion treated fishes. These results suggest that sublethal concentrations of malathion has negative impact on the behavior of *Heteropneustes fossilis*.

Key-Words: Malathion, *Heteropneustes fossilis*, Behaviour, Sublethal concentrations

Introduction

Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment: (Little and Brewer 2001). Alterations in fish behavior, particularly in non-migratory species can provide important indices for ecosystem assessment. Any change in the behavior of fish indicates the deterioration of water quality, as fish are the biological indicator and hence index of environmental suitability and the cost of survival (Halappa and David, 2009). Pesticides in sublethal concentrations present in aquatic environment are too low to cause rapid death directly but may affect the functioning of the organisms, disrupt normal behavior and reduce the fitness of natural population (Susan *et al.*, 2010). Malathion is a non-systematic, wide spectrum organophosphate insecticide. This is used for agricultural and nonagricultural purposes. Once malathion is introduced into the environment, usually from spraying on crops, droplets of malathion in the air fall on soil, plants, water or manmade surfaces. While most of the malathion will stay in the areas where it is applied, some can move to areas away from where it was applied by rain, fog and wind. In water malathion breaks down quickly by the action of water and bacteria in the water (Patil and David, 2008).

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Malathion may cause serious intimidation to the aquatic organisms and is notorious to cause severe metabolic disturbances in non-target species like fish (USEPA, 2005). Pesticidal impact on the life of aquatic organisms is often acute resulting in mass mortality or chronic changes in behaviour. Such behavioural alterations are very sensitive indicators of stress imposed on fish by the environment (Cooke *et al* 2000). Fish behavior under stress conditions provides important information for aquaculturists (Kristiansen *et al* 2004). Methods of monitoring and quantifying the behavioural response have become potential alternatives for assessing stress, disease, water pollution and toxic material in water (Kane *et al* 2004). In the present paper an attempt has been made to study the toxic effect of different sublethal concentrations of malathion on behavioural parameters of *Heteropneustes fossilis* for 21 days.

Material and Methods

Live specimens of *Heteropneustes fossilis* (10-12 cm in length and 4-6 gm in weight) were collected locally. Fishes were washed with 0.5% KMnO₄ solution for five minutes to remove external infections and then acclimated in dechlorinated tap water under laboratory condition for a period of 15 days prior to experiment. The physico-chemical characteristics of water which was used for acclimation and experiment were – pH 6.6±0.06, alkalinity 5.52±0.56 mg/l, dissolved oxygen 10.5±0.18 mg/l, temperature 27±0.22°C and free

carbon dioxide 4.32 ± 0.88 mg/l. Commercial fish food was given to fishes. The three sides of the aquarium and bottom were made opaque by placing white thermocol sheets to avoid the mirror image of test organism and visual disturbances (Nimila and Nandan, 2010). Three different sublethal concentrations $2.13 \mu\text{L/L}$, $1.07 \mu\text{L/L}$ and $0.71 \mu\text{L/L}$ ($1/10^{\text{th}}$, $1/20^{\text{th}}$ and $1/30^{\text{th}}$ of 96 hr LC_{50}) were taken for behavioural studies. Group of ten fishes along with one replicate were exposed to these three concentrations. Control was also maintained throughout the experiment. During the experiment fishes were fed once a day and uneaten food was removed 30mins after feeding. Water exchange was made at three day intervals with fresh test solution in each experimental aquarium. Resting period between each swimming action was calculated in seconds (Gupta and Dua 2010). Opercular movement was recorded for one minute and gulping of air calculated for fifteen minutes (Gupta and Dua 2010). S jerk i.e. movement of body sequentially from head to tail, threat i.e. movement of a fish towards another fish and burst swimming i.e. sudden and rapid movements (Nimila and Nandan 2010; AL-Aker and Shamsi 2000) were recorded for 15 mins. In addition to the above mentioned behavioural parameters some other general activity like food sensitivity, habit, death, body position and body colour of the fishes were also recorded throughout the experiment. The frequency of occurrence of different behavior was counted for 10 fishes together. All the parameters were recorded for 21 days and the results of the observations are expressed in mean values and standard deviations.

