Contemporary Health Issues and Environmental Impact

Edited by

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FROM THE EDITOR'S DESK

Editorial

Over the last two to three decades, natural environment has been persistently contaminated by Urbanization and industrialization. Pesticides, xenobiotics, heavy metal toxicity, cattle feed, fertilizers, drugs and various pathogens alter the biological equilibrium of both land and aquatic ecosystem. *The book Contemporary Health Issues and Environmental Impact* is a collection and compilation of some valuable research papers, review articles and case studies dealing with these current environmental problems.

The first paper by Mukherjee *et al.* discusses an important polyphagous pest *Aulacophora foveicollis which* attacks more than 81 important plant species. The pest developed resistance to highly toxic monocrotophos (an organophosphate insecticide) by the detoxifying enzymes is the important venture of this study.

Fish accumulates heavy metals within tissue through absorption and humans can be exposed to heavy metals via food chain. This can cause acute and chronic health hazards in human. The paper by Sarbajna deals with heavy metal (As. Cd, Pb and Hg) contamination of different fish species and human health risk. So regular monitoring is necessary to identify these toxic elements in water bodies and protective measure against excessive exposure to these toxic substances is essential. The metals, viz., As, Cd, Pb and Hg are most toxic to human beings, animals, fishes and environment.

Bakra in her reviewed article" Arsenic toxicity through Dietary Intake pathways in the villages of West Bengal, India" discusses Ganga-Brahmaputra plains in India and the Padma-Meghna plains in Bangladesh. They together constitute the most wide spread arsenic-affected area in the World. She illustrates beautifully the pathways of arsenic exposure through drinking water and through different vegetables, pulses and rice and how it poses a risk for adults, infants and children.

Protein phosphorylation pattern of murine peritoneal macrophages during attachment with *Leishmania donovani* promastigotes were investigated by Das (Ghosh) .PKC mediated specific proteins were phosphorylated with exogenous orthophosphate when murine peritoneal macrophages were stimulated with *L. donovani* attachment. Thus she explained that phosphorylation of these proteins might be a unique event associated with the recognition and uptake of *L. donovani* by macrophages and phosphorylation of these proteins were not required for intracellular replication of these pathogens.

Climate change with anthropogenic activities create conditions favourable for the parasite to thrive and flourish, leading to serious clinical conditions .The paper written by Biswal discusses this serious problem and I think it would create awareness among the readers regarding the same.

Commercial shrimp culture is one of the major aquaculture activities in the coastal areas of West Bengal. Mandal stated that various anthropogenic activities alter the biological equilibrium of the coastal zones. This situation allows the pathogenic organisms which normally exist in the dormant state in water to become multiply faster. Infection from these pathogens can be effectively prevented by maintaining proper water quality in a shrimp culture system.

Dey and De performed a case study on some wetlands of Murshidabad and observe the environmental impact of Arsenic contaminant in lentic ecosystem, in surface water and ground water and how it enters the food grains.

Natrum Sulphuricum-200 can be used for the treatment of liver disorder including neoplastic growth. An ameliorative effect in mice was observed after treatment with Natrum Sulphuricum -200. The authors Bhattacharjee and Khuda-Bukhsh have suggested that this homeopathic medicine can be used as a supportive medicine.

Sarkar (Paria) *et al.* have clearly explained why the intensity of Aedes mosquito will increase in near future and climatic factors are capable of assisting or interrupting the biology and population dynamics of vector mosquitoes, thereby influencing abundance and distribution. The same factors also play a crucial role in the survival and transmission rate of mosquito-borne pathogens. Dr. Sarkar *et al.* presented a climate model for the dengue vector which is useful for management purposes, particularly with regard to the climate change in the future.

Ghosh and Homechaudhuri have shown the seasonal disease occurrence, percentage of mortality and survival of adult and fingerlings of *Channa panctatus* with sub lethal dose of aeromonad bacteria. The authors discuss on the environmental deterioration coupled with increased virulence of pathogen on a susceptible host. Motile aeromonads is the most common bacteria found in freshwater habitats and frequently cause disease among cultured and feral fishes.

Roy explains the role of modern environment on cancer. She suggests cancer to be a 'modern 'disease because of its high incidence in modern times which might cause by environmental and life style changes and also due to the changes in diet pattern. According to W.H.O, people should be educated so that they can recognise early signs of cancer and seek prompt medical attention for symptoms.

From the paper 'Dietary Phytoestrogens-Boon or Bane' by Majumdar we came to know the mechanism of action of phytoestrogen. She has nicely described the beneficial and adverse effects of these hormones. The question whether phytoestrogens are beneficial or harmful to human health remains unsolved.

Dalui *et al.* have discussed on contact dermatitis. The causative agent is fruit borer *Autocharis albizonalis* which at the time of infecting mango, releases urushiol from mango peel causing dermatitis.

I assure the readers that the wide ranging topics covered in the papers of this collection would serve as a timely intervention in creating awareness on common health hazards ensuing from pathogenic infection to heavy metal toxicity, drug resistance insecticides and many others. Here I end with the hope that our collective efforts would be beneficial for you.

Dr. Mala Bose, Dr. Soma Aditya (Bandyopadhyay), Dr. Sandeep Poddar

Mukherjee et al.
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Chapter 1

Monocrotophos Resistance in Aulacophora Foveicollis

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Monocrotophos resistance in *Aulacophora foveicollis*

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Abstract

Persistence use of chemical pesticides produces pesticide resistance in many pest populations. *Aulacophora foveicollis* is a polyphagous pest, feed voraciously on leaves, flower buds and flowers. Damage may reach upto 35-75% at seedling stage. In some cases, the losses by this pest have been reported 30-100% in the field. Many pesticides-permethrin, cypermethrin, monocrotophos are used extensively to control these pests' populations. But they may have developed resistance to insecticides by using the activity of detoxifying enzymes-Esterases, Catalases, Glutathione S-transferases (GSTs), Cytochrome P450 monooxygenases (CYPs). Monocrotophos is an organophosphate insecticide. It is relatively cheap and highly toxic. In this work different days (1 day, 7 days and 14 days) of stress by the exposure of the sublethal concentration (0.001mg/ml) of monocrotophos were applied to *A. foveicollis* for determining the activity of detoxifying enzymes and NO generation. The activity of the enzymes and NO generation is significantly higher in stress conditions than control. This result indicates that in *A. foveicollis* the enzymes may provide resistance to monocrotophos.

***Keywords*:** *Aulacophora foveicollis*, Monocrotophos, Detoxifying Enzymes, Nitric Oxide.

Introduction

Aulacophora foveicollis is a polyphagous pest and attacks more than 81 plant species including pumpkin, squash, cucumber, bottle gourd, wax gourd, snake gourd, water melon etc. and a wide range of food crops. The adult beetles feed voraciously on leaves, flowers, and flower buds. It can be called as one of the most serious pest of cucurbits because it attacks every stage of cucurbits and it can cause heavy loss to all cucurbits except bitter gourd.[1] *Aulacophora foveicollis* uses enzymes like- Glutathione S-transferase (GST), Cytochrome P_{450} monooxygenase, general esterase, catalase etc to detoxify. These enzymes detoxify toxic compounds by oxidation, reduction, hydrolysis, transferase reaction or conjugation of molecules.[2] So, the detoxifying enzymes may provide resistance of insects to insecticides. Metabolic resistance often results from overproduction of detoxifying enzymes that can metabolize many synthetic insecticides.[3] Nitric oxide (NO) is produced as a reactive nitrogen intermediate during formation of L-citruline from L-arginine by nitric oxide synthase.[4] This NO has the ability to kill foreign microorganisms by combining with superoxide to form peroxynitrite, a strong bactericidal agent.[5]

In this study, the activity of major detoxifying enzymes from tissue extracts and nitric oxide (NO) generation from haemolymph of *A. foveicollis* were estimated by exposing the insects to sublethal concentrations (0.001mg/ml) of pure monocrotophos (Sigma Aldrich, Germany) insecticides in controlled

laboratory conditions. Monocrotophos is an organophosphate insecticide which is used in India for the control of these insect pests. It is choline-esterase inhibitor.

The aim of the study is to determine the activity of the enzymes which may provide resistance to insecticide in *A. foveicollis*.

Materials and Methods

Nitric Oxide Activity Assay

At first, haemolymph was collected from four adult insects (male: female =1:1) in aeppendorf containing 0.5 ml 0.1 M sodium phosphate buffer (pH 7.0). Then 0.5 ml Griess reagent (1percent sulphanilamide, 0.1percent naphthyl ethylenediamine dihydrochloride and 5 percent orthophosphoric acid) was added to it. The contents were then incubated at 37°C for 30 min. The absorbance was recorded in a spectrophotometer at 550 nm against a standard blank. Blank was prepared with 0.5 ml distilled water and 0.5 ml Griess reagent without haemolymph.[6]

Estimation of Total Protein

In a dark room, 800 microliters Bradford reagent (100 mg Coomassie Brilliant Blue G-250 was dissolved in 50 ml 95 percent ethanol and 100 ml 85 percent phosphoric acid and diluted to 1 liter with distilled water) was added to 195 microliters 0.1 M sodium phosphate buffer (pH 7.0). Then 5 microliters tissue extract was added to it. Then the contents were incubated 20 min at room temperature. Absorbance was read at 595 nm in a spectrophotometer against blank. Blank was prepared with 800 microliters Bradford reagent and 200 microliters 0.1 M sodium phosphate buffer (pH 7.0) without tissue extracts.

Enzyme Extraction

A pool of four adults of *A. foveicollis* (male: female = 1:1) was homogenized in 3ml ice cold 0.1M sodium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 20 minutes at 4°C in high speed refrigerated centrifuge (Plasto Crafts, Rota 4R-V/FA). The resultant supernatant was kept in 1.5ml eppendorf tubes and stored at -20°C and served as the enzyme source.

Detoxifying Enzyme Activity Assay

1. General esterase activity: General esterase activity was measured using α-naphthyl acetate (α-NA) as substrate according to the method described by Van Asperen[7]with few modifications for use in a spectrophotometer. Eight hundered microliters of freshly prepared 30 mM α-NA was added to 80 microliters enzyme stock for reaction to occur. The reaction was stopped after 15 min by adding 50 microliters of staining solution prepared fresh by mixing two parts 0.1 percent Fast Blue BB salt with five parts of 5 percent sodium dodecyl sulphate (SDS). After 5 min, absorbance was read at 590 nm against blank in a spectrophotometer. The change in absorbance was converted to end product (α-naphthol) using the standard curve of α-naphthol. Blank were set at the same time using a reaction mixture without enzyme stock.[3]
2. Cytochrome P450 monooxygenase activity: Most of the classic methods to evaluate oxidase activity with chromogenic substrates require the purification of microsomal fractions, but we are unable to use these methods to estimate the differences in oxidase activity in single small insects.[8] The alternative method is to measure the level of haem-containing enzymes, which includes the cytochrome oxidase enzymes.[9] Peroxidation of tetra-methyl benzidine (TMBZ) is catalysed by microsomal proteins with hydrogen peroxide as co-substrate. The amount of

oxidase enzymes is correlated with the peroxidase activity of the haem groups. Such a technique would be helpful for estimating large scale differences in oxidase activity characteristic of insecticide resistance and oxidase induction.[9]

Cytochrome P450 activity was estimated by measuring haem peroxidase activity.[10] As haem constitutes the majority of cytochrome P450 of non-blood fed (herbivorous) insects, quantification of haem activity can be expressed as cytochrome P450.[9]

For cytochrome P450 activity, 60 microliters enzyme stock was taken and 600 microliters of 6.3 mM TMBZ solution (10 mg TMBZ dissolved in 5 ml absolute methanol mixed with 15 ml of 0.25M sodium acetate buffer, pH 5.0, prepared fresh daily) was added to it. 240 microliters of 0.0625M potassium phosphate buffer pH 7.2 and 75 microliters of 3 percent hydrogen peroxide were then added. Blank was prepared with 60 microliters of homogenizing buffer and with all the ingredients except the enzyme source. Absorbance was recorded at 630 nm after 30 min of incubation in a spectrophotometer agaist blank. A standard curve for haem peroxidase activity was prepared using different concentrations of cytochrome C.[3]

3. Glutathione S-transferase (GST) activity: GST activity was estimated by using the protocol described by Kao *et.el.*[11] with minor modifications. Fifty microliters of 50mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 150 microliters of 50mM of reduced glutathione (GSH) were added to 2.78ml of sodium phosphate buffer (100 mM, pH 6.5). Twenty microliters of enzyme stock were then added. The contents were shaken gently, incubated for 2-3min at 30°C and then transferred into the sample cuvette of UV-Visual spectrophotometer. Absorbance at 340nm was recorded for 10-12 min employing the kinetics (time scan) menu in UV-VIS spectrophotometer (UV-1800 SHIMADZU). Blank was set at the same time using a reaction mixture without enzyme stock.[3]

   A unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of 1 µM of 2,4-dinitrophenyl glutathione per minute at 30°C using 1 mM concentrations of GSH and CDNB. Specific activity is defined as units per mg of protein. The difference in the millimolar extinction co-efficient between CDNB– GSH conjugate and CDNB is 9.6. Changes in absorbance per minute were converted into µM CDNB conjugated/min/mg protein using the formula, CDNB − GSH conjugates (*µ*mol/min/mg protein)

$$\frac{\text{Abs. increase (in 5 min)} \times 3 \times 1000}{9.6 * \times 5 \times \text{protein in mg}}$$

   (*9.6mM/cm is the extinction coefficient for CDNB–GSH conjugate at 340 nm).[12-13]

4. Catalase activity: Catalase catalyzes the following reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$

   For catalase activity, 1.0 ml of 0.059 M hydrogen peroxide was added to 1.9 ml of distilled water. Then the contents were incubated for 4-5 min at 25°C. After that 0.1 ml enzyme stock was then added to it. Absorbance was read at 240 nm for 2-3 min (time scan) in a spectrophotometer against blank.[3] Blank was prepared with all the ingredients except the enzyme stock. $\Delta A_{240/min}$ was calculated from the initial (45 second) linear portion of the curve.

$$\text{Units/mg} = \frac{\Delta A_{240/\text{min}} \times 1000}{43.6 \times \text{mg enzyme/ml reaction mixture}}$$

Statistical Analysis

Comparisons of data on enzyme and nitric oxide activities among different days of exposure in *A. foveicollis* populations was done by one-way analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) test using SPSS 21 (IBM) software. Data are presented as the mean±standard error.

Results

Nitric oxide (NO) activity in *A. foveicollis* was studied on control and stress conditions (1day, 7days, and 14days). NO generation is increasing with increasing stress. Significant difference occurs between control and in different stress conditions ($F_{0.05}$=129.209, df=3, *P*< 0.001). Tukey test indicated significant differences of NO generation between control and 1 day stress [mean difference=|(I)-(J)|=|q|=1815.4180, *P*< 0.001]; control and 7 days stress [|q|=2276.5110, *P*< 0.001]; control and 14 days [|q|=2324.2852, *P*<0.001]; 1 day stress and 7 days stress [|q|=461.0930, *P*<0.039]; 1 day stress and 14 days stress [|q|=508.8672, *P*<0.024]. But the difference is not significant between 7 days and 14 days stress [|q|=47.7742, *P*<0.984] (Figure 1).

In *A. foveicollis* total protein gradually decreases with stress [$F_{0.05}$ =55.767, df=3, *P*< 0.001. Tukey test indicated significant differences of total protein between control and 7 days stress [|q| =0.2530, *P*<0.018]; control and 14 days [|q|=0.7583, *P*<0.001]; 1 day and 14 days stress [|q|=0.6640, *P* <0.001; 7 days and 14 days stress [|q|= 0.5052, *P*<0.001]. But the variations are not significant between control and 1 day stress [|q|= 0.0942, *P*<0.494]; 1 day and 7days [|q|=0.1588, *P*<0.138].(Figure 2)

General esterases activity increases in different days of exposure ($F_{0.05}$=101.878, df=3, *P*<0.001). Tukey test indicated significant differences of esterase activity between control and 1 day stress [|q|= 0.1567, *P*<0.003]; control and 7 days stress [|q|= 0.3825, *P*<0.001]; control and 14 days stress [|q|=0.4386, *P*<0.001]; 1day and 7 days stress [|q|=0.2258, *P*<0.001]; 1 day and 14 days stress [|q|=0.2818, *P*<0.001]. But the variation is not significant between 7 days and 14 days stress [|q|=0.0560, *P*<0.276]. (Figure 3).

Cytochrome P450 monooxygenases activity increases in stress conditions ($F_{0.05}$=44.214, df=3, *P*<0.001). Tukey test indicated significant differences of cytochrome P450 monooxygenase activity between control and 1-day stress [|q|= 0.6664, *P*<0.001]; control and 7 days stress [|q|= 0.7297, *P*<0.001; control and 14 days stress [|q|=0.9538, *P*<0.001]; 1 day and 14 days stress [|q|=0.2874, *P*<0.044]. But the variation is not significant between 1 day and 7-day stress [|q|=0.0633, *P*<0.885]; 7 days and 14 days stress [|q|=0.2241, *P*<0.122]. (Figure 4).

Glutathione S-transferases showed higher activity in stress conditions ($F_{0.05}$=633.201, df=3, *P*<0.001). Tukey test indicated significant differences of GST activity between control and 1 day stress [|q|=11.8489, *P*<0.001]; control and 7 days stress [|q|=28.4923, *P*<0.001]; control and 14 days stress [|q| =66.4130, *P*<0.001]; 1day and 7 days stress [|q|=16.6434, *P*<0.001]; 1 day and 14 days stress [|q|=54.5641, *P*<0.001]; 7 days and 14 days stress [|q| =32.9207, *P*<0.001]. (Figure 5)

Catalase activity increases with stress conditions ($F_{0.05}$=268.136, df=3, *P*<0.001). Tukey test indicated significant differences of catalase activity between control and 1 day stress [|q| = 14.9900, *P*<0.001]; control and 7 days stress [|q| = 25.1967,

P<0.001]; control and 14 days stress [|q| =53.4733, *P*<0.001]; 1 day and 7 days stress [|q| =10.2067, *P*<0.003]; 1 day and 14 days stress [|q| =38.4833, *P*<0.001]; 7 days and 14 days stress [|q| =28.2767, *P*<0.001]. (Figure6) [|q|=0.9538, *P*<0.001]; 1 day and 14 days stress [|q|=0.2874, *P*<0.044]. But the variation are not significant between 1 day and 7 day stress [|q|=0.0633, *P*<0.885]; 7 days and 14 days stress [|q|=0.2241, *P*<0.122]. (Figure 6).

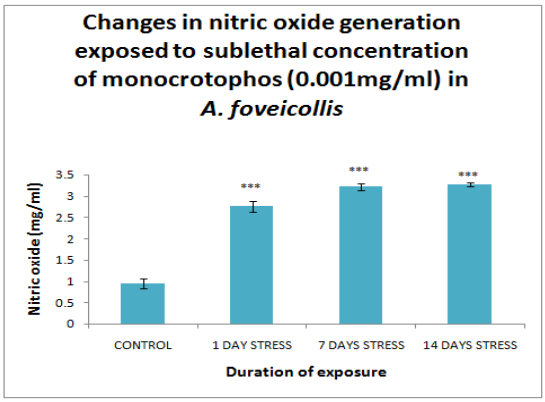

Figure 1. NO generation in *A. foveicollis* exposed to sublethal concentration of monocrotophos (0.001mg/ml). Data are presented as mean± standard error (n=3). **p*<0.05, ***p*<0.01, ****p*<0.001

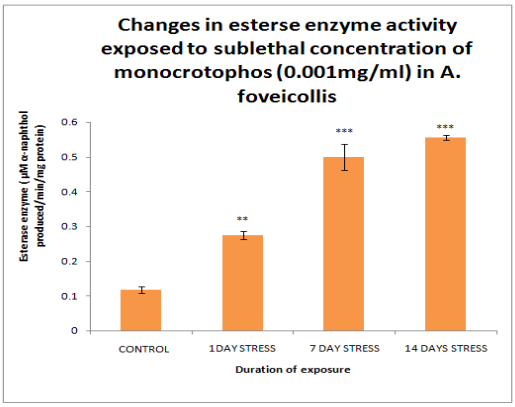

Figure 3. Esterase enzyme activity in A. foveicollis exposed to sublethal concentration of monocrotophos (0.001mg/ml). Data are presented as mean± standard error (n=3). *p<0.05, **p<0.01, ***p <0.001

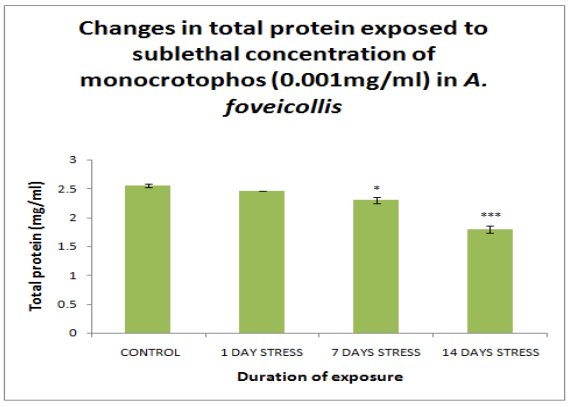

Figure 2. Total protein in *A. foveicollis* exposed to sublethal concentration of monocrotophos (0.001mg/ml). Data are presented as mean± standard error (n=3). **p*<0.05, ***p*<0.01, ****p*<0.001

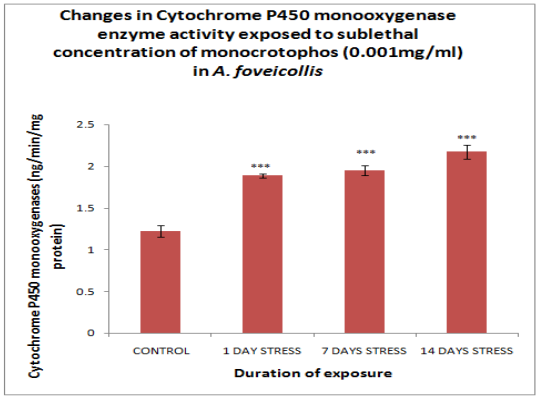

Figure 4. Cytochrome P450 enzyme activity in *A. foveicollis* exposed to sublethal concentration of monocrotophos (0.001mg/ml). Data are presented as mean± standard error (n=3). **p*<0.05, ***p*<0.01, ****p*<0.001

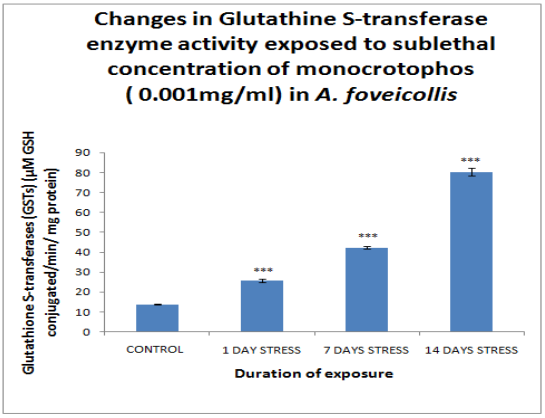

Figure 5. Glutathione S-transferase enzyme activity in *A. foveicollis* exposed to sublethal concentration of monocrotophos (0.001mg/ml). Data are presented as mean± standard error (n=3). **p*<0.05, ***p*<0.01, ****p*<0.001

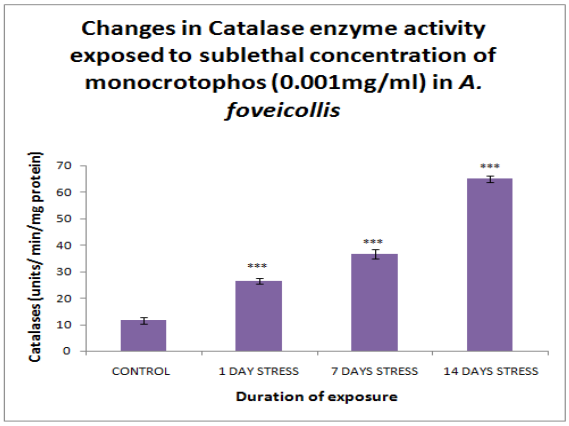

Figure 6. Catalase enzyme activity in *A. foveicollis* exposed to sublethal concentration of monocrotophos (0.001mg/ml). Data are presented as mean±standard error (n=3). **p*<0.05,***p*<0.01,****p*<0.001

Disscussion

Polyphagous insects have developed diverse resistance mechanism to cope with plant chemical defenses and synthetic insecticides.[14] Some detoxifying enzymes are involved in plant toxins and insecticide metabolism.

Phagocytic activity is expressed by the generaration of nitric oxide. Chakraborty *et.al.*[6] found an elevation of NO generation in the haemocytes of *Lamellidens marginalis* by the exposure of lowest concentration of sodium arsenite. The present result also found an increasing NO generation in the haemolymph of *A. foveicollis* by the exposure of the sublethal concentration of monocrotophos.

Esterase enzymes catalyse hydrolysis reaction of detoxification. In *Trichoplusia ni* and *Spodoptera littoralis* esterases hydrolyze the trans isomer of permethrin and cypermethrin more extensively than the cis-isomer.[15-16] When *A. foveicollis* treated with sublethal concentration of monocrotophos, esterse enzyme activity increases because the enzyme hydrolyses the insecticidal activity of monocrotophos. So, our result is similar with the previous report.

Glutathione (GSH) is a tripeptide consisting of glutamic acid, cysteine and glycine residues. Glutathione S-transferases catalyse the conjugation of GSH with a wide range of xenobiotics. In insects the enzyme plays an important role in the detoxification of insecticides. Kostaropoulos *et.al.*[17] found high GST activity against some organophosphorous insecticides-parathionmethyl, paraoxon-methyl in yellow mealworm, *Tenebrio molitor*. The present result shows similarity with this report because in *A. foveicollis* GST activity increases against monocrotophos.

Cytochrome P450 monooxygenase is a major detoxifying enzyme. It catalyses all the phase 1 reactions such as hydro-xylation, dealkylation and oxidative deamination. Brogdon *et.al.*[9] found an elevated cytochrome P450 mono-oxygenase activity in permethrin treated mosquitoes. The present result

also showed higher activity of this enzyme in *A. foveicollis*, treated with monocrotophos.

Catalase is a common enzyme found in nearly all living organisms. It helps decomposition of hydrogen peroxide to water and oxygen. So, it is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). The intracellular and extracellular activity of catalase was increased in the haemolymph of *Crassostrea gigas*, exposed to leucine-enkephalin (L-ENK).[18] Our result also showed higher activity of catalase in *A. foveicollis* in different days of exposure.

