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INCIDENCE OF MENTUM DEFORMITY OF CHIRONOMID LARVAE (DIPTERA: CHIRONOMIDAE) PREVALENT IN RICE AGRO-ECOSYSTEM OF HOOGHLY DISTRICT, WEST BENGAL: A SIGN OF PESTICIDE INDUCED STRESS?

DEBNARAYAN SAHA[1*] AND ABHIJIT MAZUMDAR[2]
[1,2]Entomology Research Unit, Department of Zoology,
The University of Burdwan, Burdwan - 713104, West Bengal, INDIA
[1]Raja Rammohun Roy Mahavidyalaya, Radhanagar, Hooghly - 712 406, West Bengal, INDIA.
e-mail: debnarayansaha@gmail.com

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***Corresponding author**

ABSTRACT
The mouthpart deformities of *Chironomus* sp. (Diptera: Chironomidae) larvae, particularly in the mentum teeth, have been proposed as a bioindicator of sediment quality and environmental stress. Larvae of *Chironomus* sp. were collected from rice fields located at Rishra, Serampore and Khanakul (District- Hooghly, West Bengal, India). Physico-chemical parameters of water and sediment were recorded at each site. The agronomic practices revealed an excessive application of pesticides in the rice field for better yield. Field data exhibit high incidence that percentage of deformity in Rishra compared with Serampore and Khanakul. Laboratory experiments demonstrated a correlation between field concentration of pesticides and occurrence of deformed larvae. Among the various head capsules structures, deformity of the mentum was strongly reflective of pesticide induced environmental stress.

INTRODUCTION

Mouthparts deformity of Chironomid larvae represents more traditional and successful methods for biological assessment of water quality. The relationship between morphological deformities and the occurrence of heavy metals and pesticides within the sediment was reported by Hamilton and Sæther (1971). Bhattacharya *et al.,* (1999) recorded high incidence of deformity of mouthparts of Chironomid larvae occurring within the river Damodar flowing through the industrial zone of West Bengal. Deformities could provide a useful tool for assessing aquatic pollution, specifically that relates to industrial wastes and agricultural runoff (Saha and Mazumdar, 2013 Wiederholm, 1984; Warwick, 1985; Janssens de Bisthoven *et al.,* 1999; Vermeulen, 1995; Bhattacharya *et al.,* 2005, MacDonald and Taylor, 2006 and Al-Shami *et al.,* 2010). The occurrence of natural abnormalities must be considered when using chironomid mouthpart deformties as an index of environmental degradation (Bird, 1997). Efforts to demonstrate the relationship of deformity incidence in chironomid larvae with water and sediment quality have been made (Vermeulen, 1995). Hamalainen (1999) suggest that deformity incidence of chironomid larvae was a potential indicator of the impact of contaminants in aquatic systems. The application of chironomid deformities as bioindicators of pollution stress has been reviewed and shown primarily for bioassessment purposes (Midya *et al.*, 2013, Bhaduri *et al.*, 2011, Amiryan, 2010 and Rosenberg, 1992). In this context, many researchers have developed several indices deformity based on chironomid larval deformities to better understand the causes (Hamalainen, 1999).

Chironomids are most important macroinvertebrates in rice agro-ecosystem, they are most widespread and often abundant group of insects in freshwater environments (Pinder, 1986). The members of genus *Chironomus* sp. is considered ecologically versatile due to colonizing ability in most varied conditions and increase response to organic enrichment (Stevens *et al.,* 2006). Chironomids were considered as one of the most useful groups in assessing the quality of running waters because of their abundance, diversity and colonizing ability (Sæther, 1980).

The chironomids have been recorded in rice fields throughout the world including India, Australia and the USA (Stevens *et al.,* 2006). The rice field is a unique man-made environment with huge diversity of aquatic organisms and this diversity are closely related to the environmental changes of rice agro-ecosystem (Saha and Mazumdar, 2013 and Ali, 1996). Fernando (1993) reported that presently rice cultivation is highly mechanized and uses high fertilizer and pesticide for enhance production. Routine agricultural practices including ploughing, draining, fertilizer and pesticide applications and wet and dry climate cycles influenced the diversity of aquatic community. Generally, rice fields show a wide variation of water parameters such as temperature, pH, dissolved oxygen, conductivity, nitrate and phosphate (Lim, 1990). Rice fields are dynamic systems of particular environmental concern because they exhibit a high capacity to concentrate and deliver pollutants.