Results and Discussion

Changes in behavioural responses of fishes started 30mins after dosing. Fishes exposed to malathion showed speedy movements as compared to control. This resulted in decrease in resting period. The normal resting period between each swimming action in control was 252.02 ± 2.88 seconds whereas this period decreased with an increase in pesticide concentrations. This increase in swimming activity may be due to disruption of shoaling behavior which occurs because of the stress of the toxicant (Venkata *et al.*, 2008). Fast swimming was also observed by Yaji *et al.* (2011) in *Oreochromis niloticus* treated with Cypermethrin. Similar observation found by Ramesh and Saravanan (2008) in *Cyprinus carpo* exposed to Chlorpyrifos. The opercular movement per minute showed increasing trend with the increase in concentration of toxicant. Under toxic condition the oxygen supply becomes deficient and so the fish breathe rapidly (Susan *et al.*, 2010). Increase in opercular movement has also been reported by Omitoyin *et al.*, (2006), Koprucu *et al.*,

(2006) and Srivastava *et al.*, (2010) in *Clarias gariepinus*, *Silurus glanis*, and *Heteropneustes fossilis* exposed to Lindane, Diazinon and Dimethoate respectively. Rapid opercular movement was also confirmed by Wasu *et al.*, (2009) in *Clarias batrachus* treated with Carbaryl and malathion. Shivakumar(2006) also observed increased opercular movement in *Cyprinus carpio* exposed to endosulfan, cypermethrin and fenvalerate. Malathion exposure caused hypoxia which was reflected in the number of air gulps per 15 mins. Number of air gulps increased from 1.15 ± 0.36 in control group to 5.76 ± 0.52 , 3.61 ± 0.50 and 2.52 ± 0.45 in the group exposed to malathion. Gulping of air may help to avoid contact of toxic medium (Katja *et al.*, 2005). Similar observation has been reported by Patil and David (2008) in malathion treated fish *Labeo rohita* and by Parithabhanu (2013) in cypermethrin treated *Oreochromis mossambicus*. Movements like S jerking, threat and burst swimming were increased in the experimental fishes when exposed to malathion. Similar observations were reported by Nimila and Nandan (2010) in *Etroplus maculatus* when it is treated with lindane and by AL-Akel (2000) in carbaryl treated fishes. S jerk and burst swimming were also observed by Marigoudar *et al.* (2009) in *Labeo rohita* exposed to cypermethrin. In this experiment food intake was slow in malathion treated group than in control group. Reduction in feeding behavior under toxic environmental condition might be profitable to lower the energetic costs of digestion (Halappa and David, 2009). This finding agreed with the study of Halappa and David (2009) where *Cyprinus carpio* was exposed to chlorpyrifos. The fishes of control and the lowest concentration of malathion i.e $0.71 \mu\text{L/L}$ were calm and quiet and preferred to confine themselves to the bottom of the aquarium whereas $2.13 \mu\text{L/L}$ and $1.07 \mu\text{L/L}$ malathion treated fishes were found active and mostly swimming near the upper surface of water and also found hanging vertically most of the time in water. These findings can be correlated with the findings of Narwaria and Saksena (2012). Surfacing phenomenon i.e. significant preference for upper layer in exposed group might be a demand for higher oxygen level during the exposure period (Katja *et al.*, 2005). Similar observations reported by Halappa and David(2009) and Shivakumar(2006) where *Cyprinus carpio* was treated with Chlorpyrifos, endosulfan, cypermethrin and fenvalerate. Hanging vertically i.e. loss of equilibrium may be due to inhibition of acetylcholine secretion (Patil and David, 2008). Similar behavioural alteration has been reported by Ramesh and Saravanan (2008), Susan *et al.*, (2010), Yaji *et al.*, (2011) in

Cyprinus carpio, majorcarps and *Oreochromis niloticus* exposed to Chlorpyrifos, Fenvalerate and Cypermethrin respectively. Mortality also occurred in the highest concentration. Death of fish that resulted during the exposure period might be due to the inhibition of acetylcholine at the nerve ending in CNS and PNS (Shivakumar *et al.*, 2006). In this experiment fishes died with bending of the mid section of the body. Inhibition of acetylcholine during exposure to malathion can cause contraction of trunk muscles (Areechon and Plumb, 1990). Similar observation has been reported in *Oreochromis niloticus* and *Cyprinus carpio* exposed to malathion and chlorpyrifos by Carifio and Capinin(1993) and Halappa and David(2009). Before death fishes found lying down motionless at the bottom of the aquarium. Koprucu *et al.*, (2006) found the same behavior in Diazinon treated *Silurus glanis*. Most of the time fishes died by opening their mouth. Death with opening mouth was also found in *Labeo rohita* and some major carps exposed to malathion and fenvalerate by Patil and David (2008) and Susan *et al.*, (2010). Fishes of control always wanted to stay together whereas malathion treated fishes occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. Similar observation was confirmed by Marigoudar *et al* (2009) and Dube and Hosetti (2010). In this experiment fishes of 2.13µl/L concentration of malathion showed fading of their body colour from 14th to 21st day of experiment. Impact of pesticides on the body colour of fish has also been reported by Omitoyin *et al.*, (2006), Koprucu *et al.*, (2006), Wasu *et al.*, (2009) in fishes like *Clarias gariepinus*, *Silurus glanis* and *Clarias batrachus* exposed to different pesticides.