The present results indicate that *A.foveicollis* may have developed resistance to monocrotophos and this resistance may be provided by the higher activity of detoxifying enzymes. Therefore, it may be concluded that the resistance to synthetic insecticides represents vital areas of research for developing long-term sustainable insect control strategies for the effective management of pests of concern.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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Chapter 2

Metal Toxicity in Fishes: A Human Health Hazard

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Metal toxicity in fishes: A Human Health Hazard

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Abstract

Urbanization and industrialization have persistently contaminated most of the natural environment, and consequently major cities around the globe are under a budding threat of pollution. The environment is continuously loaded with foreign organic chemicals (xenobiotics) released by urban communities and industries. Among different pollutants, metal pollution of aquatic environment has become a great concern in recent years because of their non-biodegradable nature, long biological half-life and their potential to accumulate in different body parts of an organism. They can also be concentrated along the food chain, producing their toxic effect at points far distant from the source of pollution. Thus, compared to other types of aquatic pollution, heavy metals pollution is less visible but its effects on the ecosystem and humans can be intensive and very extensive. Fish are regarded as indicators of metals contamination in the aquatic ecosystem as they occupy high trophic levels. It constitutes a major part of the human diet because of its high protein content, low saturated fat and presence of omega fatty acids. But there is a growing concern that metals accumulated in fish muscle tissues may represent a health risk, especially for populations with high fish consumption rates. Excessive pollution of surface waters could lead to health hazards in man, either through drinking of water and/or consumption of contaminated fish. It has been reported in several studies that metal toxicity can result in damaged or reduced central nervous system function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Therefore, it is important through regular monitoring to identify trace elements, and to take protective measures against excessive exposure to these toxic substances during consumption of fish by humans.

Keywords: Metal Toxicity, Biomagnification, Pollution, Biotoxicity, Human Health

Introduction

The metals are considered as critical toxic contaminants of aquatic ecosystems, due to their high potential to enter and accumulate in food chain.[1] Metals may enter aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills/dumpsites and atmospheric deposits.[2] Metals like iron and manganese are required for metabolic activities in organisms, but some other elements like arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc exhibit toxicity effects on aquatic organisms.[3] In aquatic ecosystem, metals have received considerable attention due to their toxicity and accumulation in biota and fishes.[4] Deposition of contaminants in the aquatic systems, including metals, can lead to elevated sediment concentrations that cause potential toxicity of the aquatic biota.[5] Variations may be observed in accumulation pattern of heavy metals depending on varying food habit and tropic occupation of the fish. The

concentration of any pollutant in any given tissue in fish is therefore depends on its rate of absorption and the dynamic processes associated with its elimination by the fish. Fish accumulates heavy metals in the tissue through absorption and humans can be exposed to heavy metals via food chain. This can cause acute and chronic effects in humans. Studies have been taken worldwide on the contamination of different fish species to determine their heavy metal contamination and human health risk.

Metal toxicity in Fishes

Fishes are major part of the human diet due to high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health therefore, various studies have been taken worldwide on the contamination of different fish species by heavy metals.[6,7,8,9,10] Fishes have been widely used as bio-indicators of pollution by metals. Muscle tissue of fish is the most frequently used for analysis because it is a major target tissue for metal storage and is the main edible part of the fish. The presence of metals in fish species depends on the age and development fishes, and other physiological factors. The fishes are the single largest sources of As and Hg for human beings. The heavy metals released from domestic, industrial and other man-made activities can highly contaminate the aquatic systems. Such contaminations can seriously affect the ecological balance and diversity of aquatic species. For evaluation of health of aquatic systems, fishes are widely used because pollutants present in food chain cause ill-effects and death of aquatic animals. Therefore, the problem of metal pollution is one of the major health problems in the persons who eat sea foods. Due to metal pollution, cellular level damage has been observed, which possibly affect the ecological balance. Concentration of metals in fish muscle tissues of fishes from East Kolkata Wetlands can be seen in Table 1.[11] It is believed that many metals exert their toxic effects by distressing the enzyme systems of fishes. Many of them bind to specific enzymes and proteins necessary for cellular function and thus compete with other substances essential for maintenance and the continued function of cells. Thus, the metals can also have the effect of inducing mineral deficiencies. Additionally, many metals appear to assist in the formation of the paramagnetic anion, superoxide (O_2^-), which itself is toxic and seems widely responsible for the spontaneous cell death.[12]

Metals	FAO/WHO guidelines	Mean	Range	
			Minimum	Maximum
Copper	30	7.54±0.94*	2.12	27.94
Zinc	100	47.80±3.29	12.30	92.70
Manganese	1.0	4.24±0.65	NT	12.97
Iron	100	58.66±6.34	16.52	186.03
Cadmium	1.0	0.31±0.12	NT	2.99
Nickel	10	4.03±0.55	NT	8.97
Mercury	-	0.41±0.04	0.09	0.77
Arsenic	-	0.45±0.07	NT	1.22

** SE= standard deviation /√n*

Table 1: Concentration mean and range (µg g-1dry wt) of metals in muscle tissues of fishes from East Kolkata Wetlands (n=35)[11]

Metal toxicity in Humans

There are around thirty chemical elements that play a pivotal role in various biochemical and physiological mechanisms in living organisms and recognized as essential elements for life. Majority of the known metals and metalloids are very toxic to living organisms and even those considered as essential, can be toxic if present in excess. Concentrations of several toxic metal and metalloids have been largely increased as a result of human activities which cause threat to human wellbeing.

Some heavy metals (like Fe, Zn, Ca and Mg) have been reported to be of bio-importance to man and their daily medicinal and dietary allowances had been recommended and is presented in Table 2.However, some others (like As, Cd, Pb, and methylated forms of Hg) have been reported to have no known bio-importance in human biochemistry and physiology and consumption even at very low concentrations can be toxic.[13] Even for those that have bio-importance, dietary intakes have to be maintained at regulatory limits, as excesses will result in poisoning or toxicity, which is evident by certain reported medical symptoms that are clinically diagnosable.[14]

	Age (Years)	Weight (kg)	Ca (mg)	Fe (mg)	Mg (mg)	Zn (mg)
Infants	0-½	6	360	10	60	3
	½-1	9	540	15	70	5
Children	1-3	13	800	15	150	10
	4-6	20	800	10	200	10
	7-10	30	800	10	250	10
Males	11-14	44	1200	18	350	15
	15-18	61	1200	18	400	15
	19+	67+	800	10	350	15
Females	11-18	44-54	1200	18	300	15
	19+	58	800	18(10)*	300	15
Pregnant			1200	18+**	450	20
Lactating			1200	15	450	25

Table 2: Recommended (Daily) Dietary Allowances (RDA) of The Food and Nutrition Board (Published by the National Academy of Science, Washington, DC, U.S.A).

The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminium poisoning: gastrointestinal (GI) disorders, diarrhoea, stomatitis, tremor, haemoglobinuria causing a rust–red colour to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapours and fumes are inhaled.[15] The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic.

According to the International Agency for Research on Cancer (IARC), inorganic As and Cd are classified as human carcinogens.[16] As is related to cancer risk and skin damage, Cd is linked to kidney damage and cancer. Other effects such as heart diseases and blood cholesterol from Sb, Anemia from Pb, kidney and liver damage from Hg, and gastrointestinal disorder from Cu are also reported.[17]

An overview of detrimental caused by metal toxicity is enumerated in Table 3. In brief As was reported to be detrimental to the central nervous system and cognitive development in children.[18] It was also found to accumulate in fingernails and hair.[19]

Heavy Metal	Detrimental Effects/Toxicities
Arsenic (As)	Water-soluble inorganic As is readily absorbed from digestive system. Inorganic forms of As are particularly toxic. It causes irritation to lung, stomach and intestine, skin disturbances, and decreased formation of RBCs and WBCs. Very high concentrations of inorganic As can cause infertility, skin disturbances, decreased resistance to infections, heart disruptions, brain damage and death. The acute LD_{50} (oral) of As ranges from 10-300 mg/kg.
Lead (Pb)	It can enter the body through ingestion and inhalation. Its maximum allowable levels may be 5 μg/L (in bottled water) to set elemental impurities limit. It can cause disruption of biosynthesis of Hb, anaemia, high B.P., kidney damage, reproductive/fertility problems and brain or nervous system damage.
Mercury (Hg)	Its prevalence in environment can lead to biomagnification in food chain. The organic Hg, such as methyl Hg, is more toxic than inorganic Hg due to ease of absorption into human system. The toxicity of Hg include: kidney damage, disruption of nervous system, damage to brain, DNA and chromosomal damage, allergic reactions, sperm damage, birth defects and miscarriages. The LD_{50} of Hg is as low as 1 mg/kg in small animals.
Cadmium (Cd)	Cd is more readily absorbed through the lungs than the digestive system. It can damage kidneys, CNS and immune system. It can also cause bone fractures and reproductive problems. It can cause stomachaches, diarrhoea and vomiting. The LD_{50} (oral) of Cd in animals ranges from 63-1125 mg/kg.

Table 3: An overview of detrimental effects caused by metal toxicity of As, Pb,Hg and Cd

Next to As, Pb was most investigated in the context of its public health effects. Pb can affect the central nervous, renal, hemato-poietic, cardiovascular, gastrointestinal, musculoskeletal, endocrinological, repro-ductive, neurological, developmental and immunological systems.[17]

The third most frequently reported toxic metal is Cd, and it has been identified as a public health concern[17].Cadmium is a cumulative toxicant and carcinogenic that affects kidneys, genrates various toxic effects in the body, disturbs bone metabolism and deforms reproductive tract as well as endocrine system. There are several morphopathological changes in the kidneys due to long-term exposure to cadmium.An exposure to cadmium increases calcium excretion thus causes skeletal demineralization, probably leading to increases in bone fragility and risk of fractures.[20]

Mercury (Hg) and its compounds are highly toxic, especially methylmercury - a potent neurotoxin. Hg is considered a global pollutant, being deposited even in remote pristine aquatic systems, where it is biomagnified through the food chain. Minamata disease name given after of methylmercury in seafood in Minamata and Niigata in Japan in the 1950-1960's, caused the death of thousands of people.A few studies reflect that even minor increases in methylmercury exposures can cause harmful effects on the cardiovascular system, blisters in the upper gastrointestinal tract, vomiting, abdominal pain, constipation and gastritis. Renal toxicity of organic forms expressed by glomerulonephritis with proteinuria (glomerular and tubular) and nephritic syndrome.[21] Elemental Hg can be oxidized to Hg2+, which accumulates preferentially in the kidneys. The increased excretion of low molecular-weight proteins demonstrated at low-level exposure and related to damage to the renal tubes. It is a potent neuro-toxin to human due to their ability to cross the blood-brain barrier. It is absorbed in the gastrointestinal track, immediately entering the blood stream. It readily passes the placental barrier affecting the developing nervous system of the foetus.[21] Continuous exposure conditions to elemental Hg can lead to its accumulation in the thyroid. The acute exposure to elemental Hg vapours can cause "pink disease" or acrodynia.

Conclusion

When considering the metal concentrations in fish species, the most important aspect is their toxicity to

humans suitable for human consumption. The metals, viz., As, Cd, Pb and Hg are most toxic to all human beings, animals, fishes and environment. Though some metals are essential for animals, plants and several other organisms, all metals exhibit their toxic effects via metabolic interference and mutagenesis. The Pb and Hg cause severe toxicity in all. Fishes are not the exception and they may also be highly polluted with these metals, leading to serious problems and ill-effects. Therefore, it is important through regular monitoring to identify trace elements, and to take protective measures against excessive exposure to these toxic substances during consumption of fish by humans.

Conclusively, the advances of toxicology have improved our knowledge about human exposure to toxic elements (metals and metalloids) and their health effects, such as developmental retardation, several types of cancer, kidney damage, endocrine disruption, immunological, neurological effects and other disorders. Several ongoing research works throw more light onto new insights and biochemical and molecular mechanisms involved in the development of pathological conditions in human. Although metal poisoning could be clinically diagnosed and medically treated, the best option is to prevent metal pollution and the subsequent human poisoning.

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Chapter 3

Arsenic toxicity through Dietary Intake pathways in the villages of West Bengal, India: A Review

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Abstract

Arsenic possesses a severe threat to human health through exposure pathways mainly water and diet. Under different geochemical conditions, arsenic can be mobilized from sediment to ground water. Many populations have been exposed to arsenic toxicity through contaminated ground water causing arsenicosis and high risk of skin cancer. Drinking water (ground water) is not only the elevated source of arsenic in affected areas of West Bengal. Irrigation of agricultural fields with arsenic contaminated ground water has led to arsenic build-up in soils, with consecutive elevation of arsenic in crops grown up on the soils. The risks not only for adults but also infants and children, due to high As concentration in rice and rice products, crops and vegetables.

Keywords: Geochemical, Arsenicosis, Cancer, Ground water.

Introduction

Arsenic is a non-essential and non-beneficial constituent that may be present in water. The World Health Organization (WHO)[33,35] lists arsenic as one of the 10 chemicals of major public health concern. Long term consumption of drinking water contaminated with naturally occurring soluble inorganic arsenic leads to chronic arsenic poisoning also called arsenicosis [24]. Arsenicosis is a chronic illness resulting from drinking water with high levels of as over a long period of time. It is commonly known as poisoning. Arseniasis means chronic arsenical poisoning, also called arsenicalism; the term arsenicism refers to a disease condition caused by slow poisoning with As.

West Bengal is an arsenic endemic state in India, with at least 9 out of 18 districts exposed to ground water contaminated with arsenic (of geological origin) above the WHOs maximum permissible limit of10mcg/L2. In fact the Ganga-Brahmaputra plains in India (7 states) and the Padma-Meghna plains in Bangladesh together constitute the most wide spread arsenic-affected area in the World.[3,17] The Govt. of West Bengal currently estimates atleast 79 blocks (administrative units) across the state to be severely affected involving 26 million individuals across 2600 villages. Exposure primarily occurs through drinking ground water contaminated with inorganic arsenic salts, and from food prepared or crops irrigated using high-arsenic water sources.[12]

Dietary intake of heavy metals poses risks to both human and animal health. Although wide range of people is exposed to arsenic (As) through the consumption of Ascontaining water; foodstuffs (i.e., crops, vegetables)[1] are also a significant route of exposure for remarkable amount of

people. Even though diet *plays a*significant role in human as exposure, there was lack of data on inorganic As (inAs) in foodstuffs in the literature until a decade ago. According to EFSA (2009)[11] almost 98% of published studies in 15 European countries considered total As in various foodstuffs without differentiating organic and inorganic As species. Since organic as species are less toxic to the human body when compared with inorganic species, future studies should concentrate on such studies, especially on bioavailability and toxicity of As depending on its various species. Thereby, overestimation of health risks will be avoided.

Pathways of arsenic exposure

Arsenic exposure through drinking water

Groundwater with elevated concentrations of As has been recognized as a problem of global concern.[4,,7] As contamination of groundwater is one of the principal pathways of human exposure to inorganic As. Elevated concentrations of As have been reported from several regions of the world[20] resulting primarily from natural sources, such as erosion and leaching from geological formations, although sometimes from anthropogenic sources, such as uses of As for industrial purposes, mining activities and metal processing, and application of pesticides and fertilizers containing As. The risk of Ascontamination is generally much higher in groundwater compared to surface water.[4, 5]

Arsenic exposure through different vegetables and pulses

Studies were carried out in West Bengal-India, on various crops which were irrigated by As enriched groundwater andwas observed for considerable variations in the amount of As accumulation in differentvegetables.[29] Underground tuberous vegetables (such as arum, radish and potato) contained the highest As concentrations (780±243µg/ kg, 674±211µg/ kg, 291±176µg/ kg) while leafy vegetables (cabbage, spinach etc) contained the second highest As concentrations (315±69.7µg/kg, 270±182 µg /kg). However, unexpectedly cabbage within leafy vegetables showed higher As concentration than potato. The third highest group was found to be fruity and fleshy vegetables (bitter gourd, brinjal, tomatoetc.) (262±133µg/kg, 217±80µg/kg, 84.4±48.5µg/kg)and lastly the fourth highest group was pulses (lentil, pea) (24.7 ±16.7 µg/ kg , 69.2 ± 22.9 µg/ kg). On the other hand, the study at the same time showed that crops which require more water for their growth generally had higher As concentrations than crops which need less water. This finding illustrates that using of As enriched groundwater for irrigation purposes can lead to transfer of As through the water-soil-crop-food chain.

Similarly,[6] analyzed some leafy, fruity, tuberous and pulses vegetables grown in As contaminated areas in West Bengal-India and compared those collected from market basket. Thirty-two types of vegetables and seven types of pulses were collected from the agricultural areas and eighteen products (vegetables and pulses) were collected from the market. Among all cultivated vegetables tested in this study, pea and lentil from the pulses family showed the highest as concentrations with 1300-480 µg/kg and 1120 -144 µg/kg respectively. These are the highest values of As measured in all vegetables listed in this review. These values are also exceeding the WHO-recommended permissible limit (1000 µg/kg) for foodstuffs. On the other hand,

among roots and tubers, arum tuber—as expected—showed the highest concentration 558-73 µg/kg and onion bulb showed the lowest with 187-77 µg/kg. Interestingly, spinach (910 - 259 µg/ kg) had a higher concentration than tuberous vegetables. Except tomato (551-262 µg/kg) and bitter gourd (529 - 44 µg/kg), fruity vegetables showed again the lowest concentrations. The total As value of vegetables taken from the market appeared to be lower than the level observed in field vegetables. It was reported that vegetables obtained from the market were imported from nonlocal areas.

Thus, it can be concluded that only field surveys or only market surveys are not adequate to give actual photograph of As contamination of foodstuffs. Results of that study showed the highest concentrations almost for each kind of vegetables among all other results taken from different studies. Only the concentrations of As in arum and radish were found higher by Samal[29] although they both tested vegetables cultivated in Nadia District, West Bengal, can lead to transfer of As through the water-soil-crop-food chain.

Arsenic exposure through rice

Inorganic arsenic (in As) content in a food varies according to food sub-types and samples. FDA (2012) demonstrated for rice-based cereals that three different samples had different concentrations of 16%, 61% and 87% in As respectively. On the other hand, for brown rice of varying types (jasmine, long-grain, short-grain, sticky) concentrations ranged from 26[35] to 95%.[15] Besides the type of product, processing and preparation of food, washing and cooking methods are other factors that lead to a change in As concentration. The effect of preparation and cooking processes on As retention in rice is well reported in literature. A study suggested that rice and rice products such as bran and rice flours have higher in As levels than polished rice.[18] Similarly,[26] carried out some tests on white and brown rice which had same origin and saw that white rice has a lower total level of in As (160 µg/kg) than brown (400 µg/kg) rice.[26] The reason of this is that as the rice is polished to get whiter, bran which has a higher As concentration, is removed from the surface of rice, resulting in low As concentrations.[11] On the other hand,[25] and [19] suggested based on their studies that, rinsing rice with As free water before cooking might eliminate As from As laden rice, resulting in less As in cooked rice than that in uncooked rice. However, in this case essential trace elements (such as Cu, Mn and Zn) enhancing growth and human health, might also be removed along with As.[20] In contrast, it should be born in mind that Asconcentration in food can be increased by washing it with As bearing water. Availability of especially inorganic As in changing concentrations in water to be used in cooking will change the As content of the food. For example, As bearing water can alter As concentration in rice after cooking due to contaminated water taken up by rice [31,14] .

The effect of preparation and cooking processes on as retention in rice is well reported in literature.[18] suggested that rice and rice products such as bran and rice flours have higher inAs levels than polished rice. Similarly,[27] carried out some tests on white and brown rice which had same origin and saw that white rice has a lower total level of inAs (160 µg/kg) than brown (400 µg/kg) rice. The reason of this is that as the rice is polished to get whiter, bran which has a higher As concentration, is removed from the surface of rice, resulting in low As concentrations.[11,18] reviewed based on their data that cooking

rice in a high volume of water reduces the total in As by up to 20% compared to raw samples as well as samples cooked in a small volume of water.[10] tried to attract attention to rice-related danger for children and suggested that rice consumption is a significant source of As exposure for children. Infants and children are exposed to As 2–3 times more than adults, consuming rice based products such as biscuits, crackers, pasta, noodles, pudding, whole grained rice etc. during their childhood.[8]

Conclusion

In conclusion, arsenic is oneof the most important toxic and carcinogenic metalloids, which can cause serious health risks to living organisms, exposed trough soil, water, air and plants. The risks in question do not only threat adults but also infants and children, due to high As concentration displayed by rice and rice products. High As concentration on crops and vegetables were especially due to irrigation with As enriched waters. It ccan be said in general that tuberous vegetables accumulate the highest As levels and the concentration gradually decrease respectively for leafy vegetables, fruity vegetables and pulses. Following the assessment of its amount and its distribution, gaining control and applying remediation measures should continue. Even though it is impossible to get rid of natural as resources, at least anthropogenic As resources such as As containing pesticides, herbicides, chemicals etc. can be forbidden by the authorities.

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Chapter 4

Leishmania donovani - Murine Peritoneal Macrophage Attachment Induces Parasite Specific Protein Kinase C (PKC)–mediated phosphorylated proteins

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Leishmania donovani- murine peritoneal macrophage attachment induces parasite specific Protein Kinase C (PKC)–mediated phosphorylated proteins

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Abstract

Leishmania donovani infects and enters macrophages by receptor-mediated endocytosis. Incorporation of exogenously added [^{32}P] orthophosphate into murine peritoneal macrophage during attachment with *L. donovani* promastigotes induced phosphorylation of certain proteins. Addition of Phorbol 12 myristate 13 acetate (PMA) activator of PKC, induced the phosphorylations. Staurosporine, an inhibitor of PKC blocked phosphorylations of these proteins. However, infection of macrophages with *L.donovani,* significantly attenuated phosphorylations of these proteins. *Phosphorylations* of these proteins were absent during attachment with other intra-macrophage pathogen, *E. coli* or *Shigella flexneri* indicating that *L. donovani* specific signals were induced in phosphorylation. These suggest that induced PKC-mediated phosphorylations signals were *L. donovani* attachment specific and signal differ significantly with intracellular replication of the parasites.

Keywords: *Leishmania donovani*, Macrophage, Attachment, PKC, Phosphorylation

Introduction

Leishmania donovani parasite binding to macrophage and its interaction with these cells efficiently occurs with parasite binding on the surface of the host cell to mannosylfucosyl receptors and CR1 and CR3 integrin receptors.[1-3] Following binding of ligands, integrin and other receptors on the host cell surface have been demonstrated to generate Tyrosine or serine and threonine specific phosphorylation signals necessary for the uptake of various particles.[4-6] Bacterial entry in many cells were shown to occur through tyrosine specific phosphorylation signals,[7-11] however, establishment of PKC-specific phosphorylation in bacterial strains as well as during leishmanial entry has not been demonstrated. Although, reports of PKC-specific phosphorylation in phagocytosis by macrophages[12] and F_C receptor-mediated phagocytosis by monocytes was noted.[13] The present study was therefore undertaken to investigate the protein phosphorylation pattern of murine peritoneal macrophages during attachment with *L. donovani* promastigotes. And demonstrated that PKC mediated specific proteins were phosphorylated with exogenous orthophosphate when murine peritoneal macrophages were stimulated with *L. donovani* attachment. Phosphorylations of these specific proteins were compared with the macrophages after established infection. Comparison of these specific proteins were also done to investigate whether attachment of *L. donovani* associated phosphorylation signals occur in other intramacrophage pathogens such as *Shigella flexneri* and *E. coli*. The role of

these specific proteins in the context of recognition, uptake and intracellular survival of *L. donovani* promastigotes in permissive murine peritoneal macrophages were discussed.

Materials and methods

Media and chemicals

Medium 199, Penicillin, Streptomycin, Gentamycin were from Gibco Laboratories, Grand Island, NY, USA, RPMI-1640, Fetal bovine serum (FBS) (heat inactivated) was from Difco, USA. Staurosporine, Leupeptin, Aprotinin, Pepstatin, Phenyl methyl sulfonyl fluoride (PMSF), PMA, *E. coli* LPS, Cytochalasin-D, NaF, NaH_2PO_4, $NaHPO_4$ sodium pyrophosphate, EDTA, EGTA, Vanadate, ATP were from Sigma. 9,10 (n)-[^{3}H]-myristic acid is from Amersham-pharmacia Biotech. [^{32}P]-orthophosphate from BARC, India and all other reagents used were of analytical grade.

Parasites *L. donovani* virulent strains AG83 (MHOM/IN/1983/AG83) and GE-1 (MHOM/IN/89/GE-1) strains were maintained in female BALB/c mice as described.[14] Parasites were recovered from the spleen of infected mice by adding spleen to culture medium M199 supplemented with 20% FBS, 100U/ml Penicillin,100 µg/ml Streptomycin. The cultures were kept for 5 days at 22°C to obtain promastigotes which were then washed and used for infection of macrophages. Bacterial strains, *E. coli* DH-5α and *Shigella flexneri* were gifts from Indian Institute of Chemical Biology (IICB), Infectious Disease Group.

Murine Peritoneal macrophages

Macrophages were isolated as.[15,16] Briefly, thioglycollate (TG)-elicited macrophages were isolated by peritoneal lavage from male BALB/c mice. Four days after injection, cells were isolated from the peritoneal cavity, by washing with 3-4 ml of RPMI-1640 medium supplemented with 25mM HEPES, 100 µg/ml Streptomycin, 100 U/ml Penicillin and 20% of FBS. Cells were then plated onto 22mm coverslips and allowed to adhere at 37°C for 2hrs. Non-adherent cells were washed out by extensive washing with PBS. Cells, thus obtained was 80-90% viable as tested by trypan blue exclusion method.

Attachment or Infection of murine peritoneal macrophages with parasites

Murine peritoneal macrophages (10^7/ml) were treated with the drug Cytochalasin-D (1.5µg/ml) for 1hr at 37°C before infection and continued to the end of incubation with parasites. *L. donovani* promastigotes or bacterial strains *E.coli* or *Shigella flexneri* were then added to murine peritoneal macrophage monolayers at a 10:1 parasite–to-cell ratio and kept at 37°C for 1-2 hrs. For established infection, cells were incubated in absence of the drug. Non-interacted parasites were removed by three washes with warm PBS. For intracellular replication, macrophage cells were kept in culture for additional 24-48 hrs. Attachment or infection levels were determined by microscopic examination of Giemsa-stained cell

Protein phosphorylation in parasite interacted murine peritoneal macrophages

Phosphorylation of murine peritoneal macrophage proteins in response to interaction with parasite was detected as previously described[17] with minor modifications. In brief, murine peritoneal macrophage monolayers were washed four times with phosphate free DMEM or loading buffer (LB) [10 mM Hepes, 2.7mM KCl, 138 mM NaCl, 7.5 mM D-glucose] and then incubated for 4 hrs at 37°C in this medium containing 250-300µci of [^{32}P] orthophosphoric acid /ml. At the end of this incubation, macrophages were treated

with *L. donovani* promastigotes or different bacterial species for 30min-1hr in presence of Cytochalasin-D as indicated in the Figures legend. For phosphorylation in established infection, infected murine peritoneal macrophage cultures were incubated at 37°C in 5% CO2 for 24-48 hrs and then labeled with 250-300µci of [^{32}P] orthophosphoric acid/ml for 2-3hrs at 37°C. After incubation, the macrophage monolayers were washed with cold PBS and then scraped in homogenizing buffer (HB) (0.25M sucrose, 20 mM Hepes, 0.5mM EGTA, pH7.0), containing protease inhibitors (2mM Leupeptin. 0.5mM PMSF,10µg/ml pepstatin, 10µg/ml aprotinin) and phosphatase inhibitors (10mM NaF, 5mM EDTA, 1 mM ATP, 10 mM NaH2PO4, 10 mM Na2HPO4, 5mM disodium pyrophosphate). The cells were then pelleted and solubilized in sodiumdodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (18) sample buffer and then incubated in 95°C for 10min. Samples (~8x 104 cpm) were electrophoresed in 8 or 10% polyacrylamide gels containing SDS and subsequently stained with coomassie brilliant blue and destained. Dried gels were exposed to Kodak X-OMAT film with intensifying screens to detect changes in the [^{32}P] –labelling of the various proteins.