Pesticides are an integral part of rice cultivation. Despite their valuable contribution, the migration of pesticides applied in rice fields and their toxicity to non-target organisms is of some concern. Several pesticides are globally used in agriculture and may enter aquatic environments through spray drift or runoff events, drainage or leaching, resulting in contamination of surface and ground waters (Cerejeira *et al.,* 2003). Mulla and Mian (1981) reported that pesticide contaminated water can cause toxic effects. The application of pesticides in rice fields alters community structure, trophic cascade and also damage adjacent water bodies and which ultimately affected the aquatic environment (Faria *et al.,* 2007). Al-Shami *et al.* (2010) reported that application of Carbofuran on rice agro-ecosystem had a negative effect of chironomids and it was more toxic than Fipronil. Madden *et al.,* (1992) experimentally demonstrated that DDT and Dacthal pesticide induced deformities in *Chironomus* sp. and also proved the dose related mentum deformity. Warwick (1988) could demonstrate a negative relationship between DDE and antennal deformities. However, this reexamination demonstrates that Aldrin, Dieldrin, DDD and DDT all experimentally could induce deformities found in contaminated Northern Canadian lakes. Janssens de Bisthoven *et al.,* (1999) demonstrate that a positive correlations between aldrin, DDE, DDT and deformities. The aim of this study to investigate that the incidence of deformities in *Chironomus* sp. Larvae collected from three sites of rice fields in Hooghly District that differed in their contamination and larval mouthparts deformities as bio-indicators of pesticide stress.

MATERIALS AND METHODS

Study area

The study was conducted at three locations (Fig-1) of Hooghly district, West Bengal - (i) Rishra (22°42′N and 88°20′E) is an industrial town. This is polluted area and various types of industrial effluents are discharged into the rice field periodically. Beside industrial effluents the farmer uses large amount of pesticide in the rice field. (ii) Serampore (22°75′N and 88°34′E) is a pre-colonial town on the right bank of River Hooghly. (iii) Khanakul (22°71′N and 87°83′E) is predominantly agricultural area.

Test organism

The larvae of non biting midges belonging to the Genus *Chironomus* sp. (Diptera: Chironomidae) are widely used in bio-monitoring and assessment of fresh water environment (Hamilton and Sæther, 1971). The *Chironomus* sp. larvae are abundant and are close contact with the sediment of rice agro-ecosystem and develop in fourth instars with three moulting processes in between them (Chaudhuri and Chattopadhyay, 1990, and Saha and Mazumdar, 2013).

Field ccollection of chironomid midges

Samples were collected randomly at monthly intervals from three sites from July 2009 to September 2012. Adults were collected by sweeping around the rice fields with a long handled insect net. Larvae were collected from mud bottom in the rice fields with mud scrapers and a scoop sampler (Chaudhuri and Chattopadhyay, 1990). The sediment was taken in a plastic bucket, washed with water and passed through sieve (300-μm pore). The sieved material was transferred to a white enamel tray. Sufficient clean water was added to pass off the mud and to retrieve the larvae. The live larvae were handpicked and transferred to small vials. The egg masses were collected from water surface mostly adhered to twigs by the application of brush. The larvae inhabiting with tubes were gathered with the help of scalpel and small forceps. The immatures were field sorted unless the distances from the sites of collection to the laboratory are far away. In most cases sorting of immature was done near the collecting spot and in some cases brought to the laboratory in containers filled with preaerated the habitat water from following Pinder (1983). The live larvae were placed in polythene bags with amount of soft sediment and water from collecting sites in order to conduct the experiments for its ecological parameters.

Rearing of chironomids

Two separate experiment were set up for rearing of egg masses and chironomid larvae- (I) Life stage experiment and (II) Pesticide treatment experiment.

(I) Life stage experiment - This unit comprised 30-40 small sized petridishes (8-12 cm in diameter) covered with long tube like transparent plastic having a mesh lid at the top (20-25 cm tall and 8-12 cm in diameter). These small petridishes were used as containers for rearing of 4-5 small third or fourth instars larvae collected from rice field in order to obtained their life stages i.e., pupae, pupal exuviae and adults. Each petridish was filled with water medium of particular collecting sites and was regularly changed with few amount of food i.e., dust of special fish food TOKYU-Spirulina was added. The autoclaved soil from the collected site was added to the petridishes. The culture experiment was conducted in the laboratory at 26.3 ºC $\pm$0.949with 68.5% $\pm$ 2.1 humidity and light and dark cycle of 14:10h in the Programmable Environmental Test Chamber (REMI). The pH, DO, and salinity (recorded from field data) were also maintained and aerated water was added. The adult were allowed to move freely, mate and oviposit in order to repeat for confirmation of the life cycle and life stages of each species. The newly emerged male and female were collected from each of the units with hand held aspirator. The rearing technique such as Pinder (1983), Chaudhuri and Chattopadhyay (1990), and Epler (1992) were followed in this study.