Conclusion

On the basis of the observations made in the above study it is possible to conclude that impact of malathion on fish behavior is dose dependent. As the concentration increases the number of behavioural alterations also increases. The sublethal concentrations of malathion may adversely affect the survivality of the fish in their natural environment. So care should be taken regarding dosage and time of application when malathion is used for pest control in agricultural field or tea gardens surrounding the aquatic bodies.

References

1. Al-Aker A.S. and Shamsi M.J.K. (2000) Comparative study of toxicity of carbaryl and its impact on the behaviour and carbohydrate metabolism of cichlid fish, *Oreochromis niloticus* (Linnaeus, 1758) and catfish *Clarias*

- gariepinus* (Burchell, 1822) from Saudi Arabia. *Egypt. J. Aquat Biol and Fish* 4(2), 211-227.
2. APHA: Standard methods for examination of water and waste water. 19th ed. American Public Health Association, Washington (1998).
3. Areechon N. and Plumb J.A. (1990) Sublethal effects of malathion on channel catfish *Ictalurus punctatus*. *Bulletin of Environmental Contamination and Toxicology* 44, 435-42.
4. Carifio Virginia S. and Capinin Emmanuel C (1993) Toxicity of Malathion to Nile Tilapia *Oreochromis niloticus* (Linn.) fingerlings. *Science Diliman* 5(2), 51-55.
5. Cooke S.J., Chandroo K.P., Beddow T.A., Moccia R.D. and McKinley R. S. (2000) Swimming activity and energetic expenditure of captive rainbow trout. *J. Appl. Ecol* 13, 12-23.
6. Das B.K. and Mukherjee S.C. (2000) A histopathological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. *VETERINARSKI ARHIV*. 70(4), 169-180.
7. Dube P.N. and Hosetti B.B. (2010) Behaviour surveillance and oxygen consumption in the freshwater fish *Labeo rohita* (Hamilton) exposed to sodium cyanide. *Biotechnology in Animal Husbandry* 26(1-2), 91-103.
8. Gupta N. and Dua A. (2010) Mercury induced behavioral alterations in *Channa punctatus*. *Poll Res.* 29(4), 721-723.
9. Halappa R. and David M. (2009) Behavioural responses of the freshwater fish, *Cyprinus carpio* (Linnaeus) following sublethal exposure to chlorpyrifos. *Turk. J. Fish. Aquat. Sci.* 9, 233-238.
10. Jensen C.S., Garsdal L. and Baatrup E. (1997) Acetylcholinesterase inhibition and altered locomotor behavior in the carabid beetle *Pterostichus cupreus*. A linkage between biomarkers at two levels of biological complexity. *Environ. Toxicol. Chem.* 16, 1727-1732.
11. Joshi N., Dharmalata and Sahu A.P. (2007) Histopathological changes in liver of *Heteropneustes fossilis* exposed to cypermethrin. *J. Environ. Biol.* 28(1), 35-37.
12. Kane A.S., Salierno J.D., Gipson G.T., Molteno T.C.A. and Hunter C.A. (2004) Video based movement analysis system to quantify behavioural stress response of fish. *Water Res.* 38(18), 3993-4001.
13. Katja S., Georg B.O.S., Stephan P. and Christian E.W.S. (2005) Impact of PCB mixture (Aroclor 1254) and TBT and a

