RESEARCH PAPER | Environment | Volume : 3 | Issue : 3 | March 2013 | ISSN - 2249-555X

Acute Toxicity and Behavioral Effects of Neonicotinoid Insecticide Formulation in Paratelphusa (Barytelphusa) Jacquemontii (Rathbun)

KEYWORDS: acute toxicity, insecticide, Neonicotinoid, crab.

V. N. Patil
Department of Environmental science, Shivaji University, Kolhapur (MH)- 416004

P. D. Raut
Department of Environmental science, Shivaji University, Kolhapur (MH)- 416004

ABSTRACT *Toxicological effects of synthetic chemicals such as pesticides are becoming apparent due to their indiscriminate use. Pesticides are presenting as pollutants of biologically active nature in all the ecosystems. Increasing evidences of their potential hazards to human and wildlife are suggesting importance of their toxicity studies. Neonicotinoid (NNT) is a novel class of insecticides used world wide. Similarly these are found to be associated with mass death of honey bees in many countries. Aquatic organisms also get exposed to these class of insecticides as these insecticides are used on crops grown in riverine region. Present study was carried to determine acute toxicity and to assess behavioral effect of a commercial formulation 'Admire' NNT group insecticide on freshwater crab. LC0, LC50 and LC100 values for 96 h of this insecticide were observed to be 4.38, 37.12 and 100.46 ppm respectively. Significant behavioral changes were observed with increase in dose concentration.*

Introduction:
Present day intensive farming for food and heavy industrialization for production of goods to meet the need of growing population has led to problem of pollution. Environmental pollutants are becoming toxicants due to their adverse effects on living beings. Toxicity studies of pollutants in environment are gaining immense importance. Some of the examples of xenobiotics are toxic chemicals used in industries, substances from wastewater, heavy metals in different applications, and contaminants in air, water and soil those are released by human activities but not present in natural condition. Toxicity of xenobiotics is complex and dosage is most important factor which determines behavior and extent of toxicity to the exposed organism. These xenobiotics may unrecoverable harm to organisms coming in their contact. Toxicity effect of these toxicants varies depending upon route of exposure, dosage and status of target organism. Pesticides and heavy metals used in their manufacture are dangerous and abundant environmental pollutants. They enter aquatic water bodies via industrial and agricultural activities. Human may get exposure to these through food and other media. Shrinavasa and Bhadane, (2002) have observed trace metals like Cd, Hg, Cr etc. in sheep and buffalo milk grazing in industrial area.

Chemical pesticide is common approach in pest control in agriculture and horticulture (Suryavanshi et al., 2009). Aquatic pollution due to pesticide residues has seriously affected the living resources. Pesticides make their ways to nearest water bodies in the area of their excess application. Bhatnagar et al., (1992) have reported that 70% agrochemicals potentially affect non target organisms in rivers, estuaries and aquaculture ponds. They may accumulate in living organisms in the form of residues without showing harmful effects on them. They affect fishes, crustaceans and other animals. Knowledge regarding mechanism of reacting and recovering of aquatic communities from pesticide particularly residue is lacking despite of its importance in environmental impact assessment of pesticides (Hayasaka et al., 2012). These organisms get direct exposure to these pesticides. Wannee et al., (2002) has reported that operational formulations are more toxic than technical formulations and are directly used by farmers in the field. This suggests importance of toxicity study of commercial grade pesticides over analytical grades.

Neonicotinoid (NNT) is a novel and major chemical class of insecticide. Neonicotinoids are a class of pesticide compounds registered on a wide variety of commercial agricultural crops. It is a group of neurotoxic insecticides got developed in past three decades replacing concurrent banned Organochlorine and some Organophosphorous group pesticides. These have great potential of crop protection with systemic action. Insecticides among this class are effective against piercing and sucking insects and fleas on cats and dogs hence these have both agriculture and domestic application. Admire is a commercial product of this class containing Immidacloprid as an active ingredient. It is flowable insecticide used to control insect pest on crops like rice, strawberries and also on vegetables grown in riverine region of our country. EPA registration code of this commercial product is 264-758 and has got approved in June of the year 2003. This product is introduced in India in the year 2006 and became most used insecticide in short span of time. Arthropods such as honeybees, parasitic wasps, and predaceous ground beetles are sensitive to Immidacloprid which is active ingredient of insecticide under the study. Federoff, (2008) has stated that testing is required for immidacloprids having outdoor uses. It can enter aquatic body by runoff and it is necessary to study the fate of immidacloprids in soil and water. Feng et al., (2007) has stated that there is need of toxicity studies of imidacloprid containing agrochemicals on non target organisms. In the present study insecticide from NNT class i.e. Admire was used in present investigation as it is widely used product against insect control on vegetables grown on the banks of the river in the locality. Test organism used is fresh water crab Paratelphusa (Barytelphusa) jacquemontii (Rathbun) which is important food value and at the same time these are exposed to agrochemicals. Decapod crustaceans are threatened by wide range of pesticides contaminating aquatic environment (Kaushik and Kumar, 1998).