(II) Pesticide treatment experiment - This unit comprised of three large plastic tray (38 cm x 30 cm x 7 cm) and 8-10 small plastic tray (25cm x 20 cm x 5 cm) was designated to investigate the effects of two common pesticides (A = Methyl Parathion & B = Chlorpyrifos) separately. Chironomid larvae were exposed to 0 (Control), 1, 5, 10, μg/L of pesticide. The controls contained 1 ml of acetone instead of pesticide. The concentration of pesticide was based on the laboratory study made by Hamilton and Sæther (1971). First instars larvae (25 no.) were added to each tray and with gentle aeration was provided. Dried, granulated fish food 0.2 mg per larvae per day was added for the midges. Physical and chemical parameters controlled according to life stage experimental unit. Each treatment was replicated four times. The method for assessing the effects of pesticides on *Chironomus sp.* was based on the experiment described by Agra and Soares (2009).

Collection of physico-chemical data

The physico-chemical parameters were recorded random at regular monthly intervals from the rice field during 2009 and 2012 according to Bhattacharya *et al.,* (2005) and Chaudhuri and Chattopadhyay (1990). Parameter like pH, water and air temperature, dissolved oxygen (DO) and humidity were recorded at the sampling sites with the pocket pH tester (pH-ep, HI-98107, HANNA), thermometer, digital-DO-meter (LT-Lutron-DO-5509) and digital humidity meter respectively. BOD was calculated by standard method of APHA (1998).

Preservation, mounting and identification

The egg masses, larvae, larval exuviae, pupae, pupal exuviae and adults were collected both from field and from experimental unit were most conveniently preserved and stored in 70–90% ethyl alcohol. Since alcohol preserved poised specimens certain drawbacks such as distortion of original colour and difficulty in mounting and a prolonged preservation of specimens, Kahle's solution (30 pt. 95% ethanol, 12 pt. formaldehyde, 4 pt. glacial acetic acid and 60 pt. distilled water) was sometimes used as preservative for both larvae and adults in order to retain normal colour of the specimen.

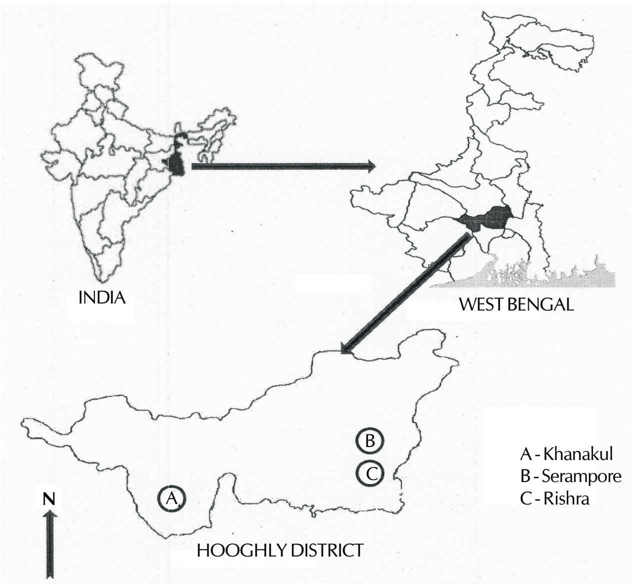

Figure 1: Map showing the location of sampling sites

The phenol-balsam technique of Wirth and Marston (1968) was mainly applied for the microslide preparation of larvae, pupae and adults. In case of larva, the head capsule was removed from the body and treated with a warm 10% KOH solution. Thereafter, these were washed in distilled water and put into 70% alcohol and transferred to alcoholic phenol. The larval head capsules were dissected and spread on the slide as per the recommendation of Warwick (1985).

Adult and immature stages were identified following Sæther (1980); Oliver and Roussel (1983); Langton (1991); Epler (1992); Pinder (1983) and Cranston *et al.,* (1989). Deformities of chironomid larvae were evaluated on the basis of Warwick (1985). Measurements of different parts of larvae and adults were made with the help of a compound microscope and expressed in micrometer (μm). Photograph of various parts of larvae were taken for deformity analysis by the help of Trinocular Microscope (MLX-TR, Magnus). The collected data were determined adopting statistical methods by using the statistical software SPSS programme, version 17.