- mixture of both on swimming behavior, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). *Aquat.Toxicol.* 71, 49-59.
14. Koprucu S.S., Koprucu K., Ural M.S., Ispir U. and Pala M. (2006) Acute toxicity of organophosphorus pesticide Diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochemistry and Physiology* 86, 99-105.
 15. Kristiansen T.S., Ferno A., Holm J.C., Privitera L., Bakke S., and Fosseidengen J.E. (2004) Swimming behavior as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus* L.) reared at three stocking densities. *Aquaculture*, 230 (1-4), 137-151.
 16. Little E.E. and Brewer S.K. (2001) Neurobehavioral toxicity in fish. In *Target Organ Toxicity in Marine and Freshwater Teleosts New Perspectives: Toxicology and the Environment*. Vol. 2. Systems. Schlenk, D. and W. H. Benson (eds). Pp 139-174. Taylor and Francis, London and New York.
 17. Little E.E. and Finger S.E. (1990) Swimming behavior as an indicator of sublethal toxicity in fish. *Environ. Toxicol. Chem.* 9, 13-19.
 18. Marigoudar S.R., Ahmed R.N. and David M. (2009) Impact of Cypermethrin on behavioural responses in the freshwater teleost, *Labeo rohita* (Hamilton). *World J. Zool.* 4(1), 19-23.
 19. Narwaria Y.S. and Saksena D.N. (2012) Acute toxicity bioassay and behavioural responses induced by sodium fluoride in freshwater fish *Puntius sophore* (Bloch). *Fluoride* 45(1), 7-12.
 20. Nimila P.J. and Nandan B. (2010) Effect of organochlorine pesticide lindane (γ -HCH) on behavior of *Etrophus maculatus* (Bloch, 1795). *Poll Res.* 29(4), 577-582.
 21. Omitoyin B.O., Ajani E.K., Adesina B.T. and Okuagu C.N.F. (2006) Toxicity of Lindane (Gamma Hexachloro-CycloHexane) to *Clarias gariepinus* (Burchell 1822). *W.J.Zoology*, 1(1), 57-63.
 22. Parithabhanu A. (2013) Pesticide toxicity and behavioural responses in the fish *Oreochromis mossambicus*. *International Journal of Fisheries and Aquaculture Sciences* 3(2), 161-164.
 23. Patil V. K. and David M. (2008) Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). *Turk. J. Fish. Aquat. Sci.* 8, 233-237.
 24. Ramesh M. and Saravanan M. (2008) Haematological and biochemical responses in a fresh water fish *Cyprinus carpio* exposed to Chlorpyrifos. *Int. J. Integrative Biol.* 3(1), 80-83.
 25. Shivakumar R., Mushigeri S.B. and David M. (2006) Toxicity of Endosulfan, Cypermethrin and Fenvalerate to freshwater fish, *Cyprinus carpio*. *J.Ecobiol.* 19(4), 313-316.
 26. Srivastava A. K., Mishra D., Shrivastava S., Srivastav S. K. and Srivastav A. K. (2010) Acute toxicity and behavioural responses of *Heteropneustes fossilis* to an organophosphate insecticide, Dimethoate. *Int. J. Pharma Bio Sci.* 1(4), 359-363.
 27. Susan A.T., Sobha K. and Tilak K.S. (2010) A study on acute toxicity, oxygen consumption and behavioural changes in the three major carps, *Labeo rohita* (ham), *Catla catla* (ham) and *Cirrhinus mrigala* (ham) exposed to Fenvalerate. *Bioresearch Bulletin* 1, 33-40.
 28. Tadehl H. and Hader D.P. (2001) Automated biomonitoring using real time movement analysis of *Euglena gracilis*. *Ecotoxicol.Environ.Saf.* 48, 161-169.
 29. USEPA. United states environmental protection agency. (2005).
 30. Venkata Rathnamma V., Vijayakumar, M. and Philip, G.H. (2008) Acute toxicity and behavioural changes in freshwater fish *Labeo rohita* exposed to Deltamethrin. *J.Aqua Biol.* 23(2), 165-170.
 31. Wasu Y.H., Gadhikar Y.A. and Ade P.P. (2009) Sublethal and chronic effect of carbaryl and malathion on *Clarias batrachus* (Linn). *J.Appl. Sci. Environ. Manage.* 13 (2), 23-26.
 32. Yaji A.J., Auta J., Oniye S.J., Adakole J.A. and Usman J.I. (2011) Effects of Cypermethrin on behavior and biochemical indices of freshwater fish *Oreochromis niloticus*. *EJEAFChe.* 10(2), 1927-1934.