Results

Protein phosphorylation of murine peritoneal macrophages during *L. donovani* attachment

Prior treatment of Cytochalasin-D, inhibitor of actin polymerization followed by infection of murine peritoneal macrophage monolayers for 1-2hrs with a virulent strain, GE-1 of *L.donovani* promastigotes(Figure-1.1) triggered phosphorylation of multiple proteins, including 29-, 45-, 67- and 95-kDa proteins (Figure-1.2). However, 29- and 45 kDa bands were obtained from phosphorylation of most prominent proteins. Phosphorylation profiles were slightly better in DMEM than in loading buffer. Addition of PMA, an activator of PKC further induced the phosphorylation of these proteins. Qualitative scanning (pixel intensity, Figure-1.3) of the autoradiograph and the summary of the scan (Table-1) demonstrated that the pixel intensity of 29 kDa protein in PMA-induced *L. donovani* attached macrophages was 119± 7.12 (n=4) (in DMEM) and 85 ±3.51 (in loading buffer) compared to 50.25± 3.92 (in DMEM) in normal macrophages. Similarly, pixel intensity for 45-kDa protein in PMA-induced *L. donovani* attached macrophages was 58.5 ± 3.16 compared to not detectable in normal macrophages. Thus, attachment of *L. donovani* GE-1 strain in unstimulated or PMA-stimulated murine peritoneal macrophage cells triggered selectively the phosphorylation of 29- and 45-kDa proteins.

Table-1[#]

Lane	Cells+Treatment	Band (Kd)	Pixel Intensity±S.D*
1.	Macrophage (Normal)	29	50.25± 3.92
		45	-
2.	Macrophage+PMA+Cyto D+*L. donovani*(GE-1)	29	119 ± 7.12
	(DMEM medium)	45	58.5 ± 3.16
3.	Macrophage+PMA+Cyto D+*L.donovani* (GE-1)	29	85 ± 3.51
	(Loading Buffer)	45	34.5 ± 2.26
4.	Macrophage+Cyto D+*L. donovani*(GE-1)	29	85.25 ± 2.62
	(DMEM medium)	45	36.25 ± 2.58
5.	Macrophage+Cyto D+*L. donovani* (GE-1)	29	70.87 ± 4.41
	(Loading Buffer)	45	29.75 ± 2.70

#Summary of the Fig. 1.3

* Pixel intensity values are mean of four different autoradiographs ± S.D;

Cyto.D-Cytochalasin D

Fig. 1.1 Peritoneal Macrophages in attached condition with *L. donovani* promastigotes.

Attachment levels were determined by microscopic examination of Giemsa-stained cells and photographed under 100x oil immersion.

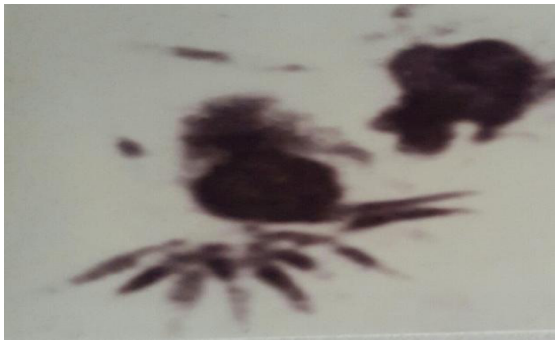

***Fig:1.1 Peritoneal Macrophages in attached condition with L.donovani promastigotes*.**

Fig. 1.2 Protein phosphorylation in murine peritoneal macrophages during *L.donovani* attachment**.** Macrophages were then pretreated with Cytochalasin-D and then subjected to stimulation with or without PMA. The cells were then subjected to SDS PAGE followed by autoradiography (see Materials and Methods). Lane-1, normal macrophage cells; Lane-2, *L. donovani* attached PMA-stimulated cells in DMEM; Lane-3, same as Lane-2 but in Loading buffer; Lane-4, *L.donovani* attached but without PMA-stimulated cells in DMEM; Lane-5, same as Lane-4 but in loading buffer.

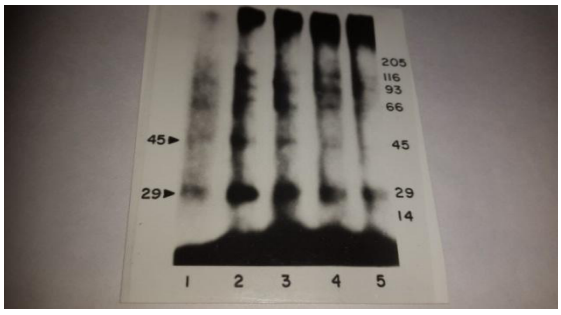

Fig:1.2 Protein phosphorylation in murine peritoneal macrophages during L. donovani attachment

Arrows indicate the lanes as in Fig.1.2 and molecular sizes of the proteins, [5] indicates 29 kd proteIn, [4]45 kd , [3]67 kd, [2] 95 kd and [1] 116 kd protein and similarly for other lanes.

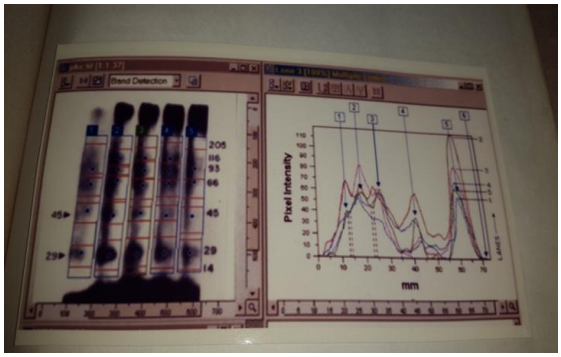

Fig:1.3 Qualitative scanning of the autoradiograph, Fig.1.2, analysed by Image Master Elite.

Attachment versus established infection: comparison of protein phosphorylation

It was then examined whether parasite attachment signal in phosphorylation differs or not with murine peritoneal macrophages after established infection. To develop established infection, murine peritoneal macrophages were infected with *L.donovani* virulent strain GE-1 for approximately 24-48hrs and the level of infection is shown in (Figure-2.1). Infected murine peritoneal macrophages were then labeled with [^{32}P]-orthophosphate and phosphorylation was compared with cytochalasin-D treated *L. donovani* attached macrophages. Phosphorylation of both 29-and 45- kDa proteins were significantly down regulated in infected macrophages(Figure-2.2). Comparison of scanning (Figure-2.3) of attachment versus established infection indicated that pixel intensities for 45 kDa proteins were 85.93 for attachment and 35.87 and 20.34 respectively for 24 hrs and 48 hrs infection. Similarly, for 29-kDa protein the pixel intensities were 126.67 for attachment compared to 80.75 for 24hrs infection and 51.25 for 48 hrs infection

(Table-2). These results therefore suggested that although the phosphorylation was induced during attachment of *L.donovani* promastigotes to the surface of the murine peritoneal macrophage,the phosphorylation of these proteins drastically reduced during the growth of the parasites inside macrophages.

Table-2#

Lane	Cells +Treatment	Band (Kd)	Pixel Intensity±S.D
1.	Macrophage (Normal)	29	67.00 ± 1.09
		45	13.01 ± 1.69
2.	Macrophage+Cyto D+	29	126.67 ± 1.78
	L. donovani(GE-1)	45	85.93 ± 1.78
3.	Macrophage +*L. donovani* (GE-1)	29	80.75 ± 0.91
	(24 hrs)	45	35.87 ± 2.07
4.	Macrophage+*L. donovani*(GE-1)	29	51.25 ± 1.66
	(48 hrs)	45	20.34± 1.28

#Summary of the Fig. 2.3

* Pixel intensity values are mean of four different autoradiographs ± S.D;

Cyto.D-Cytochalasin D

Fig: 2.1 Murine peritoneal macrophages infected with *L.donovani* parasites for 48 hrs.

Infection levels were determined by microscopic examination of Giemsa-stained cells and photographed under 100x oil immersion.

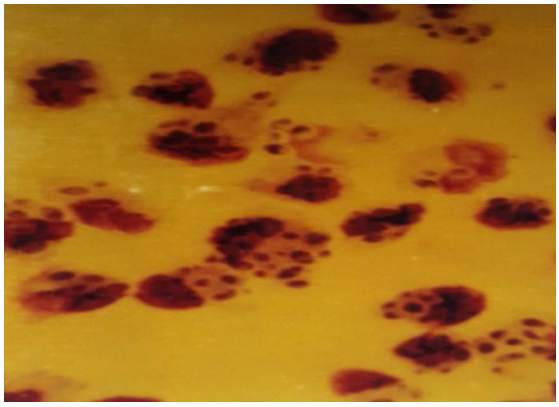

Fig: 2.1 Murine peritoneal macrophages infected with L.donovani parasites for 48 hrs.

Fig.2.2 Attachment versus established infection: comparison of protein phosphorylation.

Protein phosphorylation in murine peritoneal macrophages during *L.donovani* attachment or after 24-48 hrs of infection(see Materials and Methods). The cells were then solubilised in Laemmli buffer and subjected to SDS PAGE followed by autoradiography. Lane-1, normal murine peritoneal macrophage cells; Lane-2, *L.donovani*-attached macrophages; Lane-3, 24hrs infected macrophages; Lane-4, 48 hrs infected macrophages.

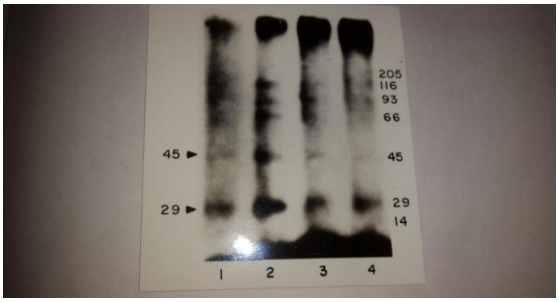

***Fig: 2.2 Attachment versus established infection: comparison of protein phosphorylation*.**

Fig: 2.3 Qualitative scanning of the autoradiograph, Fig.2.2, analysed by Image Master Elite. Arrows indicate the lanes as in Fig.2.2 and molecular sizes of the proteins,[5] indicates 29 kd proteIn, [4] 45 kd, [3] 67 kd [2] 95 kd and [1] 116 kd protein and similarly for other lanes.

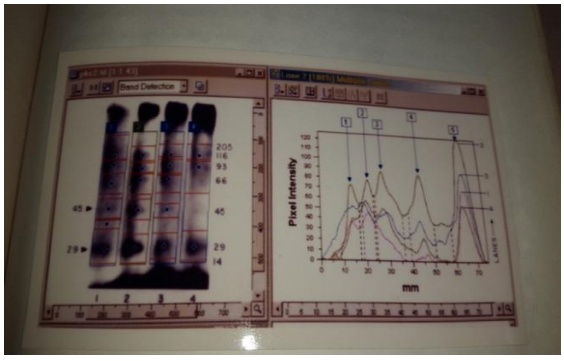

Fig: 2.3 Qualitative scanning of the autoradiograph, Fig.2.2, analysed by Image Master Elite

Inhibitor of protein kinase C blocks protein phosphorylation

To confirm the role of PKC in the phosphorylation process, phosphorylation reaction was done in presence of staurosporine, an inhibitor of PKC. Murine peritoneal macrophage cells were preincubated with staurosporine (1μM) for 15-30 min prior to further processing for phosphorylation reactions. Treatment of cells with staurosporine resulted in decreased expression of 29- and 45-kDa protein (Figure-3.1). The actual decrease in the intensity of these bands was found from the scanning of the autoradiograph (Figure-3.2). Pixel intensity values of the 45 kDa band for *L.donovani* attached macrophages was 66.78 compared to 20.05 in staurosporine treated macrophages and for 29-kDa band it was 110.52 in parasite-attached macrophages compared to 64.52 in staurosporine treated macrophages indicating the role of PKC in the phosphorylation reaction.

Fig.3.1 Analysis of PKC mediated phosphorylation in macrophages.

Autoradiograph of the Staurosporine treated phosphorylation experiment. Macrophage monolayers were pre-treated with or without Staurosporine prior to Cytochalasin-D treatment followed by infection with *L. donovani* parasites (as described in Materials and Methods). Lane-1, Normal murine peritoneal macrophages; Lane-2, *L.donovani* attached with Cytochalasin-D, macrophages cells; Lane-3, Staurosporine pretreated, *L. donovani* attached with Cytochalasin-D, macrophages cells.

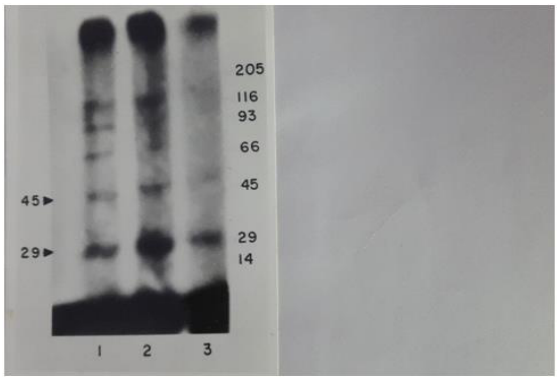

Fig: 3.1 Analysis of PKC mediated phosphorylation in macrophages

Fig. 3.2 Qualitative scanning of the autoradiograph, Fig. 3.1, analysed by Image Master Elite.

Arrows indicate the lanes as in Fig.3.1 and molecular sizes of the proteins, [5] indicates 29 kd protein, [4] 45 kd, [3] 67 kd, [2] 95 kd and [1] 116 kd protein and similarly for other lanes.

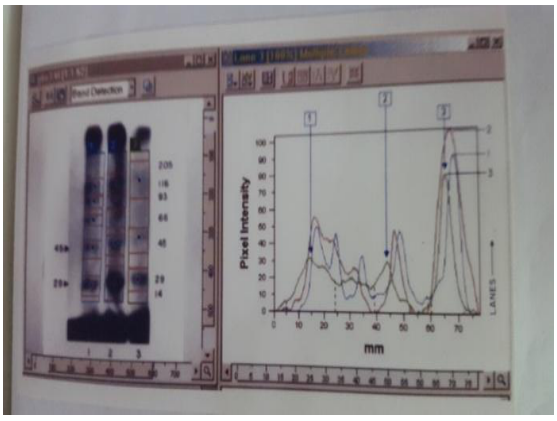

Fig: 3.2 Qualitative scanning of the autoradiograph, Fig. 3.1, analysed by Image Master Elite.

Analysis of parasite specific induction of phosphoproteins

It was next tested whether the induction in

protein phosphorylation was *L. donovani* specific or the exposure of murine peritoneal macrophages to other pathogens would also induce the observed protein phosphorylations. For this, [32P]-orthophosphate labeled murine peritoneal macrophages were incubated with either *L. donovani* promastigotes or *E.coli* (Figure-4.1, 4.2) or *Shigella flexneri* (Figure-4.3) in presence or absence of cytochalasin–D. All these species successfully internalized into macrophages [19,20]. Studies with *E.coli* or *Shigella flexneri*, using the same conditions as those with *L. donovani,* were performed for comparative purposes. Figures are representatives of autoradiographic and scanning data. These data showed that although *E. coli* internalization produced slightly more intense bands for 29- and 45 kDa proteins (Figure-4.4,4.5), attachment of macrophages with these bacteria was ineffective at inducing the phosphorylations of 29-kDa and 45 kDa proteins.

Fig. 4.1 Murine Peritoneal Macrophages in attached condition with bacterial strain of *E.coli DH 5α.*

Attachment levels were determined by microscopic examination of Giemsa-stained cells and photographed under 100x oil immersion.

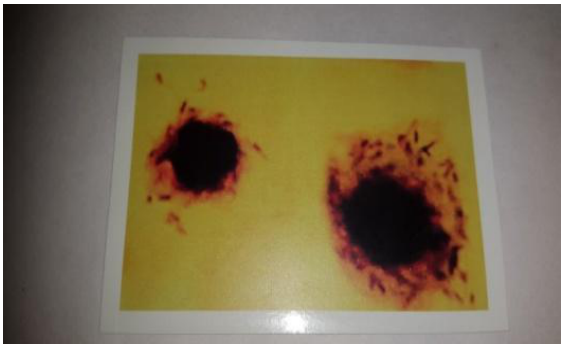

Fig: 4.1 Murine Peritoneal Macrophages in attached condition with bacterial strain of E.coli DH5α

Fig. 4.2 Murine peritoneal macrophage treated with bacterial strain *E.coli* DH 5α.

For intracellular replication, macrophage cells were kept in culture for additional 2hrs. Infection levels were determined by microscopic examination of Giemsa-stained cells and photographed under 100x oil immersion.

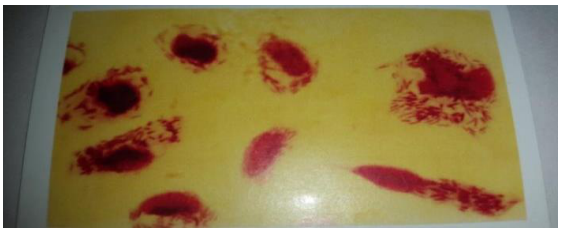

Fig: 4.2 Murine peritoneal treated with bacterial strain E.coliDH 5α.

Fig. 4.3 Murine Peritoneal Macrophages in attached condition with *Shigella flexneri.*

Attachment levels were determined by microscopic examination of Giemsa-stained cells and photographed under 100x oil immersion.

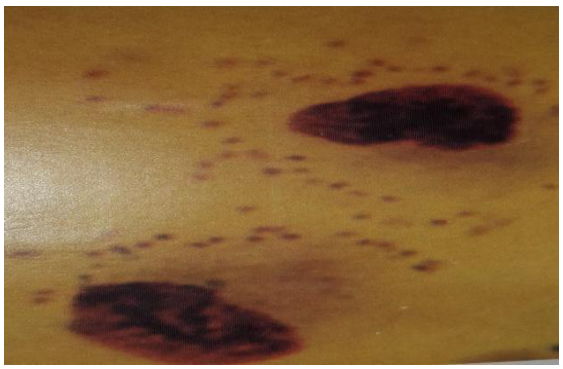

Fig: 4.3 Murine Peritoneal Macrophages in attached condition with Shigella flexneri

Fig: 4.4 Parasite specific induction of phosphoproteins.

Autoradiograph of the experiment performed with either *L.donovani* promastigotes or *E.coli* or *Shigella flexneri*

in presence or absence of cytochalasin–D. Lane-1, normal macrophages; Lane-2, *L.donovani* attached macrophages; Lane-3, *L. donovani,* 2hrs infected macrophages; Lane-4, *E.coli* attached macrophages; Lane-5, *Shigella flexneri* attached macrophages; Lane-6, *E.coli*, 2hrs infected macrophages.

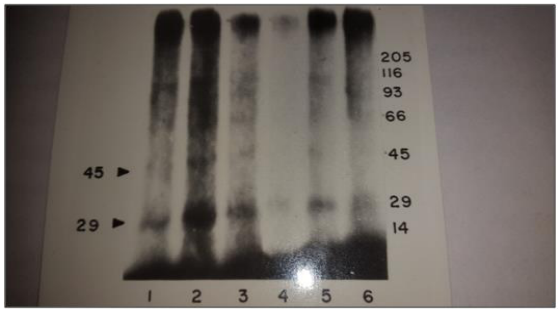

Fig: 4.4 Parasite specific induction of phosphoproteins

Fig. 4.5 Qualitative scanning of the autoradiograph, Fig.4.4, analysed by Image Master Elite.

Arrows indicate the lanes as in Fig.4.4 and molecular sizes of the proteins,[5] indicates 29 kd proteIn, [4] 45 kd, [3] 67 kd, [2] 95 kd and [1] 116 kd protein and similarly for other lanes.

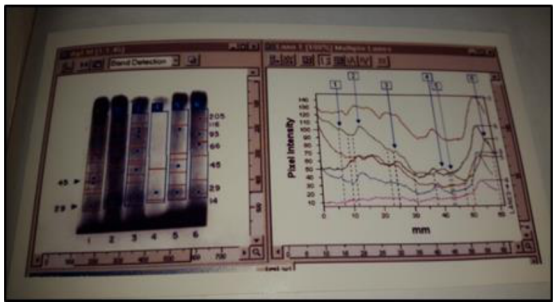

Fig: 4.5 Qualitative scanning of the autoradiograph, Fig.4.4, analysed by Image Master Elite

Discussion

Protein phosphorylation and dephosphorylation regulates various functions of host cells in response to different stimulus such as bacterial lipopolysaccharides to bacterial pathogens. Binding of the stimulus (ligand) to the surface receptor of various kinds of immune cells including macrophages has been demonstrated to induce phosphorylations of certain cellular proteins [(21)]. *L. donovani* uses several macrophage surface receptors including MFR receptor, advanced glycosylation end products and integrin receptors for their *attachment* and entry [(1-3, 22)]. Both PKC and PTK-mediated phosphorylated proteins through integrin receptors interaction had been demonstrated for bacterial entry in many systems [(4-6, 23),] this study revealed that attachment of a virulent strain GE-1 of *L. donovani* in presence of cytochalasin-D (Figure-1.1) induced phosphorylation of at least two proteins 29- and 45-kDa of macrophages as confirmed by Figure and its corresponding scanning (Figure 1.2, 1.3, Table-1). Addition of PMA, an activator of PKC further induced the phosphorylation of these proteins (Figure-1.2, 1.3, Table-1) and staurosporine an inhibitor of PKC blocked the phosphorylation (Figure-3.1, 3.2) of the proteins, confirming the role of PKC in the process. It has been reported in many systems that during bacterial entry involvement of tyrosine specific phosphorylation signals were witnessed [(4-6)], however, recent reports on various particles [(12,13)] and few pathogens including *E.coli* invasion in HEp-2 cells [(24)] and *Legionella pneumophilla* entry into human monocytes[(23)] shown to be PKC-mediated. It was thus suggested that interaction of *L. donovani* promastigote with probably integrin receptors activated PKC and in turn induced the phosphorylation of 29- and 45-kDa proteins. This was further supported by the previous report (25) that *Leishmania* attachment is associated with increased intracellular Ca^{+2} influx and DAG, which

subsequently may activate PKC, and in agreement with previous observation that *L.donovani* attachment induces PKC-mediated oxidative events [26,27]. In contrast, other intramacrophage pathogens such as *Shigella flexneri* and *E. coli* could not induce the PKC-specific phosphorylation (Figure-4.1, 4.2) as those observed with *L.donovani* attachment indicating that observed phosphorylations of 29- and 45-kDa proteins might be selectively associated with the attachment by the virulent strain of *L.donovani*. However, after established infection, the expression of these proteins were significantly attenuated (Figure-2.2, 2.3, Table-2) and as reported by others (28, 29) suggesting that PKC-mediated protein phosphorylation was not required for parasite replication once they entered the host cell.

Summary

The data obtained in this study suggested that the process of *L.donovani* attachment generate a variety of PKC-mediated inductive molecular signaling within the macrophages and these events significantly down regulated after established infection. Indeed, it was found the inductions in 29-kDa and 45-kDa phosphorylated proteins during *L.donovani* attachment but not in macrophages attached to *E.coli* or *Shigella flexneri*. These results indicates that the phosphorylations of these proteins might be a unique event associated with the recognition and uptake of *L.donovani* pathogens by macrophages and phosphorylations of these proteins were not required for intracellular replication of these pathogens.

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Chapter 5

Impact of Climate Change on Parasitic Diseases: Do We Need to Bother?

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IMPACT OF CLIMATE CHANGE ON PARASITIC DISEASES: DO WE NEED TO BOTHER?

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Anthropogenic activities over the last few decades have seriously aggravated the climatic conditions and have accelerated the climatic change pushing the entire race on the brink of a perilous situation. The direct impact of such changes observed in the form of global warming have attracted human attention but the indirect consequences are far more dangerous because they don't produce direct tangible results. Effect of climatic change on parasitic diseases is one such indirect consequence. Studies show that the increase in temperature and alteration of weather patterns can cause changes in the spatial distribution pattern of various disease vectors. The abundance of vectors, rates of biting, survival rates and the range of reservoir hosts also tend to increase in warm climates. The current work seeks information regarding various effects of climatic changes on parasitic diseases and their outcome.

Keywords: Climate change, parasitic diseases, Vectors, Anthropogenic activities, Positive feed-back loop

Introduction

Climate may be defined as the average state of the lower strata of atmosphere that includes land and/or water and their interactions therein. It is usually referred on a regional basis with a time span of several years. Population explosion in the last decade has forced the human civilization to forage into previously undisturbed areas in search for food, water and shelter for the millions. This had resulted in habitat encroachment and destruction of the biotic structure by the aid of technological masterminds. These anthropogenic activities have accelerated the rate of climate change in its wake that would have been otherwise had the nature been left to itself.

The impact of climate change has far-reaching effects that have become evident only lately after they have silently crept in without any warning. The scientific community has revealed astounding results regarding the effects of climate change that is much beyond the direct environmental impacts of global warming alone. The studies show that the increases in temperature and changes in weather pattern brought about by climate change may change the spatial patterns of several disease vectors as well as human population.[1] Moore *et al.* stated that increased temperatures favour the development of parasite-carrying arthropod vectors and the parasites as well.[2] Warm climates reportedly serve to increase the range of reservoir hosts, the abundance of vectors and the transmission rates of

several parasites by vectors like mosquitoes and ticks.[3]

The tropical and sub-tropical regions of the world are a haven for the parasites and their vectors because of its ambient temperature and climatic conditions that promote their survival and breeding.[4] According to Daszak et al. the reasons for the survival of the parasites can be traced to the complex interactions between the hosts.[5] These interactions are subject to alteration by climate changes.[5] Therefore climate changes have the potential to expand the geographical distribution of the hosts and the parasites (via the hosts) to regions which were otherwise not suitable for their survival.[6] The life cycles of the parasites are also affected by temperature and other weather conditions which in turn determines the prevalence of the vector and/or the parasites in a particular region.[6]

The current investigation summarizes the effects of climate change on parasitic diseases and their possible outcomes. The discussion has broadly been done (and restricted) under two heads namely, effects of changes in temperature and effect of changes in rates of precipitation.