RESULTS AND DISCUSSIONS

Physico-chemical parameters in rice field were measured during July 2009-September 2012 (Table 1). The Physico-chemical parameters were more variable in three sites. The mean water temperature was (1-2°C) higher in Rishra compared with the Khanakul and Serampore. The mean value of water pH in three sites ranges from 6.9-7.7. The Biological Oxygen Demand was higher in Rishra in compare to other two sites. Faria *et al.* (2007) demonstrated that the temperature and pH were higher in highly contaminated site than low contaminated site. This indicates that Rishra site were highly polluted than other two sites.

Deformities in the head capsules of *Chironomus* sp. larvae in the mentum, mandibles and antenna were describe by Warwick (1985 and 1988), Janssens de Bisthoven *et al.,* (1999), Al-Shami *et al.* (2010), Bhattacharya *et al.* (1999) and Veroli *et al.* (2012). Hamilton and Sæther (1971) and Wiederholm (1984) also reported that morphological deformities of various chironomid genera occur when they are exposed to contaminants. Chironomids are present in a wide variety of habitats and environmental conditions (Pinder, 1986) and thus it has been used as bio-monitoring agents. In this study 920 *Chironomus* sp. larvae were collected (Fig.2) from three sites of rice agro-ecosystems and the incidence of deformities were recorded. The result shows that out of the total deformities highest deformities obtained from Rishra rice field (55.10%)

Table 1: Physico-chemical characteristics (Mean ± SD) at three sampling sites during 2009-2012

Parameters	Khanakul			Serampore			Rishra		
	Jul	Aug	Sept	Jul	Aug	Sept	Jul	Aug	Sept
Water temp. (°C)	24.7±0.823	24.5±0.707	23.6±0.843	26.5±1.080	25.9±0.876	25.1±0.994	26.3±0.949	25.3±0.823	24.9±0.738
Water pH	6.9±0.163	7.3±0.235	7.5± 0.124	7.4± 0.362	7.3±0.244	7.5±0.133	6.9±0.298	7.5± 0.419	7.7± 0.326
DO (mg/l)	7.7±0.326	7.9±0.133	8.1±0.205	7.3±0.244	7.5±0.133	7.4±0.362	6.6±0.368	7.6± 0.326	7.4±0.182
BOD (mg/l)	4.5±0.326	4.0±0.182	4.0± 0.200	5.8±0.323	4.0± 0.226	3.9±0.226	5.0±0.194	4.0±0.200	4.4± 0.323
Air temp. (°C)	27.8±0.789	27.6± 0.516	26.6±0.699	28.3±0.949	28.1±0.738	27.3±0.823	30.2±0.789	29.3±0.949	28.2± 1.033
Relative humidity	70.1± 1.449	68.7±1.636	68.2± 1.476	67.4±1.713	66.8±1.398	66.6±1.350	68.5±2.121	67.6±1.350	66.7± 1.160

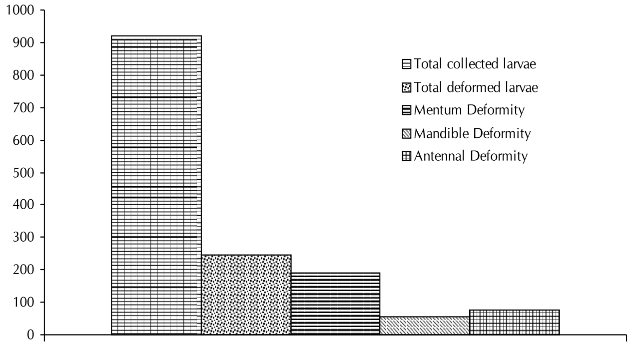

Figure 2: Cumulative deformity incidence of *Chironomus* sp.