Table 1: Effect of malathion on different behavioural parameters of *Heteropneustes fossilis*

Parameters	Control	Solvent control	2.13µl/L	1.07 µl/L	0.71 µl/L
Resting period (Seconds)	252.02±2.88	251.24±1.64	184.84±2.15	198.15±4.04	224.93±2.48
Opercular movement (1 min)	43.51±3.03	43.92±2.31	54.84±2.23	51.9±2.17	46.14±3.99
Air gulp (15 mins)	1.15±0.36	1.12±0.31	5.76±0.52	3.61±0.50	2.52±0.45
S-jerk (15 mins)	20.53±1.37	20.07±1.97	35.07±2.06	31.56±1.44	23.05±1.09
Threat (15 mins)	17.43±2.34	16.37±1.96	36.06±1.09	33.32±0.85	26.49±2.02
Burst swimming (15 mins)	26.52±1.70	27.05±1.13	35.43±2.04	32.07±1.77	30.63±1.41
Food sensitivity	Full response	Full response	slow	slow	Slow
Habit	Calm quiet	Calm quiet	Active, mostly swimming	Active, mostly swimming	Calm quiet
Death	No mortality	No mortality	Death with bending of mid section of the body and opening mouth	No mortality	No mortality
Body position	At bottom of the aquarium	At bottom of the aquarium	Mostly swim near the upper surface of water	Mostly swim near the upper surface of water	At bottom of the aquarium
	Horizontal	Horizontal	Mostly vertical	Mostly vertical	Horizontal
	Tend to stay together	Tend to stay together	Spreading throughout the aquarium	Spreading throughout the aquarium	Spreading throughout the aquarium