Effects of changes in temperature

I) *Alteration in the length of the season of transmission*: It has been reported that when a vector thrives in an environment of low mean temperature a small increase in temperature may aid in the enhanced development of the vector and the parasites developing within them.[7] Thus rise in temperatures even slightly in otherwise cold regions or delay in onset of winters on even rainfall in late spring all account for prolonged season of transmission thereby increasing the chances of encounter between the host and parasite.[8]

II) *Affects geographical distribution of the vector and/or parasite:* In recent times the prevalence of several parasites in regions where they were not previously found is an effect of climate changes. It has been hypothesized that the vectors adjust to alterations in temperature by altering their geographical distribution.[9,10] Increased temperatures also stimulate an evolutionary change in certain vectors that allow them to adjust genetically to the changing ambience.[11] Greater selection pressure on the population of mosquitoes living in higher latitudes has been cited as an example for such microevolutionay process.[11] By default, the parasites they carry within them also can extend their geographical horizons.

   It has also been stated that these microevolutionary processes may help the vectors and the parasites within to adapt themselves to altered seasonal patterns and is one of the key reasons for changes in the seasonal patterns of some.[11, 12]

III) *Behavioural changes of vectors*: Changing ambient temperatures and/or humidity have been found to affect the behaviour of many vectors. At temperatures above 30^0 C and low humidity levels the triatomine insects reportedly feed more frequently in order to prevent dehydration.[13] These not only affect the survival rates of the vector themselves but also the parasites they carry within them.

IV) *Changes in rates of development and transmission cycles*: Rates of development have been found to be

related with changes in temperature. It has been found that warm climates favour better synchrony between the larval and nymph stages of ticks that increases the rate of transmission of disease and aid in better survival of more virulent strains of the parasites in tick vectors.[14] Higher temperatures have also been reported to quicken the development of larvae within the hookworm eggs that hastens the formation of the infective stage.[15,16] The development of *Trypanosomacruzi*in some vectors is also favoured by increased temperatures.[17] The lifecycles of triatomine insects is shortened by increased indoor temperatures that help them to increase their population density at a faster rate and enhancing their chances to spread the disease.[13] The shortening in the length of life-cycles of insects feeding on human blood encourage them to have frequent blood-meals increasing their chances of host-contacts in warmer weathers and also the chances of disease-transmission.[18] Snails increase their populations at faster rates in warm waters.[19,20] Hence rise in temperature of water due to global climatic change coupled together with decreasing the water currents by anthropogenic activities like construction of dams help snail populations to thrive and serve as intermediate hosts to helminths like *Fasciola* sp. and *Schistosoma* sp.[19, 20]

The development of the trematodes have been found to be closely related to temperature.[21] It has been observed that the production of cercariae in a snail host is increased by an increase of the Surrounding temperature.[22] The emergence of these cercariae is also enhanced by such temperature rise.[23] The overall effect is a net increase in infective cercariae within the aquatic habitats highly increasing their chances of infection.[23,24] Reduced transmission cycles of trematode parasites is alarming since it is not only a major human and veterinary health issue but also because they play important roles in building of animal communities.[22]

V) *Use of alternate intermediate hosts*: In case of parasites like *Echinococcus granulos us* that do not have any specific intermediate hosts may use alternative hosts like racoon dog (as its definitive host) thereby expanding its geographical range.[19]

VI) *Impact on some life-cycle stages*: Parasites that have to spend a part of their life outside the host are more susceptible to environmental factors. The cyst and the oocyst stages of the protozoan parasites have been seen to thrive well in warmer climates.[15,25] This results in greater survival rates and thereby their transmission. Similarly in case of the soil-transmitted helminths like hookworms the survival of the eggs and larval stages requires moist soil and warm temperature.[15] Hence warmer temperatures may increase the survivability and transmission rates of such soil-transmitted helminths.

VII) *Shifts in balance between co-evolved hosts and parasites*: A significant effect of temperature rise on the growth rates of fish parasites has been reported. It has been observed that the parasites infecting fish modify their behaviour in such a way that the fish-host search for warmer temperatures thereby creating a positive feedback loop maximising the outcomes of global rise in temperature.[26]Many such studies have

shown that temperature rise may cause shifts in the balance between co-evolved hosts and parasites accelerating the speed of development of parasites with shortening lengths of life-cycle.[26] These factors ultimately may result in increased parasitism of natural animal populations.

Effects of changes in rates of precipitation

I) *New breeding grounds and larval habitats*: The effects of changes in the rates of rainfall is pronounced considering its potential to generate new breeding grounds and support the survivability of the larval forms.[27]It has seen that conditions of high temperatures and reduced rainfall coupled together support new habitats for *Phlebotomus* sp., the causative agent of leishmaniasis.[28]Studies have shown that reduced rainfall favour the breeding of *Culexpipiens* in urban drainage systems leading to an outbreak of epidemics.[29]The rates of precipitation also determine the distribution of lymphatic filariasis because it is directly linked to the breeding grounds of the mosquitoes.[30]

II) *Lengthened seasons of transmission*: Like temperature precipitation rates of precipitation plays an equally important role in lengthening the seasons of transmission of vector-borne diseases like dengue and malaria that ultimately increase the chances of infection.[31]

III) *Increase in population of vertebrate reservoirs*: Higher rainfall may favour increased productivities and increased food supplies which may help the vertebrate reservoirs to flourish.[32] This helps in the better survivability of the parasites and their chances of transmission.

IV) *Increased chances of vector-human contact*: Sudden heavy and untimely rainfalls may lead to flooding obliterating the larval habitats and making conditions unsuitable for vertebrate hosts to thrive.[32]Such events of habitat destruction by flooding may force the insect and /or rodent vectors to take shelter inside houses increasing the probability of vector-human contact.[32]Reports from Brazil show that intensive unseasonal flooding had triggered the epidemics of leptospirosis, a rodent-borne disease.[33] It has also been reported that heavy rainfall stimulate the blood-feeding habits of *Culexnigripalpus*i increasing the frequency at which they visit the human host increasing their chances of spreading infections.[34]

V) *Problem of unseasonal drought*: Unseasonal droughts in the wet regions of the tropics may lead to slowing down of rivers and creation of shallow, stagnant pools which are ideal breeding sites for different vectors.[32] Thus, such droughts may increase the prevalence of parasites.

VI) *Increased survival rates of vectors*: It has been reported that increased incidences of rainfall during late spring or early summer make the ticks more active and increase their rates of survival.[35]In addition to this, it has been observed that change in behaviour of the hosts according to the changing weather conditions may also expose them to greater chances of infections by ticks. Randolph reported that higher rainfalls favour the growth of

mushrooms in Poland followed by their harvesting after the rains. However the activity of the ticks is also much higher during the time period as a result of which the harvesters have a greater risk of getting bitten by them.[36]

VII) *Increased flush of cysts and oocysts of protozoan parasites*: Use of fertilizers to increase crop productions have become a common trend. In some cases animal fertilizers including human biosolids are also used.[37]These may contain cysts and oocysts of protozoan parasites which may be washed down by heavy rains into the local waterways contaminating drinking water and leading to epidemics like those of cryptosporidiosis and giardiasis.[37,38] The peak prevalence of cryptosporidiosis have been reported during the hot humid months of the rainy seasons in India, Bangladesh and Guatemala.[39,40,41]

VIII) *Impact on soil transmitted helminths*: Frequent rainfall events may prevent the desiccation of helminth eggs/larvae especially in case of soil-transmitted helminths increasing their survival rates in the external environment thereby increasing their chances of transmission to vertebrate hosts.[15]

Conclusion

It is to be kept in mind that climatic changes alone do not pose serious threats regarding parasitic diseases and its transmission. In fact it is very difficult and will be grossly erroneous to assess the impact of climate change alone. Climatic changes together with anthropogenic changes create conditions favourable for the parasites to thrive and flourish.[42]The greatest example of this is high rates of human migration and global travel as a part of cultural dissemination, civic unrest, commercial purposes or whatever have led to distribution of some diseases from endemic regions to the non-endemic regions of the world.[43,44]The most important outcome of such events is brow-raising as the population of the non-endemic regions are immunogenicallynaïve to the parasites and lack the preparedness to face them often leading to serious clinical complications.[45] Another such example is construction of dams and re-direction of streams for irrigation purposes which slow down flowing streams and generates stagnant water-bodies that forms the breeding havens for vectors.[42, 46]They become the nurturing grounds for the parasites. Such water-bodies also support snail populations which in turn are intermediate hosts of various helminth parasites.[42]

Lack of proper sanitation, open defecation and ill-maintenance of urban drainage system are other important issues of particularly the developing countries that favour the survival and reproduction of the vectors and/or the parasites.[47]These issues multiply by times uncountable when combined with effects of climatic change. Careless dumping of wastes and improper clearing of the garbage from places of human habitation encourage the survival of vectors with container-breeding habits.[32]

Poverty, illiteracy and lack of proper health facilities are the vicious cycles in developing countries that serve as positive feedback loops for various parasitic diseases.[6]They manage to survive and become a source of perpetual trouble for the entire nation.

Thus it is seen that climatic changes alone are not the major key-players of persistence, proliferation and transmission of parasitic diseases. The anthropogenic

activities are equally responsible and they cannot be considered one without the other. In short, the antagonistic effects of human activities on the effects of climatic changes and thence the parasitic diseases cannot be negated.

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Chapter 6

Brackish Water Shrimp Disease and Its Remedy by Maintaining Water Quality in The Coastal Belt of East Midnapur District West Bengal

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BRACKISH WATER SHRIMP DISEASE AND ITS REMEDY BY MAINTAINING WATER QUALITY IN THE COASTAL BELT OF EAST MIDNAPUR DISTRICT WEST BENGAL

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Abstract

Commercial shrimp culture is one of the major aquaculture activities in the coastal areas of West Bengal. Shrimp farming has become a very lucrative business because of the short duration of the crop, quick turn over, ready market and availability of suitable brackish water area in the country. Rapid and unplanned expansion of shrimp farming has recently led into a number of environmental problems. During the last half of 2000, a major loss came to the shrimp farming in India in the form of epizootic disease outbreak. In West Bengal, its worst hit was caused to coastal belt of East Midnapur District such as Rasulpur, Digha, Sankarpur and Dadanpatrabar. Over stocking, poor feed and water quality management are said to be the reason of such disease outbreak. Shrimps and microbes co-exist if the conductive environment is provided. The heavy application of feed, fertilizer, drugs and chemicals into the culture system alters the biological equilibrium of the costal zones. This situation allows the pathogenic elements which are in the dormant state in water to multiply fast. Disease can be effectively prevented by maintaining proper water quality in a shrimp culture system. From a practical point of view more attention should be paid to culture conditions, with special attention to water quality. Where adequate filtration is not possible, treatment of water is suggested to lower the bacterial and fungal load of the water. The present communication aims at giving an overview of the physical and chemical parameters as well as biological parameters maintained in the different shrimp farms in the coastal belt of East Midnapur District of West Bengal. Data from these farms revealed that they were able to arrest the disease problem by avoiding overstocking and through feed and water quality management.

***Keywords*:** Brackish water, Coastal belt, Water quality Management

Introduction

Commercial shrimp culture is one of the major aquaculture activities in the coastal areas of West Bengal. There is a growing concern about the environmental degradation of the areas where aquaculture practices were under taken over the years[1]. Recently, shrimp farming has become a very lucrative business because of the short duration of the crop, quick turnover, ready market and availability of suitable brackish water area in the country[2]. With the largest pool of brackish water in the country nearly 30% of the total resources, west Bengal is the second highest producer of shrimps at 33,685 metric tons, according to the last available data published in 2009 – 2010. This accounted for nearly 35% of the total shrimp production of India (95,919t) (www.undercurrent news, 2014)[3]. Rapid and unplanned expansion of shrimp farming has recently led into several environmental

problems. During the last half of 2000, a major loss came to the shrimp farming in India in the form of epizootic disease outbreak[4]. In West Bengal, its worst hit was caused to coastal belt of East Midnapur District such as Rasulpur, Digha, Sankarpur and Dadanpatrabar. Disease can be effectively prevented by maintaining proper water quality in a shrimp culture system[5]. From a practical point of view more attention should be paid to culture conditions, with special attention to water quality. Where adequate filtration is not possible, treatment of water is suggested to lower the bacterial and fungal load of the water.

Materials and methods

The selected farm is located about 40 km. away from Digha and 8km from Contai being about 1km away from the sea shore of Bay of Bengal. A map of the study area (not to scale) is shown in Fig.1. Location of study site.

Fig. 1: Location of study site

Selected shrimp farm of this area is taken into consideration for detailed investigation. The shrimp farm is located at the village – Soula in the East Midnapur district. The Soula canal that meets the Bay of Bengal serves as the brackish water source. The major parameters of water were analyzed with the help of methods adopted in APHA (1992)[6]. The design and facilities of the selected shrimp farm along with water input through soula canal from Bay of Bengal is shown in Fig. 2. Design of the Shrimp farm.

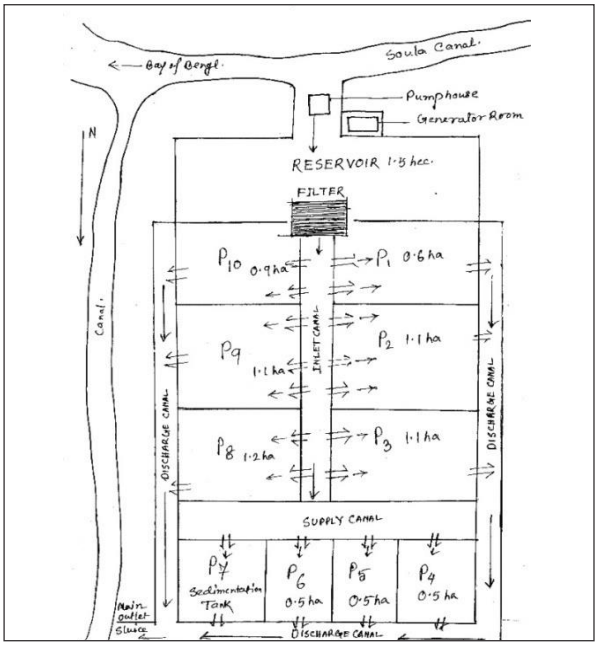

Fig. 2. Design of the Shrimp farm

The study was undertaken during summer period of the year 2016 from the period of February to May. For preparation of appropriate culture conditions in ponds, a series of activities were undertaken. The pond bed was prepared by application of quicklime. Fertilizers like urea and single superphosphate is added to the pond water for plankton bloom. The moderate stocking density @ 6 – 10 pieces/sqm. area) with periodic aeration and feed addition was maintained. Periodic water exchange was made. The detailed steps of activities

involved in the shrimp farming are shown in Table – 1.

Location of study site

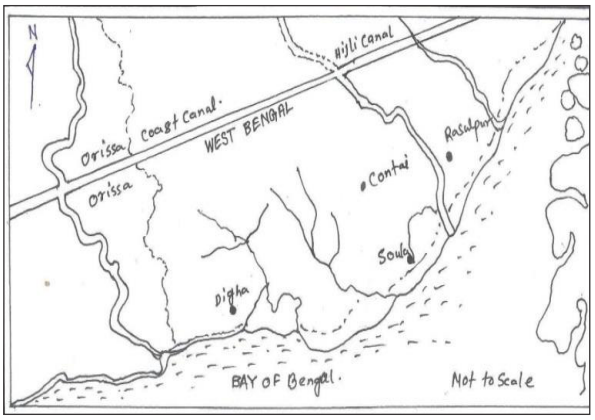

Table 1: Activities of shrimp farming

(A) Before Stocking	(B)After stocking
1.Soil preparation: (a) clean the pond; (b) Sundry (c) Pond bottom distillation and disinfection	**1.Water management:** (a) water analysis cand quality control; (b) water exchange and aeration; (c) Salinity maintenance.
2.Water quality maintenance: (a)Monitoring of source water quality; (b)Filling of ponds with water and adding the fertilizer for plankton bloom; (c)Monitor the plankton quality and load.	**2.Feed management & growth data monitoring:** (a)Daily feed requirement assessment and feeding control; (b)Fry survival rate assessment and biomass assessment.
3.Seed selection/ stocking: (a)Selection of fry; (b)Fry transport and stocking; (c)Regular inspection and maintenance of water quality	**3.Disease incidence monitoring:** (a)periodic check up of disease incidence; (b)therapeutic management.

The general characteristics of this modified extensive farming are also shown in Table – 2. (M. Mandal, M. Dev and S.C. Santra, 2005)[7]

Table 2: General characteristics of modified extensive farming

S. No. Characteristics	Requirement status
1. Pond size (ha)	1.0-1.5
2. Stocking density (nos/m2)	5-10
3. Survival rate (%)	70-80
4. Feed Frequency (no. of times/	1-4
5. water exchange (% per day)	10-15
6. water depth (m)	0.8-1.5
7. Harvest size (gms)	20-50
8. Production level (kg crop/ha/season)	1500-3000
9. Level of pond Management required	Moderate but systematic

A variety of chemicals that were used as input for farming is monitored along with periodic monitoring of culture pond water quality, discharge water quality and fish growth.

Result

A good number of chemicals were used (as standard dosage) during shrimp farming as input material. The details are given in Table - **3.** The average daily growth rate was monitored. It varies from 0.12 to 0.45gm/day

Table 3: Input materials used in framing

S.No.	Chemicals	Composition	Dosage	Purpose of use
1.	Quick lime	Calcium oxide(CaO)	6-7 tons/ hectare/cropping (in phases)	Control pH and alkalinity, develop
2.	Dolomite	calcium-magnesium carbonate, $CaMg(CO_3)_2$	70-100kg/ hectare.	plantonic growth
3.	Zeolite plus	Sodium-calcium-aluminium silicate and neem oil mixture	80Kg/hectare	Zeolite absorbs NH_3,SO_2 and\ H_2S through its cation exchange properties, Neemoil acts as antifungal, anti-bacterial and antiviral agent
4.	Mustard seed cake	-	12Kg/hectare	Acts as a fertilizer for plankton bloom
5.	Urea- singal super Phosphate mixture (6:1)	-	5 tons/ hectare (m phases) before stocking	Acts as fertilizer for plankton cake
6.	Bleaching powder	$Ca(OCl)_2$	250Kg/hectare	Acts as disinfectant
7.	Potassium permangenate	$KMNO_4$	0.5-1.0 Kg/hectare	Do
8.	Feed (artificial shrimp feed)	Fish meal, squid meal, soyabean meal, cod liver oil, broken rice, wheat Flour, cholesterol, vitamin etc.	2.5-3.5 MT/hectare (in phases)	To maintain the growth and survivability of fry
9.	Medicine(anti-biotics) & vitamins	Oxytetracycline, Erthromycin, Ascorbic acid	2.5- 4.5 gms/ Kg of feed for each	Do

After stocking was monitored, the data of water quality is presented in **Table 4**.

Table 4: water quality status of culture ponds and reservoir

SL. No.	Parameters	Reservoir BS AS	Pond-1 BS AS	Pond-2 BS AS	Pond-3 BS AS
1	Temperature (^{0}C)	23 25	24 26	24 26	25 26
2.	pH	8.0 8.2	8.4 8.3	8.1 8.2	8.4 8.3
3	Alkalinity (mg/L)	200 130	240 220	200 150	240 140
4	Salinity (ppt)	24.0 26.0	16.0 25.0	26.2 26.0	26.2 26.0
5	Secchi transparency (cm)	55 52	53 50	53 50	52 50
6	Dissolved oxygen (mg/L)	5.6 5.3	5.5 5.2	5.3 5.2	5.4 5.6
7	BOD (mg/L)	2.0 2.5	2.5 2.6	2.5 2.6	2.8 3.0
8	COD (mg/L)	24 40	25 42	35 42	36 45
9	Hardness (mg/L)	6500 5500	6600 5400	6200 5200	6500 5700
10.	Suspended solids(mg/L)	10 18	10 15	18 22	16 20
11.	Phosphate (mg/L)	0.62 0.55	0.64 0.54	0.65 0.54	0.64 0.55
12.	Nitrate (mg/L)	0.52 0.40	0.51 0.32	0.48 0.37	0.66 0.53

NB: BS= Before Stocking; AS = After Stocking

The quality of water periodically discharged for water exchange requirement and also final discharged after crop harvesting from culture ponds was also monitored. The details are given in Table – 5.

Table 5: Characteristics of discharge water from Culture ponds (average values)

S No	Parameters	1st exchange	2nd. exchange	3rd. exchange	Final discharge	CPCB Standard*
1	Temperature(^{0}C)	24.0	24.5	25.5	25.0	-
2	pH	8.1	8.3	8.5	8.5	5.5 – 9.9
3	Alkalinity (mg/L)	390	350	340	350	-
4	Salinity (ppt)	28	30	28	30	-
5	Secchi transparency (cm)	42	36	35	34	-
6.	Dissolved oxygen (mg/L)	4.1	4.0	4.6	4.8	≥3
7	BOD (mg/L)	3.7	3.6	4.5	4.8	20
8	COD(mg/L)	52	63	70	70	-
9	Hardness(mg/L)	6500	5800	7500	7500	-
10	Suspended solids(mg/L)	34	42	45	45	100
11	Phosphate(mg/L)	0.34	0.34	0.20	0.20	-
12	Nitrate(mg/L)	0.37	0.42	0.50	0.52	-

N.B.: *CPCB – State Pollution Control Board.

Discussion

While alkalinity, phosphate and nitrate decreased in the reservoirs and ponds after stocking suspended solids and COD showed substantial increase after stocking. A high alkalinity is observed due to the liming process before stocking. The decrease in nutrients is due to the consumption of plankton. A high suspended solid is due to presence of feed particles, plankton etc. an increased COD may be attributable to the organic matter so formed owing to various stocking activities.

Changes in discharge water quality with respect to input water in shrimp farming process it shows that pH and alkalinity increased slightly due to application of lime. Suspended solids increased slightly due to impurity of lime and feeds.

Dissolved oxygen (Do) increased due to blooming and artificial aeration. BOD increased slightly due to growth of microbes and hardness decreased slightly due to use of calcium and magnesium by plankton. Nitrate increased slightly due to the presence of artificial feed residue while phosphate decreased slightly due to utilization by plankton.

Conclusion

Though the discharge was containing fairly less pollutant load yet, there is need for chemical treatment by alum or specialized biological treatment before reuse as reservoir water. The existing farm can be more affective in respect of maintenance of water quality with slight modification in the farm design with reasonably low-cost. The main reservoir can be divided into three parts, for more affective water treatment.

Water can be used in culture and stocking ponds. The used water can be reused after filtration as shown in Fig.3.

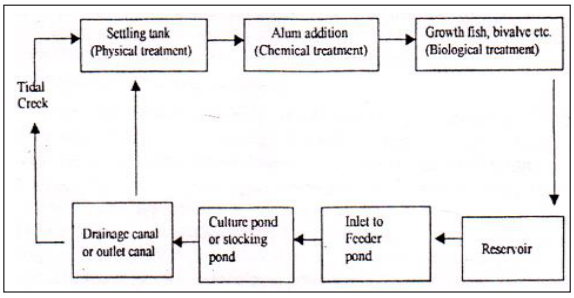

Fig.3: Scheme for treatment of discharged wastewater for reuse

The modified design will promote secondary aquaculture like bivalves, algae, fish etc. The secondary aquaculture will open a new avenue of additional income.

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Chapter 7

Arsenic Contamination in the Lentic Ecosystem and its Environmental Impact: Case Study on Some Wetlands of Murshidabad

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Arsenic contamination in the lentic ecosystem and its environmental impact: Case study on some wetlands of Murshidabad

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Abstract

Increasing exposure to heavy metal pollution is one of the biggest concerns for public health, water quality and ecosystem conservation. West Bengal is well known for severe Arsenic (As) contamination. People unknowingly consume arsenic contaminated rice and vegetables and become victims of arsenic toxicity. Increased consumption of As-enriched water may result in skin lesions, chromosomal changes, as well as skin, lung and bladder cancer, disturbance of the cardiovascular and nervous system functions, and eventually death. Arsenic (As) may occur in surface freshwater ecosystems as a consequence of both natural contamination and anthropogenic activities. In this study in Murshidabad district, West Bengal, India it was found that arsenic contamination is present on the lentic ecosystem. Water samples collected from five lentic water bodies Chaltia Beel, Bishnupur Wetland (Beel), Indraprastha Beel, Dhupghati Beel and Laldighi were analysed for arsenic contamination. Tests were done for both pre and monsoon samples. The surface water bodies are also contaminated in varying degree (0.0014 to 0.0188 mg/L). As aquatic ecosystems are not simply isolated bodies or conduits but are closely connected to terrestrial environments the contamination is likely to spread to the surrounding terrestrial ecosystem through plants and animals. The biological magnification poses greater threat to human being. The probable source of contamination is the ground water. Huge amount of ground water is taken out by pumps for household purpose. This water is used then drained back into the wetlands without any treatment

Keywords: Arsenic, Lentic Ecosystem, Wetlands, Surface water, Bio magnification

Introduction

Arsenic (As) enrichment in groundwater and its significant adverse impact on human health is a great concern in many places around the world. Over past three decades arsenic contamination in groundwater appears to be major calamity in Bengal delta. Of these, West Bengal is well known for severe Arsenic contamination, with large numbers of exposed individuals within the Ganga-Meghna-Bramhaputra basin. Around 9.5 million people are at risk of consuming Arsenic. The rural people in this area depend on groundwater for drinking, cooking and other domestic purposes. In addition, groundwater is continuously being used for irrigation to cultivate a variety of crops and vegetables. Irrigation with As-enriched groundwater is therefore the main pathway for Arsenic to enter the human food chain.

Arsenic can find its way into the grains of plants, such as rice and wheat, and into some vegetables and fruit plants through irrigation with arsenic-contaminated water.[1] It has been reported that accumulation of 1.7 mg kg^{-1} of arsenic in three rice samples from Bangladesh which is much above the permissible limit in rice, 1.0 mg kg^{-1} according to WHO recommendation.[2] Rice grain has been reported to accumulate arsenic up to 2.0 mg kg^{-1} by Islam and co authors in their study in Gangetic flood plains of Bangladesh.[3]

Environmental impact of Arsenic contamination in the lentic ecosystem

Mass poisoning of human populations from drinking water sources containing high arsenic (As) concentrations have been reported globally[4]. Increasing exposure to heavy metal pollution is one of the biggest concerns for public health, water quality and ecosystem conservation.[5] Ecotoxicological and bioaccumulation effects of arsenic pollution have been largely explored through experimental tests and short-term surveys[6,7] but little is known of its long-term impacts on freshwater ecosystem health. Biomagnification is a process where the concentration of certain substances, such as toxic chemicals, heavy metals and pesticides, increase as they move up in a food chain. Arsenic contamination in surface soil and in plants is elevated using arsenic contaminated ground water for irrigation in crop fields. The source of irrigation in most of the arsenic affected areas is groundwater from shallow aquifer within 100 m bgl. In the absence of alternate source of arsenic free irrigation water, rural people continue to tap arseniferous aquifer resulting in further aggravation of the problem in different forms, such as, mobilization of arseniferous groundwater to freshwater zones, spreading of the sources by the cycling process of water and use of fertilizers and pesticides, transport through food chains, etc. The productive wetland ecosystem plays a significant role in the ecological sustainability of a region. Being an essential part of civilization meeting many crucial needs for lives on earth, such as source of drinking water, protein production, water purification, energy, fodder, biodiversity, flood storage, transport, recreation, research education, sinks and climatic stabilizer it acts both directly and indirectly in the ecosystem. Yet wetlands are the primary receptacles for agricultural discharge containing agro-chemicals, it has brought the crisis of non-point source pollution into the forefront. Unregulated use of fertilizers and pesticides is already having telling effects on human health.