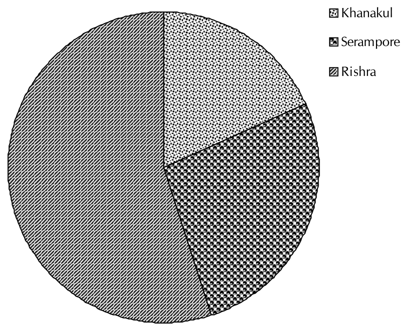

Figure 3: Percentage of deformities at three sampling sites

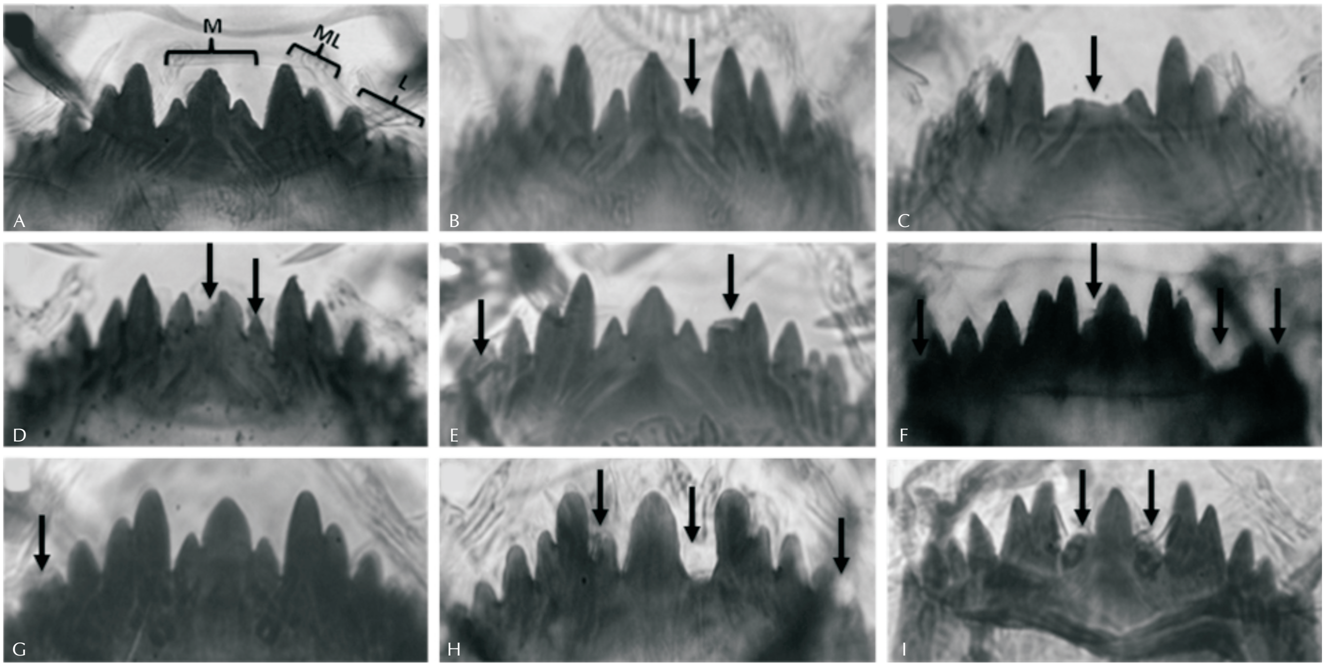

Figure 4: A-Normal mentum (M - median teeth, ML- mediolateral teeth and L - lateral teeth), B-I - deformed mentum. Arrows indicate deformities

and other two Serampore (26.53%) and the Khanakul (18.37%) (Fig. 3). However Wiederholm (1984) reported that deformity rates up to 17% in polluted environment. This indicate that the *Chironomus* sp. normally respond to pollution. Overall, this incidence of deformity was highest in the larvae collected from Rishra, the most polluting site and influenced by that effluents discharged from the industrial region.

In this study morphological deformities were observed in mentum, mandible and antennae, of which the mentum deformities were the most common deformity of the larvae. The mentum of *Chironomus* sp. has dark teeth. Median tooth trifid, 2 smaller outer toothlets may be fused with central or quite separate, two pairs of mediolateral teeth and four pairs lateral teeth (Fig. 4). Al-Shami *et al*. (2010) demonstrated that median teeth deformities were the most frequent compare to mediolateral and lateral teeth. Deformities in mentum range from the loss of inner lateral or median teeth to massive disorganization and loss of symmetry (Warwick, 1985).

The deformities of the mentum of *Chironomus* sp. were classified into three classes.

Class I - Deformity in Median tooth. The median teeth located between the mediolateral teeth. We found that this tooth is either totally lost or broken at the tip and asymmetrical in shape.

Class II - Deformity in Medio-lateral tooth. The mediolateral teeth are found in both right and left side of the median teeth. We found that the mediolateral tooth is missing or broken and another cases there were also reduction in the size of some of them.

Class III - Deformity in Lateral tooth. The lateral teeth are situated at the lateral side of the mentum. Loss of teeth or broken in various positions of the lateral teeth and gap in between the lateral teeth was noticed.