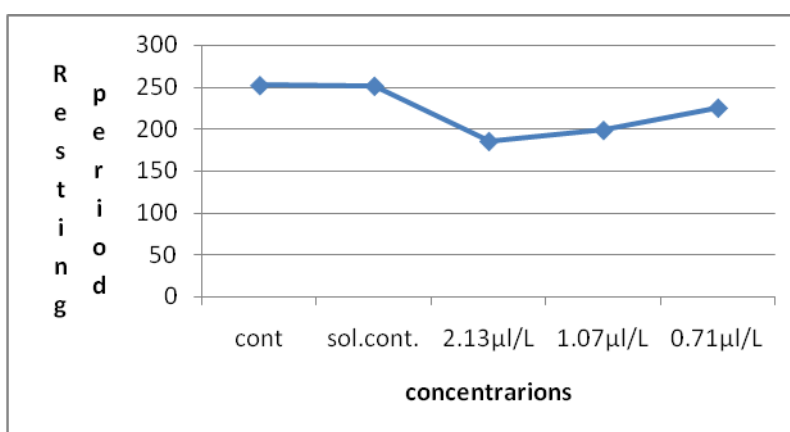


Fig. 1: Average frequency of resting period during exposure to malathion

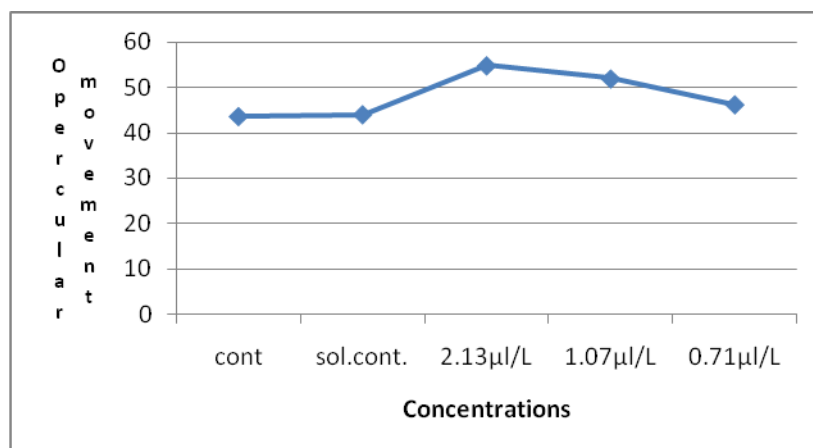


Fig. 2: Average frequency of opercular movement during exposure to malathion

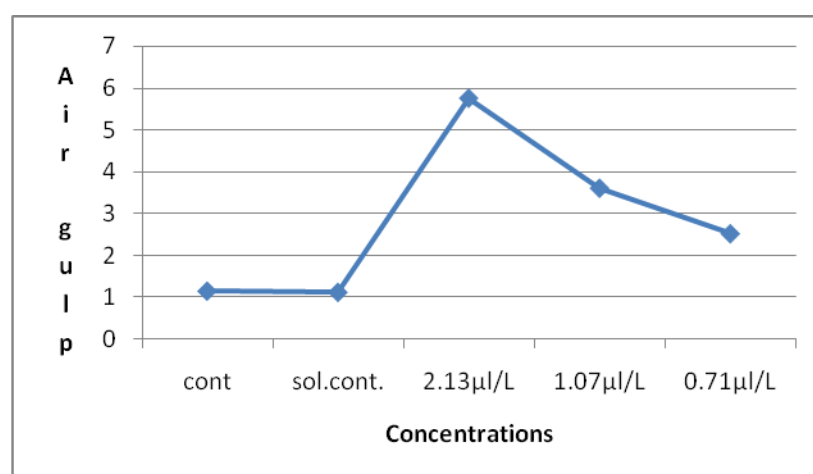


Fig. 3: Average frequency of air gulp during exposure to malathion

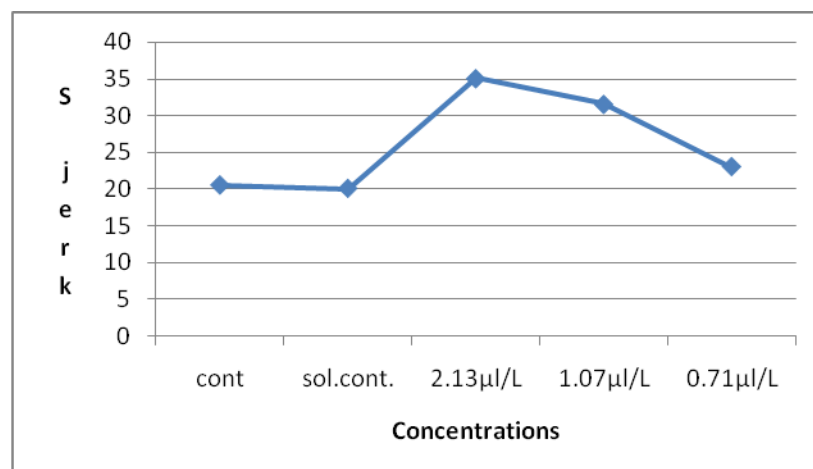


Fig. 4: Average frequency of S jerk during exposure to malathion

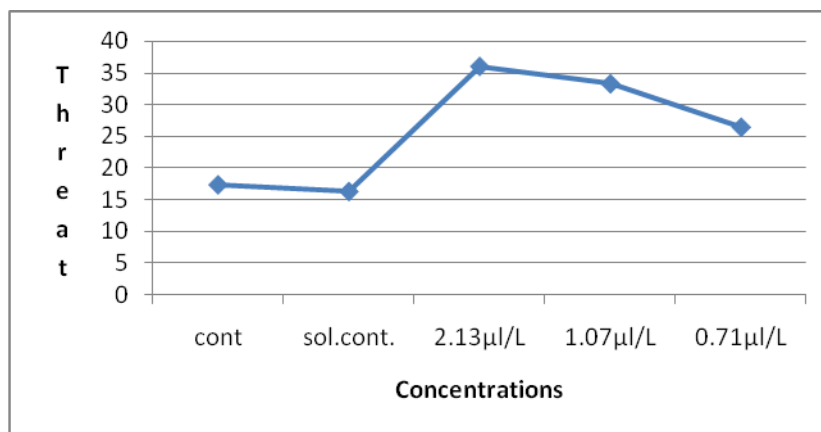


Fig. 5: Average frequency of threat during exposure to malathion

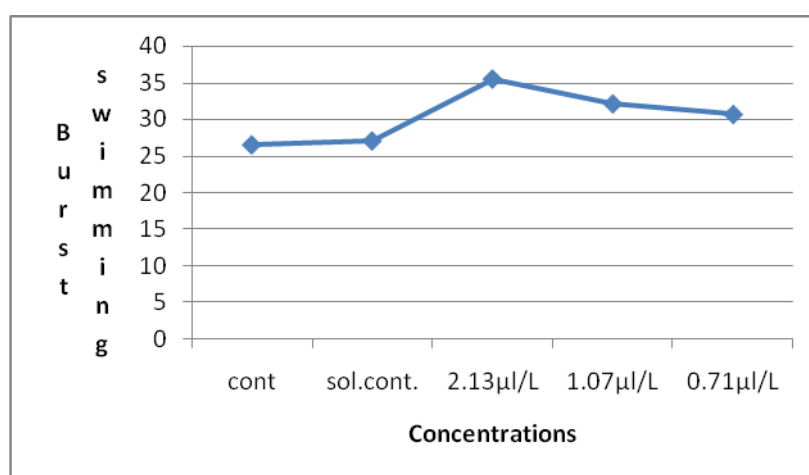


Fig. 6: Average frequency of burst swimming during exposure to malathion

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