Sources of Arsenic in Ganga–Brahmaputra Aquifers

The Ganga River basin (GRB) is a part of the Ganga-Brahmaputra-Meghna (GBM) river basin, draining 1.08 million km2 in Tibet, Nepal, India, and Bangladesh; it covers nearly 26% of India's land mass and is home to a population of over 630 million[8]. There is no proof regarding the natural emission of As in the Ganga–Brahmaputra plains so far. However, the release of As, by the natural processes in groundwater, has been recognized, from the Holocene sediments comprising sand, silt and clay[9, 10] in parts of the Bengal Delta Plains (BDP), West Bengal and in the Gangetic plains of Bihar. It is also to be understood that the arsenic contamination of groundwater in the Bengal Delta Plains (BDP) is the result of interaction of the aquifer lithology and aquife waters in flux in a complex evolutionary sequence in the mid-Holocene to the present times.

Sources and Mechanisms of Groundwater Arsenic Contamination in the GRB as indicated by research states that all rivers, susceptible to arsenic contamination through arsenic containing sediment loads origin in the Himalayan mountains and Tibetan plateaus.[11] It is considered that arsenic, which is present in aquifer sediment with iron oxides, has been released by microbially-driven reductive dissolution in an organic-rich environment.[3, 12]

Arsenic in surface water

In Tibet, arsenic contamination was first reported in river water due to wastewater discharge from a geothermal power plant.[13] Also in Tibet, the arsenic levels up to 5985 μg /L in hot springs was found, and also 10,626 μg /L in alkaline salt lakes in western Tibet.[14] Studies in Tibet have shown that the arsenic contamination in surface water, as well as groundwater sources,were linked with the carbonite abundance.[15] The presence of arsenic (As) and related elements such as fluoride (F^-) and vanadium (V) have been well documented in groundwater.[16] Nevertheless, studies of their presence in surface water are scarce. High levels of natural contamination with As and other trace metals (F^- and V) in lotic and lentic ecosystems to the southwest of Buenos Aires Province.[17,18]

Mechanisms of Arsenic uptake

Arsenate [As(v)] is the main As species in aerobic soils. It has a strong affinity for iron oxides/hydroxides in soil; thus the concentrations of arsenate in soil solutions are usually low. However arsenite (As III) is the dominant As species in reducing environments such as flooded paddy soils.[19] Flooding of paddy soils leads to mobilization of arsenite into the soil solution and enhanced As-bioavailability to rice plants.[20] Arsenite uptake is of particular importance of rice and other aquatic plants with their roots growing in anaerobic or semi anaerobic environments. As accumulation in rice shoots and grain over a longer growth period. It has been reported that additions of silicate inhibited As accumulation by rice when arsenate was the form of As added to the nutrient solution; yet this effect was not attributable to a direct competition between Si and arsenate because they do not share the same transporters.[21]

Human exposure to arsenic through food grains

Agricultural water requirements in most of the rural areas are met from groundwater source. In arsenic affected areas often, arsenic contaminated groundwater is used for agricultural irrigation resulting in excessive amount of available arsenic in the crops in those areas. Many researchers reported that food is the second largest contributor to arsenic intake by people after direct ingestion of arsenic contaminated water. In food, rice is the maximum sensitive to arsenic followed by vegetables. Most of the arsenic affected states use rice as its staple food.

The occurrence of arsenic in groundwater is well studied in West Bengal, the major problem is that it is coming in the food chain through water used for irrigation purpose.[22-24]

Study area

Murshidabad district is divided into 26 blocks. There are 2414 villages and wards in this district. The river Ganga separates it from Bangladesh. The Bhagirathi River divides it into two parts. To the west lies the Rarh, a high, undulating continuation of the Chota Nagpur plateau. The eastern portion,

the Bagri, is a fertile, low-lying alluvial tract, part of the Ganges Delta. The district is drained by the Bhagirathi and Jalangi rivers and their tributaries. Bhagirathi is a branch of the Ganges, and flows southwards from Farakka barrage where it originates from the Ganges. It flows southwards through the district and divides it into more or less equal halves. Covering an area of 5,341 km² (2,062 sq mi) and having a population 5.863m (according to 2001 census) it is a densely populated district and the ninth most populous in India (out of 640). It borders Malda district to the north, Jharkhand's Sahebganj district and Pakur district to the north-west, Birbhum to the west, Bardhaman to the south-west and Nadia district due south. The international border with Bangladesh's Rajshahi division is on the east. Berhampore is the headquarters of the district.

Water sampling

Lentic bodies are known as 'Beels" or 'Dighi' in the local language, Bengali. Water samples collected from five lentic water bodies Chaltia Beel, Bishnupur Beel, Indraprastha Beel, Dhupghati Beel and Laldighi were analysed for arsenic contamination.

Material and methods

Arsenic monitoring is utmost important nowadays. Palmer reported atomic spectroscopy is the most widely-used method for the arsenic determination.[25] A more practicable method for the analysis of arsenic at low detection limits on spectrophotometer was followed, which appears to be useful for all simple and modest laboratories.[26] The protocol of Narayan was also referred.[27]

Pre-sampling method Clean-up of sample container: Sample containers should be scrupulously clean so as not to introduce contaminants that could interfere with quantification of the target analyte(s). This is very important task for determination of trace or ultra-trace elements and their concentration levels. The following cleaning procedure has been set up for cleaning the sample bottles. Different type of bottles whether borosilicate glass, linear polyethylene, polypropylene, or PTFE clean by this method.

Detergent → Tap water → 1:1 HNO 3 → Tap water→ 1:1 HCl →Tap water → Reagent water

Collection and preservation of water samples Water samples of about 500 ml were collected from different water sources in polyethylene vials these vials are pre-treated with clean up procedure. Before filling, rinse the bottles two or three times with the water being collected. After collection, sample is acidified with concentrate nitric acid to a pH below 2 to minimize precipitation and adsorption of heavy metals on the container walls. After acidifying sample transfer to the lab and kept in refrigerator at 4ºC temperature until further analysis.

Results

The following results were obtained in the laboratory and confirmed by PWD laboratory

Water samples from the five study sites were analyzed by pre -calibrated spectrophotometer at 535 nm (λmax.) using the modified method on concentration mode by Tahir *et al.*[26] The results are given in Table 1 below.

Table 1: Arsenic Content in five lentic water bodies of Murshidabad

Sample Water	**Pre Monsoon (As mg/L ± SD)**	**Post Monsoon (As mg/L ± SD)**
ChaltiaBeel	0.0024± 0.0003	0.0048± 0.0008
Bishnupur Wetland (Beel)	0.0087± 0.0003	0.0133± 0.0008
Indraprastha Beel	0.007 ± 0.0001	0.0118± 0.0008
DhupghatiBeel	0.0122± 0.0004	0.0188± 0.0006
Laldighi (Control Wetland)	0.0014±0.0001	0.0018± 0.0001

Discussion

The threat of arsenic contamination derived from wastewater discharge is widespread in many industrial and urban areas around the world.[28] A number of recent investigations suggest that perennial ponds, chiefly constructed by local populations in the last 50 years and used for bathing, washing, and aquiculture, represent a source of recharge water and Dissolved organic matter (DOM) to the As-affected, shallow groundwaters, owing in part to increased flushing of the shallow aquifers that results from irrigation pumping.[29, 30] However the water from perennial ponds is an insignificant source of recharge and DOM to West Bengal groundwaters.[31]

The healthy aquatic ecosystem is depends on the biological diversity and Physico-chemical characteristics[32] Without adequate quantity and quality of fresh water, sustainable development will not be possible.[33,34] The lentic ecosystem is landlocked. So all the "Beels" or the 'Dighis"are land locked and are rainfed and there should not be any arsenic contamination in the 'Beels" from groundwater arsenic. Indiscriminate

dumping of industrial wastes into streams and rivers impair the physico-chemical composition of the receiving water and ultimately it will have an adverse effect on an aquatic life.[35, 36] The probable reason of arsenic contamination in the lentic ecosystem of Murshidabad wetlands is wastewater discharge from local industries (*viz.* Rice mills) which goes directly into the lentic ecosystems/surface water bodies.

Murshidabad district comprises of 26 blocks, 262 GPs including Municipal areas (known as wards) and 1918 inhabited villages. It was also reported that in Murshidabad the number of villages where it was found arsenic concentration above 10, 50 and 300 µg/L are 1320, 971 and 281 respectively; and 25, 24 and 17 blocks were found to be contaminated with arsenic levels above 10, 50 and 300 µg/L.[37, 38]

Human-caused arsenic contamination of surface waters and its ecological risks have received far less attention, despite the fact that ongoing industrialization and urbanization can significantly increase arsenic exposure of humans and ecosystems at large spatial and temporal scales[39, 40]. In this study it is found that often the waste water goes directly (with any treatment) into various 'Beels' in and around Berhampore, Jangipur, Raghunathgaunge in Murshidabad district. As the people of these townships are using ground water with high arsenic content[41], for industrial use, the arsenic contamination goes into the surface water of lentic ecosystems through wastewater drainage systems. So all the 'Beels" or 'Dighis' are contaminated. The drains that are carrying wastewater contain a fair amount of arsenic which contaminates

the 'Beels". In this investigation it is found that the pre-monsoon amount is low but the post monsoon amount of arsenic is high. As the soil surface in Murshidabad is highly contaminated with arsenic, the run-off water into these water bodies during post monsoon increases the amount of arsenic present.

The development of a cost-effective and reliable technique for arsenic determination by using a comparatively inexpensive instrument like spectrometer is what is needed at this time.[26] Most laboratories in these countries lack state-of-the-art equipment like the Atomic Absorption Spectrometer (AAS) or the Inductive Coupled Plasma Spectrometer (ICP) to analyze arsenic at low detection levels. So many laboratories are unable to analyze arsenic due to lack of such equipment and manpower. Spectrophotometer is a commonly available instrument in most of laboratories. Considering this fact, the modified spectrophotometric method presented here has been developed for the analysis of arsenic at low detection limit.[26] Hopefully proper treatment of waste water from industries along with awareness, a cost-effective and reliable technique for arsenic determination will go a long way in curbing the arsenic contamination of the lentic ecosystem of Murshidabad district.

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Chapter 8

Protective Potentials of Natrum Sulphuricum-200, a Homeopathic Remedy, against p-DAB induced Hepatocarcinogenesis in Mice

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Protective potentials of Natrum Sulphuricum-200, a Protective potentials of Natrum Sulphuricum-200, a homeopathic remedy, against p-DAB induced hepatocarcinogenesis in mice

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Abstract

The study was undertaken to examine if administration of Natrum Sulphuricum-200 (Nat Sulph-200), a potentized homeopathic drug can provide ameliorative effect in mice (*Mus musculus*) fed an azo-dye p-*dimethylamioazobenzene* (p-DAB), a group-2B carcinogen (initiator), and phenobarbital (PB), a promoter to induce hepatotoxicity, free radical generation, and liver tumors. Mice (*Mus musculus*) were divided into following groups of 6 mice each: normal untreated (control-1), normal+succussed alcohol (alcohol being the "vehicle" of the drug, control-2), p-DAB (0.06%)+PB(0.05%) (carcinogen-intoxicated), p-DAB+PB+succussed alcohol (carcinogen-intoxicated control-3), p-DAB+PB+Nat Sulph-200 (intoxicated drug-fed). Biomarkers like superoxide dismutase, catalase, glutathione reductase, glucose-6-phosphate dehydrogenase, gamma glutamyl transferase and lactate dehydrogenase were assayed at regular intervals. Additionally, pathophysiological parameters like serum total bilirubin, serum albumin, blood glucose, haemoglobin, blood cholesterol, serum creatinine were measured at day 30, 60, 90 and day 120. All toxicity parameters were positively modulated by the administration of the homeopathic remedy, Nat Sulph-200. Chronic feeding of p-DAB and PB triggers generation of reactive oxygen species (ROS) over the lapse of time. Nat Sulph-200 protected the cells against the effects of free radicals, thereby restricting the cellular damage. Nat Sulph-200 produced beneficial effects against the carcinogen-induced geno-toxicity, hepatotoxicity, oxidative stress and liver tumors in mice, presumably through its gene-modulatory action. Further research is warranted to examine if Nat Sulph-200 can be used as a supportive medicine in liver cancer therapy.

Keyword's: Natrum Sulphuricum-200, p-DAB, PB, Hepatocarcinogenesis, *Mus musculus*, Anticarcinogenic

Introduction

In various toxicological experiments[1-3] azo dye induced carcinogenesis has been thoroughly studied. Complementary and alternative medicine (CAM)[4,5] practices are also used against induced carcinogenesis. A hepatotoxin, p-dimethylaminoazobenzene (p-DAB) is a known carcinogen (initiator)[6] and phenobarbital (PB) which is also acarcinogen[7] having the ability to promote tumorigenesis in liver[8].

Chronic feeding of these two chemicals to mice or rats develop hepatic tumors at day 60 onward and subsequent transformation of some tumors with neoplastic characteristics was also observed. Various toxicity biomarkers

in liver have been studied after chronic administration of the carcinogens in mice.[9,10]

The homeopathic remedy, Natrum Sulphuricum 200c (Nat Sulph-200) was reported by us to combat against carcinogen-induced toxicity in mice[11]. Different potencies of Natrum Sulphuricum, Lycopodium and Cholesterinum are routinely used in treatment of various liver disorders in humans.[12,13,14,15]

The present study was an extended effort to evaluate protective potentials of Natrum Sulphuricum 200 in ameliorating oxidative stress and hepatotoxic effects during azo dye induced hepatocarcinogenesis in mice.

Methodology

Animals

Healthy inbred adult (from the same source and batch) albino Swiss strain mice (*Mus musculus*), in equal number for each sex, weighing between 25-30 g, were initially randomized from the colony. All animals were acclimatized seven days prior to the commencement of the treatment and allowed free access to food and water. Experiments on animals were performed with clearance from the Animal Ethics Committee, University of Kalyani, and under the guidelines and supervision of Animal Welfare Committee, University of Kalyani.

Source and preparation of stock solution of drug

Alcoholic preparations (90%) of Nat Sulph-200, and 90% alcohol (used as placebo) prepared by following the principle of homeopathic dilution and potentization procedure as recommended in the Homeopathic Pharmacopoeia of India, Vol. I (1971)[16] were procured from HAPCO, 165, B. B. Ganguly Street, Kolkata.

Feeding procedure and dose

Each mouse was fed with the aid of a fine pipette 0.06 ml of stock solution (1 ml of each drug or alcohol (vehicle) was diluted separately with 20 ml of double distilled water) of Nat Sulph-200, that conformed a single dose.

As per homeopathic practice, Nat Sulph-200 was fed once daily.

The doses selected for chronic feeding were derived on the basis of our earlier work. [9, 10,11]

Experimental groups

30 healthy mice of both sexes, weighing between 25-30 g were used for each of four fixation intervals – namely, 30, 60, 90 and 120 days, making a total of 180 animals for the entire study. For every fixation interval, 6 mice each were fed 5 different diets. Animals were randomly divided into the following five groups:

i) The first group of 6 healthy untreated mice reared under normal laboratory conditions with normal low protein diet (control-1; Group -1); this data can be taken as baseline data for day 0.

ii) The second group of mice fed alcohol with normal low protein diet (control-2; Group-2);

iii) The third group of mice fed diet mixed with 0.06% p-dimethylaminoazobenzene (p-DAB; initiator) (Sigma,D-6760) and provided 0.05% aqueous solution of phenobarbital (PB; promoter) instead of water (carcinogen treated; Group-3);

iv) Another group of mice chronically fed 0.06% p-DAB along with 0.05% aqueous solution of PB plus successed alcohol (as the “vehicle” of the drug was ethyl alcohol (Alc), carcinogen treated positive control-3; Group-4; as in earlier experiments in our laboratory).

v) The fifth group of mice chronically fed p-DAB+PB plus Nat Sulph-200 (treated drug-fed-1; Group-5);

Mice were sacrificed at four different intervals of fixation, viz. at 30, 60, 90 and 120 days for gamma glutamyltransferase (GGT) and lactate dehydrogenase (LDH) and two intervals, viz. 90 and 120 days, for, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD). Pathophysiological parameters like serum total bilirubin, serum albumin, blood glucose, haemoglobin, blood cholesterol and serum creatinine were measured in at day 30, 60, 90 and day 120.

Laboratory methodology

Biochemical assays

The detailed procedures for preparation of tissue homogenates, blood collection, and different biochemical assays etc. have been provided in our earlier publications.[5,9,10,11,13]

Assessment of liver function

Gamma glutamyl transferase (GGT) (E.C 2.32.2) and serum total bilirubin were estimated according to the manufacturer's protocol (Reckon Diagnostics, India).

Assessment of renal functions

Serum samples were assayed for creatinine and urea by using standard diagnostic kits (Span Diagnostics, Gujarat, India).

Assessment of pathophysiological parameters

For blood glucose determination, standard glucose test kits (enzymatic, GOD-POD method, Span Diagnostics Limited, Baroda India, was used. Hemoglobin content was determined by Sahli's method with the help of a hemometer (Marienfield, Germany). The blood cholesterol was measured following the method of Plummer.[17]

Biochemical assays

From whole blood

Glucose-6-phosphate dehydrogenase (G6PD) (E. C 1.1.1.49) was assayed from whole blood by the reagent kit (UV-Kinetic method) according to the manufacturer's protocol (Reckon Diagnostics, India).

From serum

Lactate dehydrogenase (LDH) (EC 1.1.1.27) was assayed from serum according to the manufacturer's protocol (Reckon Diagnostics, India).

Statistical analysis and scoring of data

The significance test between different series of the data was conducted by student's t-test. A value of $p < 0.05$ was considered to indicate a significant difference between groups. This experiment was done as per randomized double-blind placebo control method.

Blinding

The observers were "blinded" during observation as to whether the sample originated from the drug or placebo treated groups.

Results

On autopsy, Groups 1 and 2 showed healthy liver while liver tumors were encountered in other groups of mice receiving the carcinogens for 60 days or more. As compared to all carcinogen fed mice showing distinct sign of tumor formation in the form of pale reddish multiple nodules (more pronounced in the p-DAB+PB and p-DAB+PB+Alc fed series at 60, 90 and 120 days (Table 1), the incidence and growth of tumors found in p-DAB+PB+Nat Sulph- fed series was less, both numerically and qualitatively.

Table 1: Tumour growth

Number of mice with tumor incidence at different fixation intervals and different groups.					
Groups	Number of Specimen	Tumor Incidence and Intensity			
		30 Days	60 Days	90 Days	120 Days
Normal	24	0/6	0/6	0/6	0/6
Normal+Alc	24	0/6	0/6	0/6	0/6
p-DAB+PB	24	0/6	0/6 (3+++, 3++)	0/6 (5+++, 1++)	6/6 (6+++)
p-DAB+PB+Alc	24	0/6	0/6 (4+++, 2++)	0/6 (5+++, 1++)	6/6 (6+++)
p-DAB+PB+Nat Sulph-200	24	0/6	2/6 (1++, 1+)	3/6 (2++, 1+)	3/6 (1++, 2+)

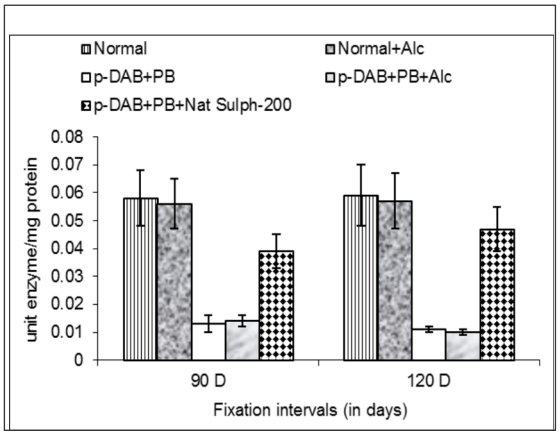

Fig 1: Histograms showing activities of SOD (Unit enzyme/mg protein) in different series of mice at 90 and 120 days fixation intervals [Data presented as mean ± S.E.]

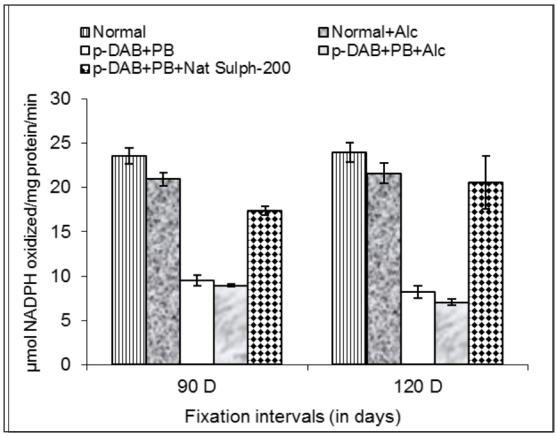

Fig 3: *Histograms showing GR activity (µmolNADPH/mg protein/min) in different series of mice at 90- and 120-*days *fixation intervals [Data presented as mean ± S.E.]*

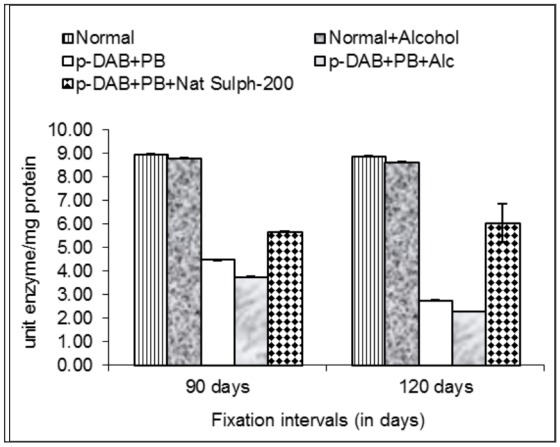

Fig 2 : Histograms showing activities of CAT (Unit enzyme/mg protein) in different series of mice at 90- and 120-days fixation intervals [Data presented as mean ± S.E.]

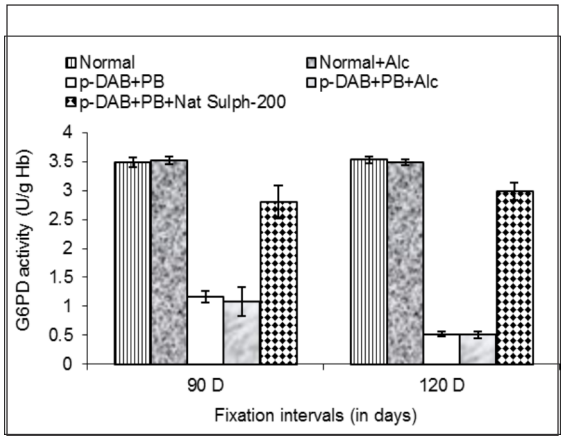

Fig 4: *Histograms showing G6PD activity (U/g Hb) in different series of mice at 90- and 120-*days *fixation intervals [Data presented as mean ± S.E.*]

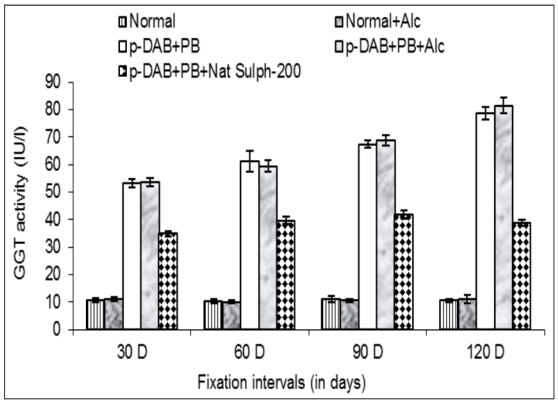

Fig 5: Histograms showing activities of serum γ-GGT (IU/L) in different series of mice at 30, 60, 90- and 120-days fixation intervals [Data presented as mean ± S.E.]

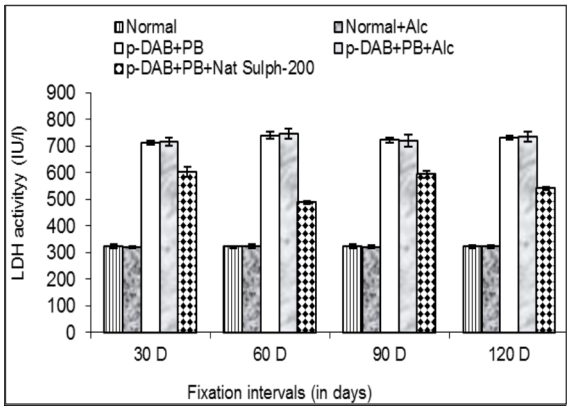

Fig 6 : Histograms showing activities of serum LDH (IU/L) in different series of mice at 30, 60, 90 and 120 days fixation intervals [Data presented as mean ± S.E.]