As shown in the Fig. 5 the pesticide A (Methyl parathion) shows higher regression coefficient (R^2) compared to pesticide B (Chlorpyrifos). In case of class I mentum deformity pesticide A shows higher regression coefficient 0.778 compared to pesticide B regression coefficient value was 0.119. Similarly the class II mentum deformity pesticide A shows higher regression coefficient 0.781 compared to pesticide B regression

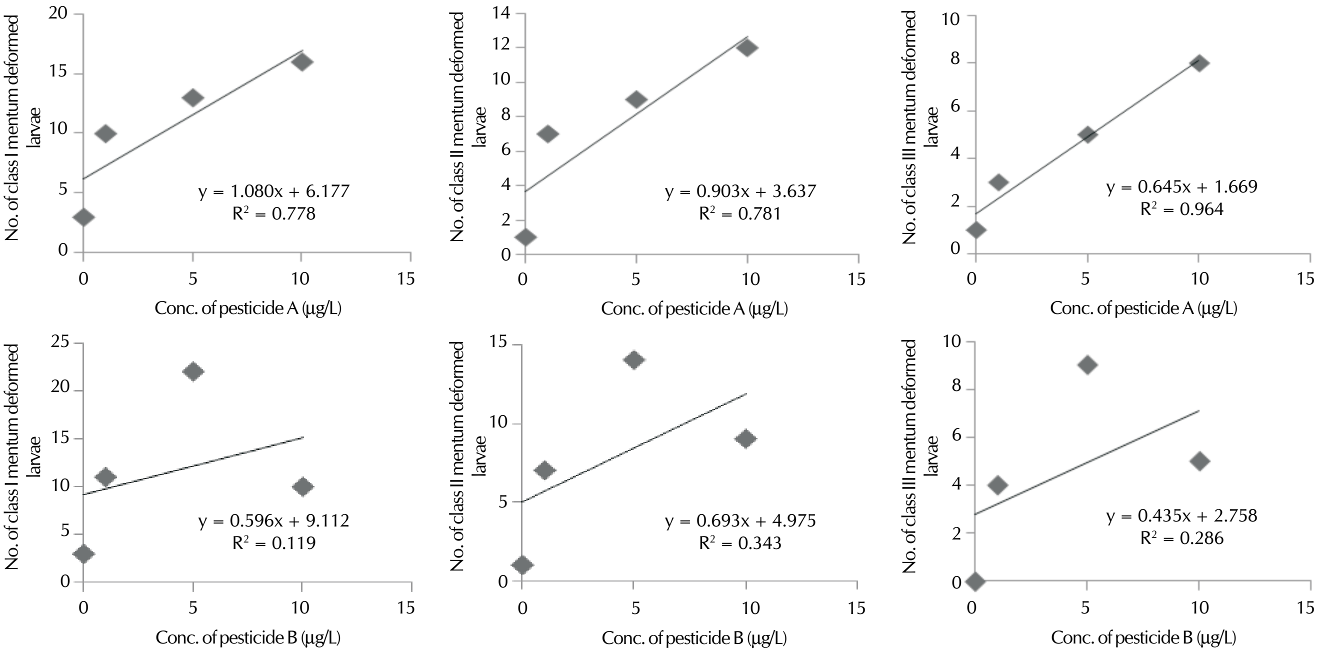

Figure 5: Relationship between mentum deformity and concentration of pesticides methyl parathion (A) and chlorpyrifos (B)

coefficient value was 0.343. In case of class III mentum deformity pesticide A shows higher regression coefficient 0.964 compared to pesticide B regression coefficient value was 0.286. This indicate that there was a significant different between two pesticides for their induction of mentum deformity.

Pesticide treatment analysis shows that the percentage of deformed menta increase as the concentration of pesticide Methyl Parathion was increased but the same relationship was not evident for the effect of pesticide Chloropyrifos. In case of Chloropyrifos, with increasing concentration up to 5 μg/L, the mentum deformity was high and then it was decrease slowly. However the percentage of deformities in all treatments was higher than the controls, thus indicating that these pesticides may induce deformities in *Chironomus* sp. This experiment also shows that Class-I- Mentum deformity was the highest percentage out of the three classes. Madden, *et al.* (1992) experimentally proved that DDT and Dacthal pesticide induced deformities in *Chironomus* sp. and also proved the dose related mentum deformity.

In this present study the main sampling sites are rice agro-ecosystem of Hooghly District in the state of West Bengal. The highest deformity was found in Rishra sampling site, this field was contaminated by different pesticides which was periodically used by the farmer for better production of crop. Despite that another main contamination receives from glass factory as industrial effluents in the adjoining rice field. Another two sampling sites are Serampore and Khanakul, which are relatively low deformity, these sites has no industry, mainly agricultural area. Morphological deformities in chironomid larvae prove that this was the main biomonitoring agent. Increasing frequencies of deformities may be caused by metals, agricultural runoff and pesticides (Bhattacharya *et al.,* 2005 and Al-Shami *et al.,* 2010).