Present paper reports acute toxicity effect of NNT pesticide in freshwater crab Paratelphusa (Barytelphusa) jacquemontii (Rathbun).

Material and methods:
An insecticide of NNT group named Admire was made available from an agro chemical shop. Commercial formulation of insecticide was preferred as it is directly used by farmers in riverine area. Fresh water crab Paratelphusa (Barytelphusa) jacquemontii (Rathbun) was obtained from local fish market. Crabs were brought to laboratory in clean plastic troughs covered with non toxic rust proof mesh by taking care that they should not get physical and mechanical stress. Water level in the aquaria was maintained for submergence of animals. Healthy crabs weighing up to 100 gm and carapace length about 8 to 10 cm were selected in six groups of ten in each. Animals were acclimatized for few days with maintaining natural photo period. Range finding was carried by exposing suitable concentration based on available litera-

Volume : 3 | Issue : 3 | March 2013 | ISSN - 2249-555X

ture. Desired concentrations of insecticide were prepared in tap water and simultaneously control set was to run to assure mortality in subsequent test period. Crabs were fed with dried shrimp and chicken liver. Water in containers was changed twice a day with addition of fresh concentrations of insecticide. Bioassay was carried according to the method described by Subrarnanian, 2001. Behavior change and death were two main end point parameters studied in present acute toxicity study. Behavioral responses of crabs were noted continuously and at the intervals of 24 hrs. They were compared with control to observe abnormal responses. Mortality was assured with isolating crab from container to differentiate narcotic effect and death. Mortalities at each concentration after completion of every 24hrs were noted. The data obtained from bioassay was evaluated by Probit method (Finney, 1964) to determine lethal values of concentration. Confirmation of the values obtained was also carried with Beheren Karber's method of cumulative toxicity assessment formulae. Trimmed Spearman-Kaber computer program (v 1.5) developed by Ecological monitoring research division of USEPA was also used to check correspondence of MLC for value for 96 hrs.

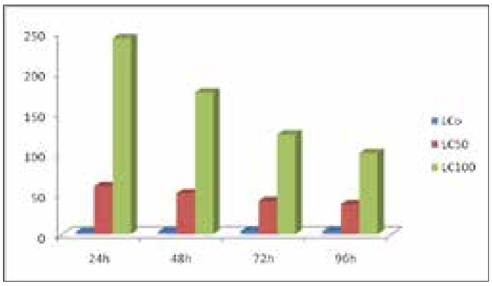
Result and discussion:
No mortality was observed in the control group during the experiment.

Sr. No.	Duration in hrs.	LC_{50} ppm	'f' value	95% UCL	95% LCL	R^2
1	96	37.12 ±5.61	1.7600	65.33	21.09	0.967
2	72	41.40 ±8.40	1.7947	74.30	23.06	0.939
3	48	50.57 ±12.50	1.8539	93.75	27.27	0.852
4	24	59.67 ±16.53	1.9089	113.90	31.25	0.757

Table-1. Median lethal concentration values of NNT insecticide to Paratelphusa (Barytelphusa) jacquemontii (Rathbun)
Approximate standard error was calculated by using Miller and Tainter, (1944) as

ASE= LC84-LC16/√2N
Where,

LC84 and LC16 are calculated for respective durations from linear equations N is number of animals in each group i.e.10



LC0, LC50 and LC100 values of NNT insecticide to Paratelphusa (Barytelphusa) jacquemontii (Rathbun)
In addition 24,48 and 96h LC50 values also evaluated according to Beherin and Karber method (Klassen,1991) with following formula

LC50 = LC100- ∑ab/n

Where, 'a' is difference between two successive concentrations 'b' is AM of mortality due to respective concentrations

Duration (hrs)	∑ab/n	LC_{100}- ∑ab/n i.e. (LC_{50} by Behren Karber's method) ppm	LC_{50} by Finney's probit method (ppm)
24	43	57.46	59.67
48	49.89	50.57	50.46
96	64.34	36.12	37.12

Table-2. Correspondence of LC50 values by Finney's method (1964) and Behrin Karber's method (Klassen,1991)
These two methods are found to be in good correspondence for medial lethality values in the present study. 96 hrs. LC50 by probit analysis method (37.12 ppm) has shown conformity with 95% upper limit of Spearman Karber method of median lethality calculation i.e. 37.64 ppm.