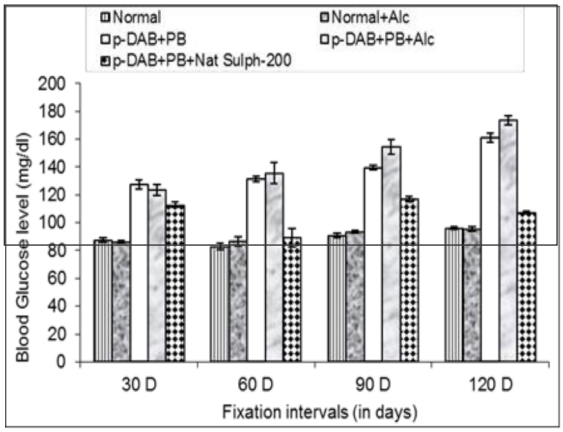

Fig 7 *: Histograms showing blood glucose level (mg/dl) in different series of mice at 30, 60, 90 and 120 days* fixation intervals *[Data presented as mean ± S.E.]*

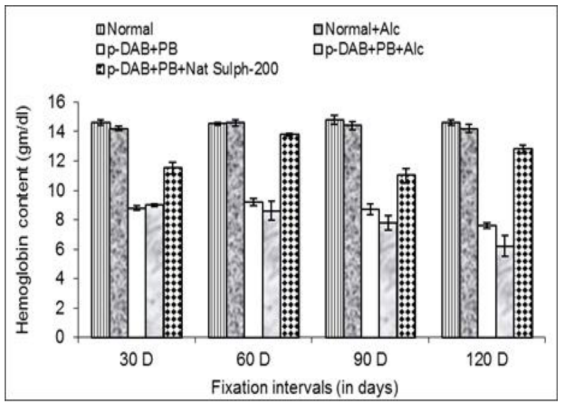

Fig 8 : *Histograms showing haemoglobin content (g/dl) in different series of mice at 30, 60, 90 and 120 days fixation intervals [Data presented as mean ± S.E.]*

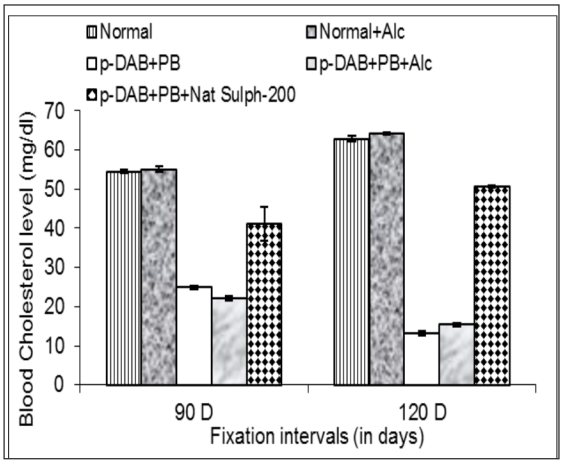

Fig 9: Histograms showing haemoglobin content (g/dl) in different series of mice at 30, 60, 90 and 120 days fixation intervals

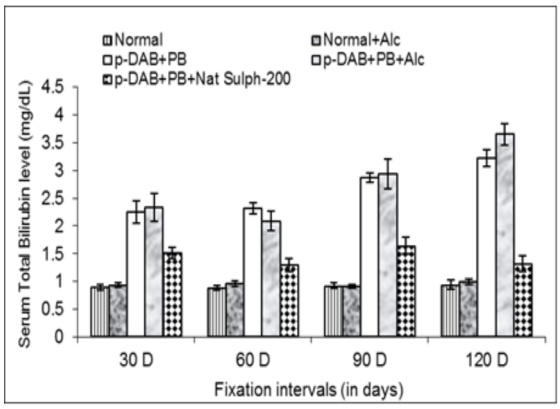

Fig 10 : Histograms showing serum total bilirubin level (mg/dl) in different series of

mice at 30, 60, 90 and 120 days fixation intervals [Data presented as mean ± S.E.]

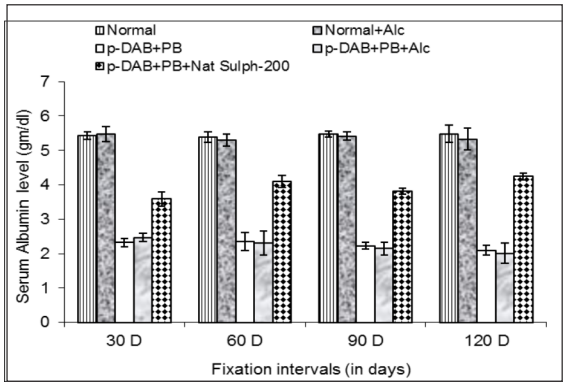

Fig 11: Histograms showing serum albumin level (g/dl) in different series of mice at 30, 60, 90 and 120 days fixation intervals [Data presented as mean ± S.E.]

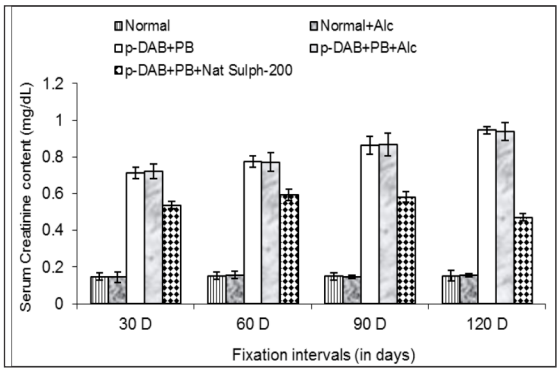

Fig.12: Histograms showing serum creatinine content (mg/dl) in different series of mice at 30, 60, 90- and 120-days fixation intervals [Data presented as mean ± S.E.]

Effect on biochemical parameters

Effect on enzymes indicative of hepatic oxidative stress

Chronic feeding of p-DAB+PB and p-DAB+PB+Alc induced oxidative stress, which was observed by decreased activities of SOD (Fig.1) CAT (Fig. 2) and GR (Fig. 3) at all fixation intervals. Administration of homeopathic remedy appeared to reverse these parameters significantly towards normal. As compared to controls, administration of p-DAB+PB+Nat Sulph-200 generally showed positive modulating abilities (p < showed positive modulating abilities (p < 0.05 through $p < 0.001$) in respect of all biochemical assays, excepting at some fixation intervals. Administration of Nat Sulph-200 produced appreciable restoration of activities of SOD, CAT and GR in liver. Administration of p-DAB+PB+Nat Sulph-200 appeared to show positive modulation as compared to that shown by carcinogen fed mice, more noticeable at longer fixation intervals.

There was generally a decline in activity of G6PD (Fig. 4) in p-DAB+PB and p-DAB+PB+Alc fed series. Compared to carcinogen fed series, an acceleration in G6PD activity was noted in all drug-fed series. In p-DAB+PB+Nat Sulph-200 fed mice, a significant ameliorative effect ($p < 0.05$ through $p < 0.001$) was noted as compared to controls.

Effect on serum GGT and LDH

The GGT (Fig. 5) and LDH (Fig. 6) activities were significantly greater in mice chronically fed p-DAB+PB and p-DAB+PB+Alc. Administration of Nat Sulph-200 separately along with p-DAB+PB brought about considerable positive modulation ($p < 0.05$ through $p < 0.001$) in the levels of serum GGT and serum LDH.

Effect on pathophysiological parameters

Effect on blood glucose, haemoglobin and cholesterol level

Blood glucose level (Fig.7) was increased in p-DAB+PB and p-DAB+PB+Alc series and decreased in the drug fed series. A significant decrease in blood haemoglobin (Hb) (Fig. 8) and cholesterol levels (Fig. 9) was observed in p-DAB+PB+Alc fed series of mice. Administration of the potentized of homeopathic remedy produced appreciable restoration ($p < 0.05$ through $p < 0.001$) in the said levels.

Effect on serum bilirubin, serum albumin and creatinine

An increase in serum bilirubin level (Fig. 10) was found in p-DAB+PB+Alc fed group of mice. Administration of Nat Sulph-200 brought down the values ($p < 0.05$ through $p < 0.001$) almost to levels comparable with those of normal controls.

A decrease in serum albumin level (Fig. 11) was observed in p-DAB+PB+Alc fed group of mice. Administration of the homeopathic remedy increased the values close ($p < 0.05$ through$p< 0.001$) to levels of normal controls.

A significant increase in serum creatinine (Fig. 12) was observed in mice chronically fed p-DAB+PB. Administration of Nat Sulph-200 along with p-DAB+PB brought about considerable positive modulation ($p < 0.05$ through $p < 0.001$) in the levels of serum creatinine.

Discussion

The elevated activities of GGT and LDH along with the decline in activities of SOD, CAT, GR and G6PD clearly showed that hepatotoxicity and oxidative stress generated by the chronic feeding of carcinogens increased during the course of carcinogenesis. In Nat Sulph-200 fed mice, the positive modulation in activities was found to be more pronounced, particularly at day 90 and day 120 in liver. Thus, Nat Sulph-200 exhibited greater modulating effects in liver particularly conspicuous at longer fixation intervals.

Homeopathic remedy can manipulate the ROS level by regulating the genes expression and related signaling pathways to keep the redox balance and cellular component integrity. ROS may induce acute alterations in cellular function, via specific covalent modifications of target molecules and leads to condition of oxidative stress. An inescapable side product of oxidative metabolism is Reactive Oxygen Species (ROS), which mediate mutagenesis and alter signaling pathways in chemically induced carcinogenesis. The incidence of tumor was much less in the drug-fed group as compared to carcinogen fed group showed anti-cancer effect of homeopathic remedy. Oxidative stress caused by reactive oxygen species (ROS) accumulate in different organ will persistently destroy the cells, which may lead to diseases.

The process of carcinogenesis [18-21] is related to the toxicity biomarkers which were used in this study. ROS is a key parameter that controls tumor progression and angiogenesis by regulating the expression of various oncogenic molecules.[22] Enzymatic antioxidants like super oxide dismutase (SOD),[23] catalase (CAT) and glutathione reductase (GR) have more effective protective effects against active and massive oxidative attack due to the ability to decompose ROS. Hepatotoxicity generates ROS and oxidative stress over the lapse of time as generation of ROS leads to damage antioxidant defences.[24] The reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) is catalyzed by Glutathione reductase.[25] A prominent cellular reductant, reduced glutathione, is related with defence against free radicals and peroxides.[26]

The increase in activities of SOD, CAT, GR and G6PD indicate the protective ability of Nat Supph-200 against induced hepatocarcinogenesis. The concomitant decline in the activities of the enzyme GGT and LDH brought about by the administration of the homeopathic remedy further supports the anti-cancer potentials of the drug.

The elevated blood glucose level is related to the occurrence of several types of cancer in men and women.[27] In

hepatocellular carcinoma decreased cholesterol level was reported.[28] Bilirubin is a yellow breakdown product of normal heme catabolism. Very high level of bilirubin is a indicator of severe liver failure with cirrhosis. Serum creatinine content is regarded as a renal function indicator. The serum albumin level has been accepted as a test for liver function.[29,30] Reduction of the level of bilirubin and rising of the level of albumin was possible only after the administration of potentized homeopathic remedy showing the hepatoprotective role of NatSulph-200. Homeopathic remedy has the potentiality to ameliorate the carcinogen induced toxicity which is supported by the results of these pathophysiological parameters.

Inside living organism p-DAB is metabolized to mono-amino azobenzene (MAB) by N-dimethylation and subsequently to amino azobenzene (AAB) which is the carcinogenic factor.[3] The homeopathic drugs could prevent the metabolism of p-DAB and thus hampering the carcinogenesis.

Khuda - Bukhsh[31-36] proposed a hypothesis that the potentized homeopathic drugs might have the ability to act as a molecular trigger" "for switching "on" and "off" certain relevant genes, and through a cascade of subsequent gene action/interaction, a series of biochemical changes could follow, that in turn could bring about the alterations observed in the parameters of the present study.

CAM therapy[37] including homeopathy is becoming popular day by day. Many independent research works are encouraged to verify and confirm (or refute) these interesting findings.

Conclusion

The results of this study would indicate a possibility of use of homeopathic remedy, Natrum Sulphuricum 200 in treating liver disorders, including neoplastic growth.

Acknowledgements

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Chapter 9

Climate Change and Aedes Mosquito Vector: A Projection towards Future Scenario of Disease Transmission

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Climate Change and *Aedes* Mosquito Vector: A Projection towards Future Scenario of Disease Transmission

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ABSTRACT

Global climate change would affect human health via pathways of varying complexity, scale and directness and with different timing. Similarly, impacts would vary geographically as a function both of environment and topography and of the vulnerability of the local population. This is no surprise since climatic change would disrupt or otherwise alter a large range of natural ecological and physical system that are an integral part of Earth's life support system. Likewise, climatic factors are capable of assisting or interrupting the biology and population dynamics of vector mosquitoes thereby influencing their abundance and distribution. The same factors also play a crucial role in the survival and transmission rate of mosquito-borne pathogens. By 2100 it is estimated that average global temperatures will rise by 1.0–3.5 °C, increasing the likelihood of many vector-borne diseases in new areas. The burden of vector borne viral disease has increased an estimated 30-fold over the past 50years. Globalization, trade, urbanization, travel, demographic change, inadequate domestic water supplies and warming temperatures are associated with the spread of the main vectors *Aedes aegypti and* Aedes albopictus Similarly, rainfall can have non-linear contrasting effects on dengue risk. Heavy rainfall may flush away eggs, larvae, and pupae from containers in the short term, but residual water can create breeding habitats in the longerterm. A dry climate can lead to human behaviour of saving water in water storage containers, which may become breeding sites for *A. aegypti.* The combined effect of temperature and humidity significantly influences the number of blood meals and can also affect the survival rate of the vector, and the probability that it will become infected and able to transmit dengue. The delayed effect (or time lag) of climatic variables on dengue incidence could be explained by climatic factors which do not directly influence the incidence of dengue but do so indirectly. This is through their effect on the life-cycle dynamics of both vector and virus. Vectorial capacity depends on six vector parameters which are highly influenced by ambient temperature, both its mean value and diurnal temperature range. Studies have shown that Dengue Epidemic Potential has a strong temperature dependence of transmission probabilities per bite in humans and vectors. The intensity and duration of dengue transmission were predicted to rapidly increase over the course of the 21st century under a scenario of high greenhouse gas emissions and subsequent increases in temperature. In a recent study it is projected that by 2061–2080, the global land area suitable for *A. aegypti* would increase 8% under moderate and 13% under high emissions pathways. Expansion is primarily expected along the current edges of dengue distribution, with contraction in some areas where conditions would no longer be suitable for *Aedes* reproduction and growth. Expansion could thus be expected to lead to a higher burden of dengue in low- and middle income countries.

Introduction

The earth's climate has always been in a state of change. For nearly three centuries it has been in a warming phase. This was preceded by a cold period, the Little Ice Age, which was itself preceded by a warmer phase known as the Medieval Warm Period, or Little Climatic Optimum. Such changes are entirely natural, but there is evidence that in recent years a portion of the current warming may beat attributable to human activities, particularly the burning of fossil fuels. The potential impact of this global warming on human health is a major subject of debate. Many of the diseases that currently occur in the tropics are mosquito borne. It is commonly assumed that their distribution is determined by climate and that warmer global temperatures will increase their incidence and geographic range. This review explores the validity of both assumptions by examining the history of three mosquito borne diseases—malaria, yellow fever, and dengue—in the context of past climates and of other factors that can influence their transmission.

Mosquitoes are found throughout the world except in places that are permanently frozen. There are about 3,500 species, of which nearly three-quarters are native to the humid tropics and subtropics. The largest populations of individual species occur in the Arctic tundra, where colossal numbers emerge in a single brood each summer from snow-melt pools that overlie the permafrost. In nearly all mosquito species, the female obtains the protein she needs for the development of her eggs by feeding on vertebrate blood. Some species are highly selective, restricting themselves to one or at most a few closely related host species. Others have a less clearly defined host preference and may alternate among birds, mammals, and even reptiles. A complex salivary secretion facilitates feeding. It is the direct injection of this fluid into the capillaries that enables several life forms—viruses, protozoa, and nematode worms—to exploit mosquitoes as means of transfer between vertebrate hosts. In nearly all cases, there is an obligatory phase within the insect. This includes a stage in which they multiply prodigiously in the salivary glands, from which they can be inoculated into a new host during a later blood meal. Although most such organisms do not appear to affect either the mosquitoes or their vertebrate hosts, some are pathogens of important human and animal diseases.

Climate change is one of the most important environmental changes populations will face in the coming decades. Understanding how it may affect human health and disease is complex and requires a thorough understanding of links between present climate and disease[1]. Links between climate and diseases with various modes of transmission (vector-, water, food-, soil-, and airborne) have been identified,[2-3] with the strongest associations being between climate and mosquito-borne diseases.[4,5,6]

Although widely held as the world's most important arbovirus, only one review of potential climate change impacts on dengue virus (DENV) transmission has been published with a focus on tools currently used to establish climate– disease associations.[7]

DENV is transmitted by *Aedes* genus mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus.* Recent analysis indicates that numbers of dengue fever (DF) cases

may be as high as 400 million/year[8]. Climate affects the DENV and vector populations both directly and indirectly[9]. Temperature influences vector development rates, mortality, and behavior[10, 11, 12] and controls viral replication within the mosquito.[13] Variability in precipitation influences habitat availability for *A. aegypti* and *A. albopictus* larvae and pupae. Temperature further interacts with rainfall as the chief regulator of evaporation, thereby also affecting the availability of water habitats. Indirectly, rainfall, temperature, and humidity influence land cover and land use, which can promote or impede the growth of vector populations. The incidence of DF has been associated with vegetation indices, tree cover, housing quality, and surrounding land cover.[14,15] Climate change can also alter how humans interact with the land, altering its use and impacting mosquito population magnitude and species composition.[16,17]

Although empirical relationships have been identified between climate conditions, DF, and DENV vectors, causal relationships have not been firmly established, thus limiting our ability to assess intervention strategies. In order to evaluate the potential impacts of climate change and better prepare mitigation strategies, we examined the strength of the evidence supporting the complex relationships among *Aedes* mosquitoes, DENV, and weather and climate. We also explored the relative utility of statistical and process-based models and their ability to identify key associations between climate and disease and to predict and simulate DENV transmission under projected climate change conditions.[18]

The dengue virus, also known as "breakbone fever," is among the leading causes of illness and death in the tropics and subtropics. Incidence of the disease has increased 30-fold over the past 50 years. Transmitted mainly by infected *Aedes aegypti* mosquitoes, the virus historically struck primarily at altitudes below 1,000 meters.Climate variables are known to influence dengue transmission patterns, but the complex relationships among the virus, mosquitoes, habitat, climate, weather, and people remain unclear, making it difficult to predict how climate change will affect the virus' spread in the future. For example, the temperatures that allow mosquitoes to thrive and multiply are not necessarily the

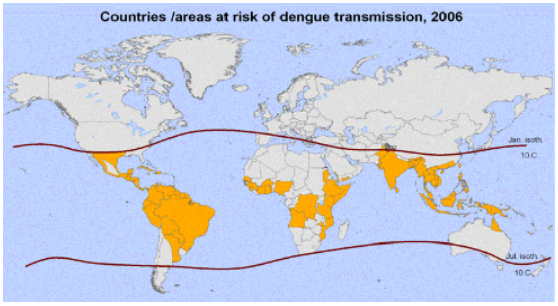

Fig 1. Countries and areas at risk of dengue (http://www.denguevirusnet.com/).

Interaction and Transmission of Dengue Virus

DENV infection begins at the cellular level when E envelope glycoproteins on the surface of a DENV virion binds to a host cell receptor initiating endocytosis. The high cell tropism of DENV has made the elucidation of a single host cell receptor difficult and the exact host cell receptor or receptors responsible for viral entry remain to found.

Once endocytosed, DENV escapes the endosome to the cytoplasm where it can immediately begin translation of its genome. Like other (+) ss RNA viruses it must transcribe a negative strand RNA template to make new copies of its genome (Fig. 2).

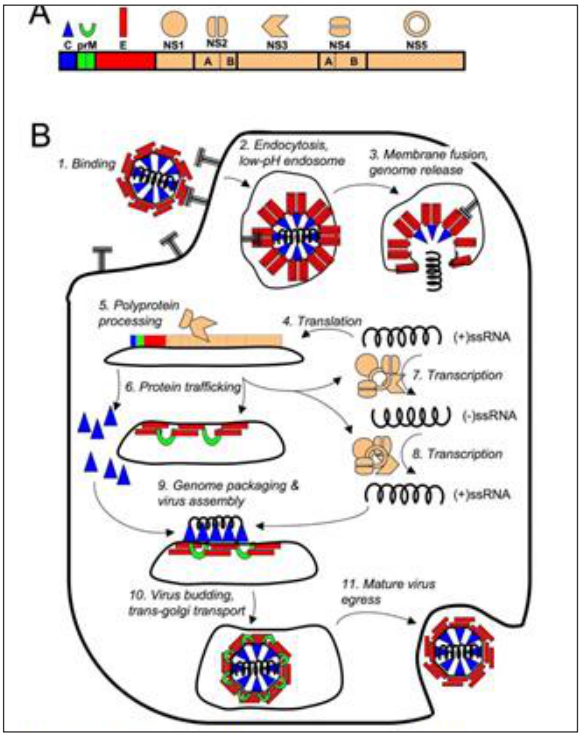

Fig 2-DENV(+)ssRNA genome and schematic of viral replication within host cell.

Co-infection of the mosquitoes with multiple DENV strains or the bacterium *Wolbachia* and interactions between DENV and the mosquitoes' innate immune system all modulate viral replication.[19] The replication kinetics of DENV in the mosquito-host has overreaching effects on the epidemiology of the disease. With a better understanding of how each of these factors affects the overall viral load and rate of viral replication within the vector. It will be possible to come up with better strategies for vector transmission control to mitigate and prevent future DENV epidemics (Fig. 3).

Distribution of Dengue Vector

Dengue disease (varying in clinical manifestations from acute febrile illness, self-limiting episodes [dengue fever, DF] to severe hemorrhagic manifestations [dengue hemorrhagic fever, DHF] and death) is caused by any one of four closely related dengue viral serotypes (DENV- 1, DENV-2,

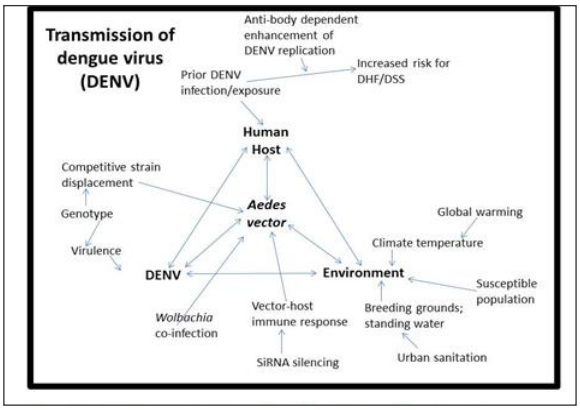

Fig 3-simplified epidemiological triad for dengue virus transmission.note the number of factors thatinfluence DENV replication in mosquito vector.

DENV-3, and DENV-4) of the genus Flavi virus, belonging to the family Flaviviridae. The worldwide distribution and incidence of dengue infections and cases are difficult to accurately establish because only approximately 20% of those infected with dengue virus exhibit apparent clinical symptoms. Disease occurs across a spectrum, and many patients with milder manifestations never seek health care. Additionally, of those patients who enter healthcare facilities, non-specific symptoms may be confused with other diseases or fail to satisfy reporting criteria: national passive surveillance systems are not designed to capture all symptomatic cases. Consistent burden estimates are elusive; from 2010 to 2013, the World Health Organization (WHO) reported an increase from 2.4 million to over3 million reported cases from the three affected regions (Americas, South-East Asia, and Western Pacific). Accordingly, their 2012Global Strategy estimated a total of 50–100 million infections per year. [20, 21]

According to,[22] areas with dengue fever are infested by *A. aegypti*. It was difficult to find information for the time before 1960 as few control activities were specifically directed at

dengue in the first half of the last century. During the Second World War, large-scale movements of susceptible populations led to a rise in dengue records. Most of the tropical and subtropical regions are now infested by dengue and its vector with Latin America, Southeast Asia and the Pacific islands reporting most of the recent dengue cases.[23] while there is a dearth of information from sub-Saharan Africa due to the absence of local mosquito laboratory facilities there.[24] Fig. 4 shows the current, global distribution of the disease.

These estimates were updated following a study in which the global distribution of dengue was modelled to map the risk of disease based on an exhaustive assembly of records of dengue occurrence. These data included environmental and socioeconomic covariates known or hypothesized to affect transmission[8]. The authors estimated that worldwide in 2010, there were approximately 390 million (range 284–528 million) dengue infections, 96 million (range 67–136 million) of which were clinically apparent. These infection rates were more than three times higher than those previously estimated by the WHO (Global alert and response, 2015), and included cases from 36countries previously considered dengue-free.[25] People in more than 125 countries, or over 50% of the world's population, were identified as being at risk of infection, including 824 million individuals in urban and 763 million in peri-urban areas.[24] Dengue was predicted to be ubiquitous year-round in the tropics, with the highest risk zones in the Americas and Asia. Asia bore 70% of the global burden of apparent infections, with India contributing 34% of the total. The America counted for 14%, with more than half occurring in Brazil and Mexico. Africa contributed 16%, with the predicted risk unevenly distributed and more widespread than previously suggested; however, documentation of data was poorest in Africa suggesting this could be an underestimate. Overall, this analysis may overestimate the number of dengue infections in some countries, such as in Hong Kong where, in contrast to a study estimate of 4300,000 episodes annually, very few cases occur, and underestimate it in others; in the USA, the study predicted zero dengue transmission whereas local transmission occurs along the US-Mexico border and in Florida.[26,27]

Suitable local temperature and high levels of precipitation were the variables most strongly associated with elevated dengue risk; in some locations, dengue is associated with humidity and vapour pressure.[8, 28] Proximity to low income urban and peri-urban centers was also associated with greater risk, particularly for those with good transport connections.[8] Climatic changes resulting in increased temperature and rainfall, together with urbanization, may therefore be associated with increased dengue incidence and outbreak risk.

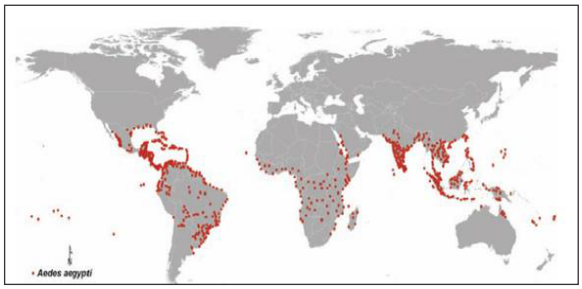

Fig.4- Global distribution of the Aedes aegypti Mosquito

In addition to the public health impacts, the economic burden of dengue can be substantial[29]. Suggested that the economic costs of endemic dengue for individual professional healthcare systems can exceed hundreds of millions of US$ annually. A review of 17 publications conducted in different geographic and health system settings reported that estimated costs for outbreaks in 2011 (in

2012 US$) ranged from US$2.8 million in the Dominican Republic to US$12 million in Vietnam.[30] Overall, the global aggregate direct (medical care and travel) and indirect (lost time and productivity) cost of dengue has been estimated as US$8.9 billion.[31]

Effect of Temperature on Denv and The Vector Ecology

Temperature is a key component in the ecology of DENV as seen from its numerous interactions with other components of the disease system (Fig 5). Most directly, ambient temperature increases are associated with a faster rate of viral replication within the vector and with a shorter extrinsic incubation period (EIP; the time required for DENV to become transmissible to another host after initial infection of a mosquito).[32] demonstrated that for both DENV-1 and DENV-4 the time between feeding and virus detection in the salivary glands of *A. aegypti* mosquitoes decreased from 9 days at 26°Cand 28°C to 5 days at 30°C.[13] more directly demonstrated that the EIP for DENV-2 virus in *A. aegypti* mosquitoes is temperature dependent by allowing infected mosquitoes to feed on monkeys. They reported that the EIP was as short as 7 days at temperatures of 32–35°C, and ≥ 12 days at30°C, whereas no virus transmission occurred at 26°C within the 25-day period of the study.[13] Using censored Bayesian time to-event models to analyze data collected from many studies,[33] estimated the average EIP to be 15 days at 25°C, and 6.5 days at 30°C:[32] estimated shorter incubation periods (5–9 days) but they defined the end of the EIP as the time when the virus was detected in the mosquito, whereas[13] defined it as the time when the mosquito transmitted the virus. Because temperature varies throughout the day in nature, [34] explored the susceptibility of *A. aegypti*to DENV infections under different diurnal temperature ranges (DTRs). They found that with the same mean temperature, mosquitoes exposed to a greater DTR were less likely to become infected than those exposed to a smaller DTR; however, the EIP was unchanged.[34] Evidence suggests that even small increases in temperatures and narrower DTRs may facilitate DENV transmission by decreasing the EIP or by increasing the susceptibility of mosquitoes to infection.