In conclusion, this study showed the impact of pesticides and industrial contaminants in terms of deformities of various structures of head capsule of *Chironomus* sp. larvae inhabiting the rice fields of Hooghly, West Bengal. The incidence of deformities in larval mentum in the rice field of industrial region is relatively high compared to non industrialized agricultural area. The identified deformities are indicative of certain environmental stresses on the studied habitats and could serve as an empirical tool for their assessment. Based on bio monitoring assessment, the study identifies the perturbations occurring in the rice field that are detrimental to inhabiting organisms, thus requiring appropriate steps for improvement of water quality. It could serve as a useful early warning tool in assessment of pollutants prevalent in rice environment receiving anthropogenic, agricultural, and industrial discharges.

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REFERENCES

Al-Shami, S. A., Salmah, M. R. C., Azizah, M. N. S., Ahmad, A. H. and Ali, A. 2010. Morphological deformities in *Chironomus* spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in the Juru River System, Penang, Malaysia. *Environ. Entomol*. **39(1)**: 210-222.

Ali, A. 1996. A concise review of chironomid midges (Diptera: Chironomidae) as pest and their management. *J. of Vec. Eco*. **21(2):** 105-121.

Agra, A. R. and Soares, A. M. V. M. 2009. Effects of two insecticides on survival, growth and emergence of *Chironomus riparius* Meigen. *Bull. Environ. Contam. Toxicol.* **82:** 501-504.

APHA, 1998. Inorganic nonmetallic constituents. In: standard methods for the examination of water and wastewater, 20th ed., Greenberg, A. E., Clesceri, L. S. and Eaton, A. D. (Eds.). *American Public Health Association, Washington DC*. pp. 4.129-4.130.

Amiryan, A. L. 2010. Morphological deformities in chironomid larvae (Diptera: Chironomidae) from Hrazdan River, Armenia. *Electronic J. of Nat. Sci.* **2(15):** 15-17.

Bhaduri, S., Sarkar, P., Ghosh, C. and Midya, T. 2011. Response of the chironomid larvae to the environmental condition: A study on the polytene chromosomes of *Chironomus striatipennis* (Kieffer). *The Ecoscan*. **5(1&2):** 75-80.

Bhattacharyay, G., Mazumdar, A. and, Chaudhuri, P. K. 1999. Incidence of deformed *Chironomus* larvae in contaminated sediment of the river Damodar, West Bengal (Diptera: Chironomidae). *Pollut. Res.* **18:** 79-82.

Bhattacharyay, G., Sadhu, A. K., Mazumdar, A. and Chaudhuri, P. K. 2005. Antennal deformities of chironomid larvae and their use in biomonitoring of heavy metal pollution in the river Damodar of West Bangal, India. *Environ. Monit. Assess*. **108:** 67-84.

Bird, G. A. 1997. Deformities in cultured *Chironomus tentans* larvae and the influence of substrate on growth, survival and mentum wear. *Environ. Monit. Assess.* **45:** 273-283.

Cerejeira, M. J., Viana, P., Batista, S., Pereira, T., Silva, E. and Valerio, M. J. 2003. Pesticides in Portuguese surface and ground waters. *Water Res*. **37:** 1055-1063.

Chaudhuri, P. K. and Chattopadhyay, S. 1990. Chironomids of the rice paddy areas of West Bengal, India (Diptera: Chironomidae). *Tijdsch. Ent*. **133(2):** 149-195.

Cranston, P. S., Oliver, D. R. and Sæther, O. A. 1989. The adult males of Orthocladiinae (Diptera: Chironomidae) of the Holarctic region. In: Chironomidae of the Holarctic region, keys and diagnoses. Part. 3. Adult males. Weiderholm, T. (ed.). *Ent. Scand. Suppl*. **34:** 165-352.

Epler, J. H. 1992. Identification manual for the larval Chironomidae (Diptera) of Florida. *Fl. Dept. Environ. Reg. Orlando. Fl.* 1.1-9.6.

Faria, M. S., Nogueira, A. J. A. and Soares, A. M. V. M. 2007. The use of *Chironomus riparius* larvae to assess effects of pesticides from rice fields in adjacent freshwater ecosystems. *Ecotoxicol. Environ. Safety*. **67:** 218-226.

Fernando, C. H. 1993. Rice field ecology and fish culture- an overview. *Hydrobiologia*. **259:** 91-113.

Hamalainen, H. 1999. Critical appraisal of the indexes of chironomid larval deformities and their use in bioindication. *Ann. Zool. Fennici.* **36:** 179-186.