Behavioral changes in animal are important parameter of toxicity study (Richmond and Dutta, 1992). Dose and duration dependent behavior responses were observed in exposed animals and they were compared with no toxicity of animals in control. These are summarized in table 3.

Sr. No.	Group	24 h	48 h	72 h	96 h
	Control	no change	no change	no change	no change
1	5 ppm	slight narcotism	narcotic effect but regained	Prolonged narcotism but resuming	remained active
2	25 ppm	Excited, slight narcotism	Less aggressive, fast movement of mouthparts	Movement of mouthparts, muscle movement stopped	movement slowed, least aggressive
3	50 ppm	more excited, mouthparts movement continuous	reduced consumption, became sedentary	feeding stopped respiration from water surface	appendages curved,
4	70 ppm	more excited, feeding stopped	Least aggressive, appendages spread, less sensitive	Sluggish, unable to revert when inverted accidently	remained inverted, muscular spasm
5	100 ppm	most excited, quick movement of mouthparts	Mouth parts movement slowed, appendages spread	muscle and mouthparts movement totally stopped, sensation lost	maximum death observed

Table-3. Time and dose dependent behavioral changes observed in Paratelphusa (Barytelphusa) jacquemontii (Rathbun) exposed to NNT formulation
Animals from control group were observed with no any behavioral changes. Observations of groups indicated that insecticide Admire has caused abnormal changes in overall responses. Behavioral response was observed after 1 hour of exposure in all concentration as animals became more active. Initially after exposure animals showed excited movements and at 5 ppm concentration animal underwent slight narcotism from which they were regaining in few minutes after exposure. Animals became sluggish after five hours of exposure period. After completion of 24 hrs from lower to higher concentrations effects observed were more excitation, narcotism, quick movement of mouthparts. After 24 hrs animals from 25 ppm concentration exposure group showed sedentary behavior while 50 ppm and above were observed with muscular spasm. After 72 hrs animals from higher concentration group became sluggish. Vijayvel et al., 2009 have observed no change in behavior of mud crab, Scylla serrata after sub lethal exposure of Nickel with no mortality for 30 days period of exposure.

Crabs were showing excited movement after few minutes of exposure. These altered behaviors are primary indicators of toxic responses. Dimethoate, an organophosphorous insecticide has caused altered behavioral responses like restlessness, hyperactivity, jerky swimming, decline in opercular movement in air breathing fish Channa panctatus (Bloch) (Pandey et al., 2005). In same fish Subathra and Karuppasamy, (2003) has studied behavior changes and mortality after cadmium exposure. Inverted position of the animals was indication end point parameter i.e. death. Animals in higher concentrations were breathing from surface of water and were showing increased activity of mouthparts. Chlorpyriphos an organophosphate pesticide has shown 0.194, 0.169, 0.154 and 0.120 ppm as LC50 concentration for freshwater crab, Spirothelphusa hydroroma in a study carried by Senthilkumar et al., (2007). In the

Volume : 3 | Issue : 3 | March 2013 | ISSN - 2249-555X

present study animals in all concentrations slowed down feeding after 24 hrs. Their response to stimulus slowed down and ceased after 48 hrs almost inn all concentration exposures. Movement of mouth parts is observed to be increased after 24hrs in concentration above 50 ppm. Similarly operculum movement increase in the fish, Channa punctatus is reported by Pandey et al., (2005). Acute toxicity of CdCl2 to water frog, Rana ridibunda (Pallas) was investigated by Mahmut et al., 2003). Slowing down of movement of mouthparts is an effect observed by Pandey, (2009) in case of organophosphate toxicity to air breathing cat fish Heteropneustes fossilis (Bloch). After exposure period of 72 hrs for 70 ppm, animals stopped movement along with loss of sensation. Appendages were seen to be curved at 96 hrs exposure period for 70 ppm and maximum death was confirmed at 100 ppm after same concentration. Insecticide is seen to be toxic in terms of mortality and behavior to the animal under study. Hence, application of this pesticide should be avoided or limited in the areas in the proximity of water bodies.

Conclusion:
It is concluded from the present study that Admire is toxic to freshwater crab, Paratelphusa (Barytelphusa) jacquemontii (Rathbun) both in terms of behavior change and mortality. Behavioral changes were manifested after one hour of exposure which suggests immediate effect of insecticide to species. Study is proved to be in agreement with EPA which has expressed concern that NNT class insecticides are highly toxic to aquatic invertebrates. It draws attention to study metabolic effects of this product on test organism under study.