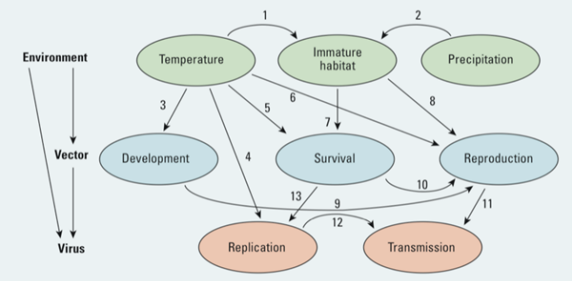

Fig. 5- Diagram of biophysical influences on DENV ecology showing the interactions between climate variables, vectors, and the virus. Numbers identify relationships between variables. Habitat availability for mosquito larvae is influenced by temperature through evaporation and transpiration (*1*) and incoming precipitation (*2*). Temperature is a major regulator of mosquito development (*3*), viral replication within infected mosquitoes (*4*), mosquito survival (*5*), and the reproductive behavior of mosquitoes (*6*). Habitat availability is required for immature mosquito survival (*7*) and reproduction of adult mosquitoes (*8*). Faster mosquito development and increased survival will accelerate mosquito reproduction (*9* and *10*). Increased mosquito reproduction enhances the likelihood of transmission by increasing the number of blood feedings (*11*), whereas faster viral replication increases transmission by shortening the extrinsic incubation period (*12*). Last, increased survival of the adult mosquito increases the amount of viral replication (*13*).

Mosquitoes of the genus *Aedes,* primarily *A. aegypti*, are the vectors for DENV; therefore, the ecology of the virus is intrinsically tied to the ecology of these mosquitoes. Egg and immature mosquito development, ovarian development, and survival at all stages of the mosquito lifecycle are governed in part by temperature.[10] In the laboratory setting[11] found that immature *A. aegypti* development rates generally increased with incubation temperatures to 34°C and then slowed. Survival through all developmental phases peaked at approximately 90% (27°C) with cooler temperatures being especially detrimental to survival [11,12] reported that *A. aegypti* egg, larvae, and pupae development rates increased at higher

incubation temperatures and ceased at< 8.3°C. Their estimated survival rates were also similar to those reported by,[12] with the ideal range for survival through all phases of development (88–93%)occurring between 20–30°C.[12] The laboratory studies discussed above yielded consistent results with little variation between trials. In the field portion of their study,[12] found that development rates accelerated in warmer water, but development was often slower and more variable in field trials than in their laboratory trials at comparative temperatures (Fig. 6).

Adult mosquito survival is important because only mosquitoes that live beyond the EIP can act as potential vectors. The first blood meal is generally taken 3 days post-eclosion (i.e., 3 days after emerging as an adult); therefore, assuming an EIPof 7–12 days (based on).[13] a minimum of 10–15 days is required for a newly emerged mosquito to become infectious. Mark–release–recapture studies have estimated that adult daily survival rates are between 86% and 91%.[35,36] Although these studies did not examine climatic influences on survivability, Christophers (1960)[10] has provided evidence of increased mortality with exposure to prolonged extreme heat (> 40°C) and cold (<0°C) in a laboratory setting. Using the ranges of adult survival rates and the estimated minimum age to complete the EIP indicated above, 10.4% (86% daily survival with a 15-day EIP) to 38.9% (91% daily survival with a 10-day EIP) of mosquitoes will survive long enough to complete the EIP and become infectious to humans, assuming they are infected with DENV when they take their first blood meal. Larger DTRs result in a shorter mosquito life span than smaller DTR surround the same mean temperature; however, larger DTRs may concurrently shorten the duration of the EIP, demonstrating the complex nature of these relationships,[7,38] recently reported that less thermal energy is required for pupation when temperatures fluctuate around a low mean value than when the temperature is constant at that value, whereas more thermal energy is required when temperatures fluctuates around a higher mean value than when the temperature is constant around that value. These results indicate that population models that assume the required the energy for pupation is constant could be over or under estimating development times, leading to inaccurate simulations. The female mosquito's reproductive cycle is also governed by ambient temperature. At< 20°C, fertilization decreases,[10,39] established that increased minimum temperatures resulted in accelerated oviposition cycles and egg laying. Female *A. aegypti* require a blood meal for ovarian development, and feeding behaviour is also influenced by temperature. Feeding activity is limited or ceases at temperatures < 15°C [10]and can also be limited at temperatures > 36°C. Multiplicity of feeding, that is the taking of blood meals from multiple hosts during a single gonotrophic cycle (blood feeding, ovarian development, and egg laying), has been associated with higher levels of DENV transmission,[40,41] found that higher temperatures were associated with higher incidences of multiple blood feedings in Thailand but not in Puerto Rico. Female size was also negatively correlated with temperature, and smaller females exhibited increased multiplicity of feeding in Thailand.[41] The effect of temperature on the ability of the mosquito to reproduce has consequences for population dynamics and range limits,[42] found that *A. aegypti* preferred shaded containers and cooler water temperatures for egg laying in Puerto

Rico. However,[43] found that containers with more sun exposure were more likely to be inhabited in Iquitos, Peru. This may be because obtaining optimal water temperature for the mosquito required heating from direct sun exposure in one location and protection from the sun in another. In both cases, however, water temperature was important for mosquito reproduction.[40] also found the presence of trees to be associated with *A. aegypti* pupal productivity; they suggested that although dense vegetation may promote growth by contributing organic material to the habitat, it can also influence water temperature and evaporation by creating shade.

Although there are similarities in the thermal characteristics of many of the variables associated with vector and viral development, the critical limiting values are not the same. Integrating all these variables together in one temperature-driven model can enable simulation of very complex dynamics. Figure 6 shows the varying effects of temperature on many of the variables discussed above. There is often little correlation in the individual responses of these variables to temperature. This indicates that although increasing temperatures may accelerate parts of the viral transmission cycle, other components may become limited by high temperatures. Thus, the overall effect of warming on DENV ecology will be context dependent.

Effect of Rainfall on Vector Population

Barrera *et al.* examined the hatching rate and the amount of eggs in containers in Chiang Mai and reported that the peak of the population was approximately 1 month after the start of the rainy season, from June until the end of September.[42]

The egg population remained low in the dry

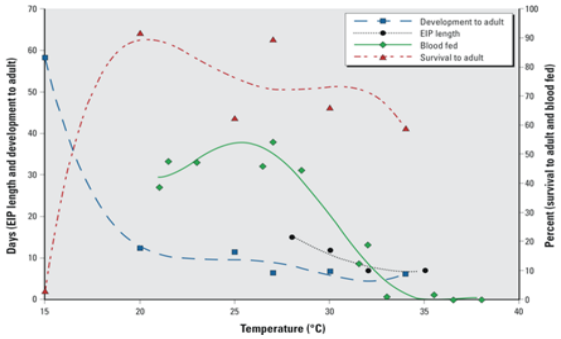

Fig. 6- Effects of temperature on variables associated with DENV transmission. Days required for immature *Ae. aegypti* development to adult (Rueda et al. 1990), length of DENV-2 EIP (Watts et al. 1987), percent of *Ae. aegypti* mosquitoes that completed a blood meal within 30 min after a blood source was made available (Morin CW, unpublished data), and percent of hatched *Ae. aegypti* larvae surviving to adulthood (Rueda et al. 1990).

season but increased exponentially during the first half of the rainy season, and then decreased sharply in the second half of the rainy season. Although the rain continued, the population of aquatic stage mosquito was actually decreasing as the food supply in containers declined and the competition among larval increased. The amount of rainfall is associated with the mosquito population by increasing breeding sites or egg carrying capacity. An equation of the population dynamics of *Aedes aegypti* is created. The egg-carrying capacity indicates the maximum population of aquatic mosquitoes (egg, larva, pupae) such that resources are sufficient and equation is as follows:

$$K(t) = \left(K_m + (1 - K_m)\sin^2\left(\frac{\pi t}{365} + \phi\right)\right)K_E$$

Where K (t) is the egg carrying capacity related to the amount of available food and space for eggs and then larvae will be able to develop, K_m is fraction of the minimum egg carrying capacity in the area, K_E is constant egg carrying capacity, ϕ is adjusted year cycle. K_E is always positive because several containers are rainfall independent. To demonstrate the mosquito

population under the influence of the rainfall and temperature, we set K_E = 100, 000, K_m = 0.18 is chosen to be the ratio between the egg hatching rate in dry and rainy seasons in Chiang Mai (183:1,023)[44].

The effects of precipitation and evaporation on available water sources can regulate the size, population, and behaviour of *Aedes*. For example, in Taiwan, the risk of dengue increased over a period of up to 15 weeks, once the daily maximum24-h rainfall reached 450 mm but there was a temporary one month decrease in dengue risk following extreme rainfall[45]. In some regions, precipitation changes with La Niñaand El Niño conditions, which affects mosquito distributions.[46]

Not surprisingly increased collections of *A. aegypti* eggs and adults coincided with the monsoon rains. In neighbourhoods near San Juan, Puerto Rico, [47] found that higher precipitation was associated with increase *A. aegypti* populations, and that man-made containers were the most important pupae habitat for producing adult mosquitoes. Intense rainfall, however, may wash outbreeding sites and thus have a negative effect on vector populations. Kolivras concluded that mosquito ranges expand during La Niña conditions (generally wetter) and decrease during El Niño conditions (generally drier).This could increase future risk of dengue fever (DF) given projected changes of El Niño Southern Oscillation (ENSO) cycles.[46]

Drier conditions, however, can indirectly expand a vector's range.[48] used a biophysical model in conjunction with an evolutionary response component to project alterations in the range of *A. aegypti* in Australia due to climate change and concluded that habitat for the mosquito will likely expand as individuals increase household water storage in response to a drier climate. Drier conditions could also cause selection pressure towards greater egg resistance to desiccation.[48,47] posited that installing domestic water reservoirs to combat drying from warmer temperatures and decreased precipitation actually provides additional breeding grounds for *A. aegypti* mosquitoes, whose range is predicted to expand with increasing temperature. Australia's risk of DF comes not only from the direct effects of climate change on mosquito population density, but also from the adaptive measures people take to mitigate its effects.[18]

Figure7 shows the average monthly dengue incidences in Chiang Mai from 2004 to 2014 with (A) the average monthly rainfall during the same period and (B) the average monthly temperature.[50]

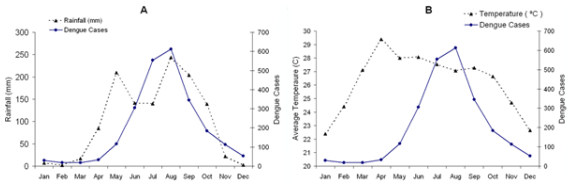

Fig. 7- The average monthly incidence rate of dengue during 2004–2014 in Chiang Mai, Thailand is indicated by the complete line.
The broken lines are (A) the average monthly rainfall and (B) the mean temperature during the same period.

CLIMATE, HABITAT AND VECTOR ECOLOGY:

Precipitation and temperature work interdependently: Increased temperatures accelerate evaporation rates and limit standing water as a potential habitat source for immature mosquitoes; although the eggs are resistant to desiccation over extended time periods[10] and thus climate effects on immature vector survival are a complex balance between precipitation and evaporation. Higher rates of precipitation combined with higher temperatures also result in increased humidity. Higher humidity is associated with increased *A. aegypti* feeding activity, survival, and egg

development[10,51] reported that daily minimum temperature and an increase in precipitation from the previous month were both associated with increased larval abundances. At the greatest extreme, complete evaporation will result in complete mortality of larvae and pupae.[18]

Weather variables, such as humidity and evaporation rate, influence vector competence, biting behaviour, and adult mosquito survival, but have received less attention. For example, in Thailand, ambient temperature appears to define a viable range for transmission, and humidity amplifies the potential within that range.[52] Eighty percent of severe dengue cases over the period 1983–2001 occurred when the temperature was 27–29.5°C and mean humidity was >75%. Given that warmer temperatures can bring higher humidity, understanding these interactions is important for early warning systems and for projecting how a changing climate could alter the future burden of dengue.[53]

Barbosa *et al.* raised immature *A. aegypti* at various densities in laboratory conditions and found that higher densities resulted in slower development, greater mortality,[54] and a lower body mass. Much of the density dependence exhibited in these studies is the result of nutritional stress in the containers. This suggests that precipitation exerts less influence on density dependent mortality than nutritional levels within the habitat. Keirans and Fay (1968)[55] subjected laboratory-reared *A. aegypti* to varying levels of food rations and concluded that oviposition and pupation were delayed under food stress. Moore and Whitacre (1972)[56] reported that nutritional levels regulate production of growth retardant factor (GRF), which can limit population growth. However a conflicting studies by Dye (1984)[57] showed that the production of GRF is strain dependent, and it probably plays only a small role in population control. Still other studies have provided evidence that shows nutrition to be important for *A. aegypti* larval development and survival.[12] discovered that containers with more organic matter resulted in larger mosquitoes, quicker development, and higher survival rates. Similarly,[42] studied larvae-inhabited containers in Puerto Rico and their results indicate the existence of food competition in most containers, although containers with a larger water volume showed reduced competition-related effects (such as retarded development and increased mortality). This may be explained by larger water bodies being less affected by evaporation, resulting in lower larvae/pupae population densities. [42] also showed that the body mass of individual mosquitoes was decreased among mosquitoes in more crowded containers. As noted previously,[41] smaller body size is associated with increased transmission potential because of the increased multiplicity of feeding in smaller mosquitoes compared with larger mosquitoes.

Climate factors may also provoke competition between species of mosquitoes. *A. aegypti* and *A. albopictus*are two DENV vectors that often have overlapping habitat distributions. [58] studied egg mortality rates of these two species in varying laboratory environments and reported that although *A. aegypti* eggs thrived across a wide range of humidity and temperature combinations, *A. albopictus* eggs experienced high mortality at conditions at <95% humidity when temperatures were > 22°C. In their southern Florida cemetery survey of mosquito presence vs. absence,[58] found a significantly lower *A. Aabopictus* presence after the dry season than during the wet

season, whereas the *A. aegypti* presence was consistent.

DISCUSSION

Previous studies have shown that climate exerts critical influence on the spatial and temporal extent of DF. However, future studies need to better capture the effects of Environmental factors on the ecology of DENV. Here we briefly discuss two examples of environmental influences that need to be resolved in future studies of climate and DENV transmission risk. The assumption that a vector will always colonize new habitats if temperature tolerances allow is simplistic. Although mosquitoes transmitting disease are unlikely to be limited by host ranges (because they often feed primarily on humans), they may be limited by competition. For example in Florida, *A. aegypti* populations have been displaced spatially and/or temporally from some locations and replaced by *A. albopictus*, especially in rural settings[59], suggesting that *A. albopictus* may be a better competitor for larval habitat under certain conditions.[60, 61, 62, 63] Although both of these mosquitoes are DENV vectors, their competence is variable. *A. albopictus* tends to be a more generalist feeder, but it prefers human hosts for blood meals when available.[64, 65, 66, 67, 68] Studies also suggest that *A. albopictus* is less susceptible to DENV infection and dissemination to the salivary glands.[69,70,37,71] Physical barriers such as large water bodies, mountains, or deserts may also restrict species dispersion.[72] The effect of climate on the virus itself also has received little attention in the literature. Vector abundance indices are generally taken as the only measure of DENV transmission potential. Because climate exerts a major impudence on mosquito population dynamics, studies focusing on climate–disease associations often choose to model vectors. However, virus dynamics within the mosquito must also be considered. Thomas *et al.,* for example, used the temperature dependence of the EIP to project DENV transmission rates in Europe under various climate scenarios.[73] Optimal temperatures for development of the vector are not necessarily the same as those for the virus. As a result, large vector populations may not be sufficient for transmitting DENV if viral replication is inhibited or if the lifespan of the mosquito is shorter than the EIP. Consequently, vector abundance/density may not always be appropriate proxy measurements of DENV transmission risk.[74, 75] The virus and vector may also adapt to changes in climate as they occur slowly over time, and thus, changes in the relationship

To determine the effect of different climatic variables on the DENV transmission different researchers have used different models to describe their views (Table 1).

Table 1. Characteristics of studies on the association between climate variable and dengue.

Study	Location (Study Period)	Statistical methods	Risk factors	Major Findings	Comments
Jonsson *et.al.*2009a	Thiland (1983-1996)	Wavelet analysis	ENSO Precipitation & Temperature	The direct relationship between ENSO & Dengue was non stationaries and ENSO appeared to be associated with local temperature and precipitation.	Further research is needed to explain the non-stationarity in ENSO & Dengue incident.
Tipayamongkholgul *et. al*. 2009.	Thiland (1996- 2005)	Poisson regression	ENSO Temperature, Relative humidity, Wind speed.	The effects of El Nino on Dengue transmission were varied according to geographical location with in Thailand.	Only southern coastal and Norther Inland regions were included.
Cazelles *et.al*. 2005	Thiland (1983- 1997)	Wavelet analysis	ENSO	The dynamics of El Nino were strongly associated with Dengue incidence but non stationary existed in this relation which might influenced by the synchrony of previous Dengue epidemic.	The magnitude of the EL Nino and dengue relationship needs to be viewed with in wider context of socio-environmental variability.
Nakhapakorn & Tripathi, 2005	Thailand (1997-2001)	Multiple regression	Rainfall, Temperat ure, Humidity,Lan d use.	Built- up area had highest risk of Dengue incidence and temperature, rainfall and humidity were likely to be key determinants of DF.	Only one province was included.
Thammapalo *et. al.* , 2005b	Thailand (1978-1997)	Linear least squre regression	Monthly total rainfall, Rain days, Daily temperature, Daily relative humidity	Increased temperature was positively associated with dengue incidence in central and northern parts of Thailand where increased rainfall was negatively associated in southern Thailand	Spatial variation was not examined
Nagao et al. 2003	Thailand (1992-1996)	Multiple regression	Rainfall, Temperature, Water wells, Tin houses	Larval abundance of *Aedes* mosquito was positively associated with house conditions, water supply and transport services. Increased rainfall in 2 months earlier and temperature were also correlated with larval indices	Only 18 province of Northern Thailand were included
Hsich & Chen 2009	Taiwan (2007)	Richards model Distributed lag model	Typhoon, Temp erature, Precipitation	The multi-wave dengue outbreak in Taiwan in 2007 was appeared to be influenced by rainfall and temperature variation as a consequence of two consecutive typhoons	Further research is required to explore the relationship between extreme weather events and dengue transmission
Wu *et al*. 2007	Taiwan (1998-2003)	ARIMA model	Rainfall, Temperature, Relative Humidity, Vector density	The incidence of DF was negatively associated with monthly temperature variation and reversely with relative humidity at lags of 2 months	Only one metropolitan city was included

Study	Location (Study Period)	Statistical methods	Risk factors	Major Findings	Comments
Wu *et al.* 2009	Taiwan (1998-2002)	Spatial analysis Logistic regression	Rainfall, Temperature, Level of urbanization, Percentage of elder population	Number of warm months and degree of urbanization were found to be associated with increasing risk of DF incidence at township level	Both climatic variables and socio-demographic factors were considered
Halide & Ridd 2008	Indonesia (1998-2005)	Multiple regression	Rainfall, Temperature, Relative Humidity	Relative humidity at 3-4 months lags and current number of dengue cases appeared to be major determinants for prediction of DF outbreak in Indonesia	Only one city was included
Arcari *et al.* 2007	Indonesia (1992-2001)	Multiple regression	Rainfall, Temperature, Relative Humidity, SOI	Rainfall and temperature observed as important predictor of DF transmission in Indonesia, although the association differed across geographical region of the country	Socio-environmental factors were not included
Bangs *et al.* 2006	Indonesia (1997 & 1998)	Descriptive analysis	Rainfall, Temperature, Relative Humidity, ENSO, House index	ENSO driven increased temperature exhibited greater impact on dengue transmission by the vector population	Only one city was included
Chakravarti & Kumaria 2005	India (203)	Descriptive analysis	Rainfall, Temperature, Relative Humidity	Temperature, Rainfall and Relative Humidity were major determinants for dengue transmission and outbreak coincided in post-monsoon period	Only one outbreak in one city was considered
Lu *et al.* 2009	China (2001-2006)	Poisson regression	Rainfall, Temperature, Relative Humidity, Wind velocity	Increase minimum temperature and decreased wind velocity were associated with increase dengue incidence	Only one province was included.
Yang *et al.* 2009	China (July-October 2004)	Descriptive analysis	Temperature, Precipitation, Humidity, Breteau index, House index	Dengue incidence seemed to have no relationship with climatic factors. However, negatively associated with vector control	Non-endemic nature of DF in Cixi city may biased the relationship between climate variables and DF
Hii *et al.* 2009	Singapore (2000-2007)	Poisson regression	Temperature, Precipitation	Weekly mean temperature and total precipitation were related to increased dengue incidence	Socio-environmental factors were not included
Hu *et al.* 2010	Australia (1993-2005)	SARIMA model	ENSO	Decreased mean of SOI at lags of 3-12 months earlier was inversely associated with dengue incidence in Queensland	Only SOI was considered
Bi *et al.* 2001	Australia (1990-1994)	ARIMA model	Temperature, Precipitation, Relative Humidity	Monthly mean minimum temperature was the major contributor for dengue outbreak in Townsville	Only 4 years dataset used

Arranged by location of study.

ENSO-El Nino-Southern Oscillation Index; ARIMA-autoregressive integrated moving average; SARIMA -seasonal autoregressive integrated moving average; SOI- Southern Oscillation Index; DF-Dengue fever

Conclusion

The climate models for the dengue vector presented here are useful for management purposes, particularly with regard to future climate change. They can be adapted for making decisions regarding allocating resources for dengue risk towards areas where risk of infection remains and away from areas where climatic suitability is likely to decrease in the future climate. These models would facilitate prioritizing dengue

management initiatives in current risk areas and those with continuing risk in the future.

Future studies should focus on addressing these issues. DENV vector dispersal ability should be evaluated by inventorying the species currently inhabiting areas of concern and then assessing the likelihood of invasion by the new vector. Current mosquito population models can be made more sophisticated by including a viral component.

Although there has been much speculation on the connection between climate and DF occurrence, in the present review we have highlighted the need for research that produces more precise and stable results. Although climate variables strongly influence DENV and its vectors, caution must be taken when using only one element or connection to predict disease occurrence.Climate influences disease ecology at many levels, and the many nonlinearities and feedbacks present in the system create complex dynamics that are not easily modelled or understood. In addition, human factors, including behaviour, immunity, and socioeconomic influences, also contributes to the complexity of these relations. Nonetheless, it may be possible to extract basic patterns and general predictions that could provide useful information for mitigating the effects of climate change on DF occurrences. Capturing all aspects of the disease is a daunting task, but newer techniques may help overcome the difficulties. Process-based models that incorporate a more holistic view of the viral ecology should be implemented as new information on the topic is obtained and computing power increases. The use of interdisciplinary approaches will ensure that studies focus on the interactions between the components of the disease system, in addition to studying each component in isolation. A better understanding of the impudence of climate on disease ecology is needed to improve projections of future disease risk, thus enabling better preparation and improved strategies to limit DENV transmission.

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Chapter 10

Seasonal Disease Occurrence, Mortality and Survival of Adults and Fingerlings of Channa Punctatus (Bloch) by Artificial Inoculations of two Strains of Aeromonads

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Seasonal disease occurrence, mortality and survival of adults and fingerlings of *Channa punctatus* (Bloch) by artificial inoculations of two strains of aeromonads

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Abstract

Motile aeromonads (Family: Aeromonadiceae, Genus: *Aeromonas*) are responsible for heavy mortality in both wild and cultured fish populations. *Aeromonas hydrophila* is an indigenous, essentially opportunist pathogen which invades the tissue of a fish and render susceptible to infection by stress factors. On the other hand, *Aeromonas salmonicida* appears to be obligatory parasite of fish. With the invasion of certain new bacterial species in Indian waters along with climatic variability, the exotics might pose a potential threat to fish diversity when present beyond the threshold level. In the present study, seasonal disease occurrence, percentage of mortality and survival of adult and fingerlings of *Channa punctatus* were investigated with chosen sublethal dose (4×10^7cfu /ml) of two strains of aeromonads (viz. *A. hydrophila* and *A. salmonicida*) injected separately and intramuscularly for a period of 15 days in different time zones of a year. The symptomatic effects of infections were also characterized after inoculations of aeromonads in both cases. It was observed that adult *C. punctatus* was prone to disease particularly in summer and monsoon periods. Similarly, highest percentage of mortality was recorded in monsoon and summer months in fingerlings of *C. punctatus* artificially inoculated with same dose of aeromonads. The onset and rates of mortality in all experimental groups of fish in this experiment possibly reflect the relative magnitude of stress due to infection of aeromonads.

Keywords: *Channa punctatus, Aeromonas hydrophila, Aeromonas salmonicida*, pathogenicity

Introduction

Bacterial infections, caused by aeromonads or motile members of the genus *Aeromonas,* are among the most common and troublesome diseases of fish raised in ponds and recirculating systems.[1] The widespread distribution of these bacteria in the aquatic environment and the stress induced by intensive culture practices predisposes fish to infections.[2] Some bacteria are considered as opportunistic pathogens. These bacteria are often present in the water and inside the fish but they usually cause no problem. In nature, fish are, in most cases, resistant to these pathogens and can seek the best living conditions available. In aquaculture, however, food fish are weakened by stress conditions including increased fish density,

inadequate nutrition, poor water quality (i.e., low dissolved oxygen, or high ammonia and nitrite), parasite infestation, and handling. Stress suppresses the immune system thereby increasing the susceptibility of fish to bacterial infections. As a result, cultured food fish are more susceptible to disease than free ranging animals. Common examples of opportunistic bacteria, which can cause disease and death of food fish, include *Aeromonas hydrophila, Pseudomonas fluorescens* etc. Some bacteria, on the other hand, are obligate pathogens which can be the sole cause of disease even in the absence of stressors. *Aeromonas salmonicida, Edwardsiella ictaluri* and *Yersinia ruckeri* are therefore considered to be obligate pathogens. The occurrence of fish disease and mortality caused by infection of aeromonads has been well documented[3]. The purpose of the present experiment was to study seasonal disease occurrence and percentage of mortality and survival of adult and fingerlings of an air- breathing fish *C. punctatus* with chosen sublethal dose of two aeromonads viz. *A. hydrophila* and *A. salmonicida* respectively injected separately and intramuscularly (im) and to characterize the symptomatic effects of infections after inoculation with aeromonads of both strains.