Hamilton A. L. and Sæther, O. A. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. *Can. Entomol.* **103:** 363 - 368.

Janssens de Bisthoven, L. , Huysmans, C. and Ollevier, F. 1999. The in situ relationship between sediment concentrations of micropollutants and morphological deformities in *Chironomus gr. thummi* larvae (Diptera, Chironomidae) from lowland rivers (Belgium): A spatial comparison. In: P. Cranston (ed.), *Chironomids: From genes to Ecosystem,* CSIRO-publication. pp. 63-80.

Langton, P. H. 1991. A key to pupal exuviae of West Palaearctic Chironomidae. *Private publication*. pp. 1-386.

Lim, R. P. 1990. Effects of pesticides on the aquatic invertebrate community in rice fields. Proceedings of the International Conference on Tropical Biodiversity-in Harmony with Nature, Malaysia. 336-352.

Madden, C. P., Sutter, P. J., Nicholson, B. C., and Austin, A. D. 1992. Deformities in chironomid larvae as indicators of pollution (Pesticide) stress. *Netherlands J. Aquat. Ecol.* **26(2-4)**: 551-557.

McDonald, E. E., and Taylor, B. R. 2006. Incidence of mentum deformities in midge larvae (Diptera: Chironomidae) from North Nova Scotia, Canada. *Hydrobiologia*. **563**: 277–287.

Midya, T., Sarkar, P., Bhaduri, S. and Mazumdar, A. 2013. Fragmentation of polytene chromosomes of *Chironomus striatipennis* (Kieffer) as a mark of heavy metal toxicity in aquatic habitats. *The Bioscan*. **8(3)**: 1075-1078.

Mulla, M. and Mian, L. 1981. Biological and environmental impacts of insecticides malathion and parathion on non-target biota in aquatic ecosystem. *Res. Rev*. **78**: 101-135.

Oliver, D. R. and Roussel, M. E. 1983. The genera of larval midges of Canada (Diptera: Chironomidae). The insects and arachnids of Canada. Part II: *Biosyst. Res. Br. Agric. Canada, Publ. no*. **1746**: 1-263.

Pinder, L. C. V. 1983. The larvae of Chironomidae (Diptera), of the Holarctic region. Introduction in Chironomidae of the Holarctic region. Keys and diagnoses. Part 1. Larvae. Weidderholm, T (ed.). *Ent. Scand. Suppl.* **19:** 7-10.

Pinder, L. C. V. 1986. Biology of freshwater Chironomidae. *Ann. Rev. Ent.* **31:** 1-23.

Rosenberg, D. M. 1992. Freshwater Biomonitoring and Chironomidae. *Netherlands J. Aquat. Ecol.* **26(2-4):** 101-121.

Sæther, O. A. 1980. Glossary of Chironomid morphology terminology (Diptera: Chironomidae). *Ent. Scand. Suppl*. **14:** 1-51.

Saha, D. and Mazumdar, A. 2013. Deformities of Chironomus sp. larvae (Diptera: Chironomidae) as indicator of pollution stress in rice fields of Hooghly District, West Bengal. *J. Today Bio. Sci. Res. Rev.* **2(2):** 44-54.

Stevens, M. M., Helliwell, S. and Cranston, P. S. 2006. Larval chironomid communities (Diptera: Chironomidae) associated with establishing rice crops in southern New South Wales, Australia. *Hydrobiologia*. **556:** 317-325.

Vermeulen, A. C. 1995. Elaboration of chironomid deformities as bioindicators of toxic sediment stress: The potential application of mixture toxicity concepts. *Ann. Zool. Fenn*. **32:** 265-285.

Veroli, A. D., Selvaggi, R. and Goretti, E. 2012. *Chironomid* mouthpart deformities as indicator of environmental quality: a case study in Lake Trasimeno (Italy). *J. Environ. Monit*. **14:** 1473-1478.

Warwick, W. F. 1985. Morphological abnormalities in *Chironomidae* (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen. *Can. J. Fish. Aquat. Sci*. **42:** 1881-1941.

Warwick, W. F. 1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress. In: M.S. Evans (ed.). Toxic contaminants and ecosystem health. A Great Lake focus. Wiley and Sons, New York. pp. 281-320.

Wirth, W. W. and Marston, N. 1968. A method for mounting small insects on microscope slides in Canada balsam. *Ann. Entomol. Soc. Am*. **61:** 783-784.

Wiederholm, T. 1984. Incidence of deformed chironomid larvae (Diptera: Chironomidae) in Swedish lakes. *Hydrobiologia*. **109:** 243-249.