Acknowledgements:
The authors express their deep sense of gratitude towards the Department of Environmental Science, Shivaji University, Kolhapur for providing laboratory and workplace facility to carry out the present study.

REFERENCE Bhatnagar M.C., Bana A.K. and Tyagi M. (1992) Respiratory distress to Clarial batrachas exposed to Endosulfan A histological approach J. Environmental Biol. 13, pp- 227-231 | Federoff N. E. (2008), EFED problem formulation for the registration of Immidacloprid, USEPA pp-10 | Feng Su, Shicui Zhang, Hongyan Li, Huaron Guo (2007) In vitro acute cytotoxicity of neonicotinoid insecticide imidacloprid to gill cell line of flounder Paralichthy olivaceus, Chinese Journal of Oceanology and Limnology v 25 No. 2, pp-209-214 | Hayasaka D, Korenaga T., Korenaga T, Suzuki K, Saito F, Sánchez-Bayo F, Goka K. (2012) Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms, Ecotoxicology and Environmental Safety, Elsevier, v 80, 2012, pp-355–362. | Kaushik N., Kumar S. (1998) Midgut pathology of Aldrin, Monocrotophos and Carbaryl in freshwater crab, Paratelphusa masoniana Bull. Environmental contamination and toxicology V 60 Springer Verlag, New York pp-480-486 | Mahmut S, Gul A. and Yilmaz M. (2002) Investigation of acute toxicity of Cadmium chloride metal salt on behavioral changes on water frog Rana ridibunda (Pallas), Chemosphere 52, Elsevier pp-259-263. | Miller LC, Tainter M.L.(1944). Estimation of LD50 and its error by means of log-probit graph paper. Proc. Soc. Exp. Bio. Med. 57:261 | Pandey S., Sharma S., Nagpure N.S., Srivastava S.K., Verma M.S., (2005) acute toxicity bioassay of mercuric chloride and malathion on air breathing fish Channa punctatus (Bloch) Ecotoxicol. And Envi. Safety,61 pp-114-120 | Pandey R. K. Singh R. N. Singh S. N. Singh N.N. Das V. K., (2009) Acute toxicity bioassay of dimethoate on freshwater air breathing catfish, Heteropneustes fossilis (Bloch), Journal of Environmental Biology, Triveni Enterprises, Lucknow (India), 30(3) pp-437-440 | Richmonds, C., Dutta, H.M. (1992) Effect of malathion on the optomotor behavior of bluegill sunfish, Lepomis macrochirus.Comp. Biochem. Physiol. 102, 523–526. | Senthilkumar P. Samayappan K. Jaykumar and Deccaraman M. (2007) Impact of Chlorpyriphos on the neurosecretory cells in freshwater field crab Spirothelphusa hydrodroma, Research J of Agri. And Biol. Sciences 3(6) INSInet publication pp-625-630 | Shrinivastava and Bhadane (2002) Effect of industrial waste on the concentration of trace metals in milk of sheep and buffalo Cheml. Environ Res, 11 pp-293-296 | Subatra and Karuppasamy (2003) Bioassay evaluation of toxicity levels of Cd on mortality and behavior responses of air breathing fish Channa punctatus (Bloch), journal of experimental Zool., India, pp-245-250 | Subramanian M. A., (2003) Toxicology principles and methods, MJP pub. Chennai, India pp-114 | Suryavanshi U. Sreepada R. A., Ansari Z. A., Nigam S., Badesab S., (2009) A study on biochemical changes in the penaeid shrimp, Metapenaeus monoceros (Fabricius) following exposure to sublethal doses of organochlorine pesticide (endosulfan), Chemosphere, vol.77(11) pp-1540-1550 | USEPA(1993) User's guide of Trimmed Spearman-Karber (TSK) program version 1.5,Ecological Monitoring Research division, Cincinnati, Ohio 45268 | Vijayvel K., Gopalkrishnan S., Thiagarajan R. and Thilagam H., (2009) Immunotoxic effects of nickel in the mud crab Scylla serrata, Elsevier (26) pp-133-139 | Wannee Jiraungkoorskul, E. Suchart Upathama, Maleeya Kruatrachu, Somphong Sahaphong, Suksiri Vichasri-Grams and Prayad Pokethitiyook, (2002) Histopathological effects of Roundup, a glyphosate herbicide on Nile tilapia (Oreochromis niloticus), Science Asia (28), ISSN 1513-1874, Science society of Thiland, pp-121-127.