Materials and Methods

Fish stock collection, acclimatization and maintenance

The 'spotted murrel', *Channa punctatus*, is an air-breathing fish and generally a freshwater inhabitant preferring from stagnant muddy pond water to canals, paddy fields, lakes, rivers etc. Live and healthy adults and fingerlings of *C. punctatus* were collected from local fish farm located at Kolkata, West Bengal, India. The specimens were transported live to the Department of Zoology, University of Calcutta, and were kept alive for one week in glass aquaria (size: 0.6m x 0.3mx 0.3m, 5-6 fish per aquarium) under controlled laboratory conditions with continuous aeration. Fishes were fed *ad libitum* with *Tubifex* sp. and larvae of *Culex* sp. during acclimatization period only. The water was renewed every day to avoid accumulation of unutilized food or metabolic waste products.

Bacterial culture collection and maintenance

Microbial cultures of two bacterial strains of Aeromonads viz. *Aeromonas salmonicida* (MTCC 1522) and *Aeromonas hydrophila* (MTCC 646) were collected earlier from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, IMTECH, Chandigarh, India and maintained in the laboratory. Those strains were received as lyophilized cultures and subsequently revived by adding Nutrient Broth (NB) and transferring the rehydrated culture to a fresh Nutrient Agar (NA) medium. Consequently, streak plate method was followed to get isolated bacterial colonies on a large part of the agar surface.

Artificial inoculation of aeromonads on C. punctatus adults and fingerlings

Both strains of aeromonads (*A. salmonicida* and *A. hydrophila*) were cultured in nutrient broth (NB) and incubated at 25°C and 37°C respectively 24h prior to infectivity testing. Bacterial cells were harvested by centrifugation at 5000 × g for 5 min and washed in physiological saline, PS (0.85 % NaCl).The strains were enumerated by correlating the OD values (600nm) of the growing culture with the corresponding colony forming units (cfu) obtained by spread plate dilution method (OD 600_{nm} 1= 2×10^9 cfu / ml). Different concentrations of bacteria ranging from 1×10^5 to 1×10^{10}cfu /ml were made up in PS (working volume: 0.5 ml /100g body weight of fish) and

injected intramuscularly (im) into different groups of *C. punctatus*, each containing 15 fish. The fish were observed for changes in their behavioral patterns as well as development of haemorrhagic ulcers and tissue necrosis. The viability of the infected fish was checked for 72 h and the corresponding LD_{50} doses were determined[4]. The seasonal disease occurrence, percentage of mortality and survival of adult and fingerlings of *C. punctatus* were studied with chosen sublethal dose (4×10^7cfu/ml) of aeromonads.

For the present experiment, a yearlong study was divided into three broad time zones: (a) from July to October, (b) from November to February and (c) from March to June. The experiments were carried out for a period of 15 days, once in a month for each strain of aeromonad (i.e. Number of experimental set up=4/time zone/strain). Before the experiment, sublethal concentrations (4×10^7cfu /ml) of both of the strains were made up separately and injected intramuscularly into 4 sets of *C. punctatus* (each set containing 15 fish). Among the 4 sets of fish studied at each time zone per strain, 2 sets were adults (length: 18.5 - 21.0 cm and weight: 78-90 g) and the remaining two sets comprised of fingerlings of *C. punctatus* (length: 8.0 - 10.5 cm and weight: 22.5 - 25.0 g). The extent of disease and mortality were studied in experimental fishes treated with aeromonads after sufficient acclimatization under laboratory conditions. The results were shown in % range.

Results

After experimental inoculation of sublethal dose of *A. hydrophila* in adults (Table 1), disease occurred in 50 - 6 % of the fish population and rest 30 - 40% survived without any external symptoms during July-October months. It was also observed that about 90-100 % of the fish died among the diseased fish. During November-February and March-June time zones, the percentage of disease occurrences was much reduced and increased respectively than monsoon months. Almost all of the fish died among the diseased fish in the November-February duration and around 80-90 % died in the March – June session. Fish infected with *A. hydrophila* showed different clinical signs which ranged from sudden death or exhibited swimming abnormalities, pale gills, and skin ulcerations. The skin ulcers occurred at multiple sites on the fish which were surrounded by a bright red rim of tissue (Figure 1 and 2).

Table 1. Percentage of seasonal disease occurrence, mortality and survival of adult C. punctatus on artificial inoculation of A. hydrophila

Months studied	Dose injected IM (cfu/ml)	Exposure Period (Days)	% of diseased fish with characteristic external symptoms	% of mortality among the diseased fish	% of survival of infected fish without external symptoms
July-Oct	4×10^7	15	50-60	90-100	30-40
Nov-Feb	4×10^7	15	20-25	95-100	75-80
Mar-Jun	4×10^7	15	60-80	80- 90	20-40

Figure 1. *Death of adult Channa punctatus caused by Aeromonas hydrophila infection.*

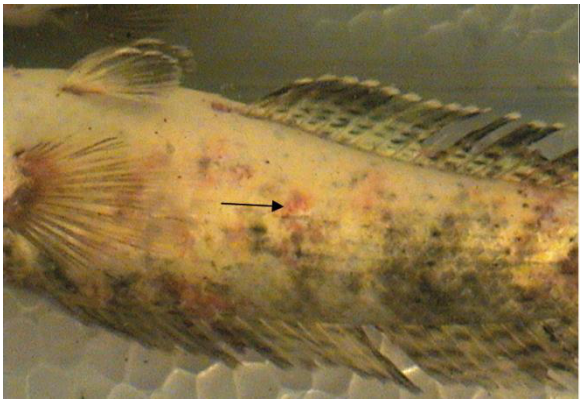

Figure 2. *A bright rim of tissue (arrow) surrounding skin ulcers produced by Aeromonas hydrophila on the dermis of* adult *Channa punctatus*.

After experimental inoculation of sublethal dose of A. *salmonicida* in adult *Channa punctatus*, percentage of disease occurrence in fish population with characteristic external symptoms in different time zones was as follows- maximum during July- October; moderate during November-February and lowest during March-June session (Table 2).

Table 2: Percentage of seasonal disease occurrence, mortality and survival of adult C. punctatus on artificial inoculation of A. salmonicida

Months studied	Dose injected IM (cfu/ml)	Exposure Period (Days)	% of diseased fish with characteristic external symptoms	% of mortality among the diseased fish	% of survival of infected fish without external symptoms
July-Oct	4×10^7	15	80-85	50	15-20
Nov-Feb	4×10^7	15	50-55	66.67- 80	45-50
Mar-Jun	4×10^7	15	0-10	90-95	90-100

Percentage of mortality among the diseased fish was maximum pre-monsoon periods. Infection with atypical strain of *A. salmonicida* did not necessarily result in the acute mortality and septicaemia characteristic of the typical furunculosis strain, but manifested more as external lesions and ulceration, often involving secondary infection. Some of the clinical signs of disease in an *A. salmonicida* infected fish included white raised patches on the skin that progressed to ragged-edged red ulcers, haemorrhages on skin and paired fin bases, ulcers most often on the upper side of the lateral line behind the head or at the base of the tail fin (Figure 3) and haemorrhages in muscles and internal organs (Figure 4).

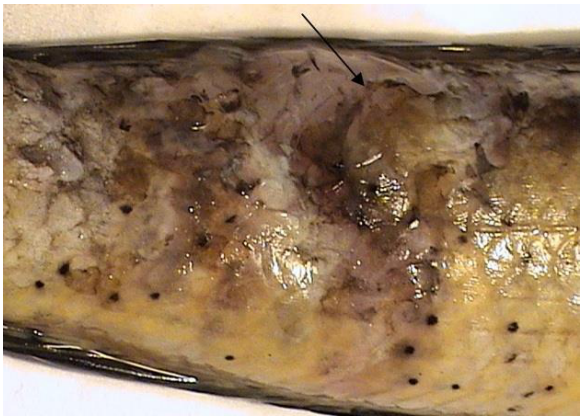

Figure 3. *Extensive lesions (arrow) produced by Aeromonas salmonicida infection in the dermis and muscles of Channa punctatus*.

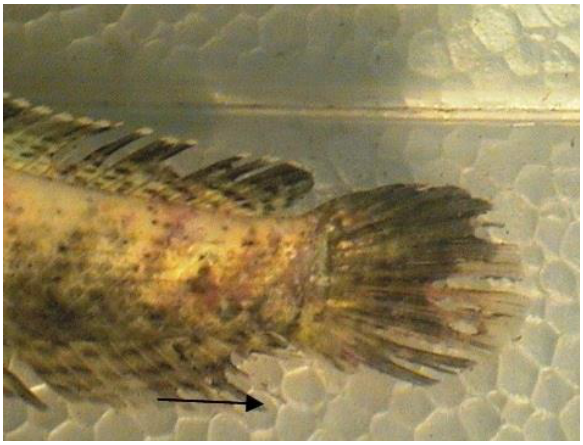

Figure 4. *Ulceration and haemorrhages (arrow) at the base of the tail fin of Channa punctatus* by *Aeromonas salmonicida*.

Table 3. Percentage of seasonal mortality and survival of C. punctatus fingerlings due to artificial inoculation of A. salmonicida and A. hydrophila

Months studied	Sublethal dose injected (IM) per strain (cfu/ml)	Exposure period (Days)	Bacterial strains	% of mortality of infected fish	% of survival of infected fish without external symptoms
July-Oct	4×10^7	15	*A. salmonicida*	10-15	85-90
			A. hydrophila	78-80	20-22
Nov-Feb	4×10^7	15	*A. salmonicida*	10	90
			A. hydrophila	10-20	80-90
Mar-June	4×10^7	15	*A. salmonicida*	37.5-40	60-62.5
			A. hydrophila	10-15	85-90

Discussion

Table 3 represented mortality and survival percentages of fingerlings of *C. punctatus* due to experimentally inoculated sublethal doses of both strain of aeromonads over the three time zones. During monsoon season (July- October), only a few percentage of the fish population died among the fishes nfected with *A. salmonicida* whereas mortality was much higher (78-80 %) of the fishes infected with *A. hydrophila.* During November-February session, percentage of mortality was reduced to both cases. During March- June period, 37.5- 40 % and 10-15 % mortality were observed in fish stocks experimentally infected with *A. salmonicida* and *A. hydrophila* respectively.

Over the years, there has been increasing concern about the number of parasitic diseases of freshwater fish constraining aquaculture production. This might be due to environmental deterioration coupled with increased virulence of pathogens on a susceptible host. Motile aeromonads are the most common bacteria found in freshwater habitats and frequently cause disease among cultured and feral fishes. *A. hydrophila* is a ubiquitous, essentially opportunist pathogen while *A. salmonicida* appears to be obligatory parasite of fish. Typical strains of *A. salmonicida* subsp. *salmonicida* causes furunculosis in salmonids whereas atypical strains cause ulcerative and generalized diseases in a wide variety of both freshwater and marine fish[5]. The incidence of bacterial infection in fish, gross pathology, disease occurrence and extent of mortality were studied by researchers. The mortalities of farmed salmonids caused by atypical *A. salmonicida* infections have been recorded 10%,[6] up to 15-20%,[7] from less than 10 % to more than 90%,[8] 50%,[9] up to 50% [10] and 10-67%.[11] The strains of motile aeromonads isolated from diseased fish were more virulent to channel catfish than were those isolated from pond water.[12] The significant differences in percentage of mortality between groups of *Ictalurus punctatus,* injected intraperitoneally with a sublethal dose of *A. hydrophila* were noted while exposed the treated groups with a variety of stressors.[13] The systemic infections were produced more readily among channel catfish (*Ictalurus punctatus)* by abrading their skin prior to exposing the fish to the bacterium, *Aeromonas hydrophila.*[14] This bacterium infected internal organs through the digestive tract or through uninjured skin under conditions of crowding and high temperature but such infections did not occur when catfish were held at a lower density and lower temperature. The spread of goldfish ulcer disease from Victoria to New South Wales, Australia, and the first isolation of an exotic atypical strain of *A. salmonicida* from wild goldfish was also reported.[15] The exposure of chinook salmon to *A. salmonicida* by gastric incubation resulted in highest prevalence of aymptomatic carrier.[16] *Aeromonas hydrophila* subsp *anaerogens* were isolated from skin lesions of air-breathing fishes, *Anabas testudineus, Heteropneustes fossilis* and *Clarias batrachus* collected from northern districts of West Bengal, India.[17] The pure culture of that aeromonad strain separated from the mixture induced superficial ulcer formation. The occurrence of different *A. salmonicida* subspecies at a number of fish farms in northern and central Finland was presented with the particular aim of assessing their importance as cause of disease in different salmonids.[18] The prevalence of motile aeromonad septicaemia in cultured and wild Nile tilapia (*Oreochromis niloticus)* was recorded as 10 % and 2.5 % respectively; it was 18.75 % and 6.25 % in cultured and wild Karmout catfish, respectively.[19] Severe eye pathology and heavy mortality among yearling and older rainbow trout was noted accompanying a severe outbreak of motile aeromonad septicaemia.[20] *Aeromonas hydrophila* was isolated from Nile tilapia, *Oreochromis niloticus* during fish disease outbreaks in various aquaculture farms and projects in Luzon, Philippines.[21] Temperature optimums may depend upon the particular strain of bacteria under investigation, but generally range from 25°C to 35°C. Most of the epizootics among warm water fishes in the southeastern United States were generally reported in spring and early summer months.[22] Summer

mortality ('die-off) was common in striped bass, *Morone saxatilis* in the San Francisco Bay-Delta region.[23] The infections of aeromonads appear with high numbers during the warmer months of the year.[24, 25] Clinical isolation of these microbes presents the same seasonal distribution.[26]

The adults and fmgerlings of *C. punctatus* is known to harbour a host of fish pathogens and is therefore, prone to disease particularly in summer and pre-monsoon months in natural water bodies of West Bengal. After experimental inoculation of sublethal dose of *A. hydrophila* and *A. salmonicida* on adult under controlled laboratory conditions, maximum percentage of disease occurrence was found in summer and monsoon periods respectively (Tables 1 and 2). On the other hand, highest percentage of mortality was recorded in monsoon and summer months respectively in fingerlings of *C. punctatus* sp. injected with same dose of *A. hydrophila* and *A. salmonicida* (Table 3). The onset of mortality was generally observed on the 6th or 7th day after sublethal exposure of aeromonads in the present study. The onset and rates of mortality in all experimental groups of fish in this experiment possibly reflect the relative magnitude of stress due to infection of aeromonads.

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Chapter 11

Role of the 'modern' Environment on Cancer

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Role of the 'modern' environment on Cancer

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Abstract

Cancer is increasingly becoming a major health issue in India for the last few years. This multifaceted disease arises from a network of extremely complicated causes. Recent studies suggest that most of the chronic diseases arise not solely due to the genetic code but due to the complex interactions of all the products of such genes with the environmental factors. Suggesting cancer to be a 'modern' disease' would not be a misnomer as the occurrence of this disease was not very evident among our recent ancestors. This indicates that the hike in cancer incidences in modern times might be caused by environmental changes and changes in lifestyle, including diet pattern. The most common lifestyle factors leading to cancer death include: tobacco, diet and obesity, infections, stress and lack of physical activity. Alcohol consumption, excessive sun tanning and occupational hazards are some other responsible factors. In India, which is a developing country, a large portion of the rural population is migrating to the cities due to the rapid growth in industrialization. Such populations are increasingly being exposed to environmental pollutants resulting from vehicular emissions, untreated industrial smoke, factory wastes, pesticides, radon exposure, radiation etc. Recent studies suggest that about 90-95% of cancer cases are caused due to the environment and lifestyle factors. Thus, there is an urgent need to look back and create a healthy state of living by finding safer alternatives to the present chemical and physical risks. Awareness, urgent actions and adoption of a healthy lifestyle are therefore the most important tools to fight against cancer and other preventable diseases of the modern day.

***Keywords*:** Cancer, Lifestyle, Tobacco, Environmental pollutants, Public awareness

Introduction

Due to the growing industrialization in India, which is a developing country, a large portion of the rural population migrates to the cities. This leads to their exposure to pollutants from vehicles, factories etc. and also changes in lifestyles. In many developing countries, a shift towards Westernized diet and lifestyle has been associated with prevalence of people being more obese.[1] Reports indicate that about 90–95% of cancer cases can be attributed to the environment and lifestyle factors.[2]

The current study emphasizes the importance of external factors like the living style of people and environmental factors on the occurrences of cancer. The inherited factors cannot be changed but the modifiable environmental factors make cancer a preventable disease to some extent. This paper reviews the external factors responsible for the prevalence of cancer and the need to look back to set a healthy state of living for the society in this age of industrialization and modernization.

Factors posing risk to cancer

Cancer is caused by both internal and external factors acting concurrently. The internal factors include genetic mutation, hormones and immune conditions, while the external or acquired factors are comprised of use of tobacco, type of diet, exposure to radiations and infectious organisms.

Environmental and lifestyle factors pose a great risk for the occurrence of various types of cancers: tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radon exposure, radiation, stress, lack of physical activity and environmental pollutants.[2]

Occupational hazards lead to about 2-20% of all cases of cancers.[3]

Lifestyle Factors

Tobacco with more than 50 known carcinogens increases the risk of developing at least fourteen types of cancer. Benzopyrenediol epoxide, a tobacco metabolite is found to be etiologically linked with lung cancer.[4] Nitrosamines and polycyclic aromatic hydrocarbons are some other notable carcinogens obtained from tobacco. Cancerous growths in the larynx, head, neck, stomach, bladder, kidney, esophagus and pancreas have also been reported to be associated with tobacco use.[5]

The present generation is inclined towards western lifestyle, which is characterized by convenience food, TV and PCs. This is taking its toll on youngsters as well as adults, by producing increased numbers of overweight and passive population with lifestyle diseases.[6] Being overweight / obese is linked with prostate, colon, breast, endometrial, and possibly other cancers.[7]

Consumption of alcohol in excessive levels has been associated with increased risk of oral, esophageal, breast, and other cancers. Physical inactivity and stress are found to be linked with increased risk of colon, breast, and possibly other cancers.

Reports suggest substantial link between faulty diet habits and cancer.[8] For example, a prudent diet enriched with fruits and refined grains, vegetables, fish and poultry is associated with decreased risk of colon cancer.[9] Consumption of coffee has found to be associated with a reduced risk of liver cancer.[10] Recent studies indicate that stomach cancer, colon cancer, breast cancer, and pancreatic cancer might have a link with consumption of grilled meat.[11] The presence of carcinogens such as benzopyrene in foods cooked at high temperatures might explain this phenomenon.

Carcinogenesis can be induced by long-term exposure to food additives such as nitrite preservatives and azo dyes.[12] Some mushrooms, termed as 'medicinal mushrooms' are known to strengthen the immune system of the body and provide anti-cancer effect by producing a group of polysaccharides, known as beta-glucans. *Agaricusblazei*, Maitake and *Trametes versicolor* are emples of such beneficial mushrooms.

Infectious agents

A number of neoplasms are found to be associated with infections. Upto 20% of human cancers on a global scale result due toviral infections.[13] These include human papillomavirus (cervical carcinoma), human polyomaviruses (mesothelioma, brain tumors), Epstein-Barr virus (B-cell lymphoproliferative disease and nasopharyngeal carcinoma), Kaposi's sarcoma herpes virus (Kaposi's Sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepatocellular carcinoma), Human T-cell leukemia virus-1 (T-cell leukemias), and *Helicobacter pylori*(gastric carcinoma).[13] HIV

is associated with Kaposi's sarcoma and non-Hodgkin's lymphoma.

Environmental factors

The era of increasing industrialization and modernization has changed the environment during the last few years leading to a rise in cancer causing substances at a rapid pace. Vehicular emissions, untreated industrial smoke, factory wastes, etc. are some of the major environmental factors that pose high cancer risks. Over the past twenty years, vehicular pollution has increased eight times, while pollution from industries has quadrupled. Air pollution associated with polycyclic aromatic hydrocarbons (PAHs) has been associated with many cancers. Some other components of air pollution mix having carcinogenic potential include soot, benzo[a]pyrene, benzene, some metals, particles (especially fine particles) and possibly ozone.Urbanization has resulted in the emergence of industrial centers without a corresponding growth in civic amenities and pollution control mechanisms. Many workers run the risk of developing cancers such as lung cancer and mesothelioma from inhaling asbestos fibers and tobacco smoke, or leukemia from exposure to benzene at their workplaces (World Health Organization, 2007). Childhood leukemia and lymphoma have been linked with indoor air pollutants such as volatile organic compounds and pesticides. With green revolution, agricultural runoff has become a major water pollutant as it contains fertilizers and pesticides. Currently very little evidence is available supporting non-ionizing radio frequency radiation from mobile phones as a cause of cancer.[14] However, sources of ionizing radiation, such as radon gas, can cause cancer. Ultraviolet radiation from the sun can lead to melanoma and other skin malignancies on prolonged exposure.

Conclusion

The environmental factors which can cause cancer can be modified to some extent and this fact points to the preventability of cancer by environmental carcinogens. More than 30% of cancer can be prevented by avoiding risk factors. Public awareness using accelerated tobacco-control programs is an efficient way to reduce the rates of tobacco-related cancer mortality. Children nowadays are increasingly being exposed to non-ionizing radiations through mobile phones, tablets, Wi-Fi devices and so on. They spend too much time slouched in front of the Television or computers. These children should be encouraged to find a physical activity or outdoor sport they enjoy. Parents should include fun exercises in family outings. A healthy lifestyle along with a proper balanced diet, physical activity and giving due respect to biological clock are sure to improve the quality of life. Evidences indicate that increased consumption of fruits and vegetables, and control of infections might reduce rates of cancer. Other factors are avoidance of intense sun exposure, increases in physical activity, and reduction in consumption of red meat and alcohol. Advances in cancer research have made a vaccine designed to prevent cancer available. A human papilloma virus vaccine, called Gardasil was approved by the U.S. Food and Drug Administration in 2006. The vaccine protects against four HPV types. An official recommendation was made in March 2007 by the US Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) that females aged 11–12 should receive the vaccine. Another HPV vaccine on the market as of October 2007 is Cervarix (National Cancer Institute). There is also a hepatitis B vaccine (HVB), which prevents

infection with the hepatitis B virus, an infectious agent that can cause liver cancer (National Cancer Institute). The use of unleaded gasoline, switch to compressed natural gas (CNG) engines, use of solar energy and biogas may be encouraged to reduce the ill-effects of pollution and hence reduce cancer risks. Organic manure and biotechnological methods can be used to minimize the use of chemical fertilizers. According to WHO (World Health Organisation) people should be educated so that they can recognize early signs of cancer and seek prompt medical attention for symptoms, which might include lumps, sores etc. Screening programs will also help in early detection of cancer thereby facilitating effective treatment. Thus, time has come that we evaluate our past and present styles of living, spread awareness and protect our environment to fight with this dreaded disease.

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Chapter 12

Dietary Phytoestrogens - Boon or Bane?

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Dietary Phytoestrogens - Boon or Bane?

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Abstract

Phytoestrogens are a highly debated topic in nutrition. That's because this group of plant compounds can mimic or block the effects of estrogen. Studies have found that phytoestrogens may offer several health benefits, including reducing the risk of heart disease, help maintain healthy bones, lowered risk of obesity, metabolic syndrome and type II diabetes, cancers such as breast cancer and prostate cancer. However, some people believe they can reduce fertility and disrupt your hormones due to their antiestrogenic properties that lead them to be an endocrine disruptor. This article looks at the benefits and risks of phytoestrogens to determine if they are healthy or harmful.

Keywords: Phytoestrogen, estrogen, isoflavone, cancer, estrogen receptor

Introduction

Phytoestrogens are plant-derived compounds that structurally or functionally mimic mammalian estrogens and therefore are considered to play an important role in the prevention of cancers, heart disease, menopausal symptoms and osteoporosis.[1, 2] Estrogens, produced in the ovaries and testis, have many biological effects in the body beyond the reproductive system. Estrogens influence the growth and functioning of female and male reproductive tissues, maintain the skeletal and central nervous system, provide cardioprotective effects in the cardiovascular system, and protect against colon cancer and aging skin.[3] The interest in plant derived estrogens—or phytoestrogens— has recently been increased by the realization that hormone replacement therapy is not as safe or effective as previously thought.[4] The isolation, identification and quantification of phytochemicals in foods and the evaluation of their potential benefits to human health has now become a major research topic. Naturally occurring estrogen mimicking compounds (i.e., phytoestrogens) are found in over 300 species of plants, including various leguminous plant foods eaten frequently by humans (e.g., soy). Eating phytoestrogens can produce either some of the same effects as human oestrogen (oestrogenic effects) or opposed effects (anti-oestrogenic effects). The effects which are triggered depend on existing levels of oestrogen in the body and how the phytoestrogens bind to receptors in the body. The biological activity of individual phytoestrogens varies and is often reported as less active than mammal or synthetic estrogens. Phytoestrogens have been studied extensively in connection with breast and ovarian cancers, with many positive results. Phytoestrogens are proven to improve heart health, specifically in postmenopausal women. They can be used to treat arteriosclerosis, a disease characterized by fatty build up within the

arteries, and seem to do so by regulating many different hormone and chemical levels within the body. Another benefit of may offer to menopausal or postmenopausal women is a reduction in bone loss, leading to higher bone density and fewer breaks, when administered in dose-specific measures alongside vitamin D. They've also been shown to regulate iron absorption into the bloodstream, offering mild anti-inflammatory effects and protecting against massive iron level fluctuations. In vitro studies using human prostate cancer cells have shown the inhibition of cell growth with high concentrations of phytoestrogens. Much research is required to clearly define the pharmacological effect of dietary phytoe-strogens. Thus, some dietary phytoestrogens may decrease menopausal symptoms without increasing the risk of breast cancer. This is just one of many opportunities that exist in the study of the endocrine effects of food components and in defining their pharmacological use. However, the (anti)-oestrogenic properties of phytoestrogens have also raised concerns since they might act as endocrine disruptors, indicating a tendency to cause adverse health effects. Altogether, the health benefits or risks of isoflavones and other phytoe-strogens are still controversial,[5, 6,7] and the question of whether phytoestrogens are beneficial or harmful to human health remains unresolved.

Dietary Phytoestrogens

In foods phytoestrogens are present as mixtures as biologically inactive glycoside conjugates containing glucose or carbohydrate moieties. Blood levels can vary widely between individuals depending both on dietary preferences as well the phytoestrogen content of a particular food product resulting from local and/or seasonal variations.[8] In the gut they are broken down by glucosidases to their respective aglycones allowing more efficient absorption, although intestinal bacteria may further metabolise these products. Once absorbed the aglycone phytoestrogens are rapidly conjugated to glucuronic acid and to a lesser extent sulphuric acid in the hepatic circulation. They are then de-conjugated prior to excretion with urinary concentrations increasing in parallel to consumption.[9] There is generally very low levels of biologically active 'free' un-conjugated phytoestrogens in the circulation (< 3% of the total) and blood levels are in the ng/mL range or lower.[10,11] Thus not only will dietary factors contribute to phytoestrogen intake but also individual variations in metabolism contribute to their levels in the blood of organisms.

Classification of phytoestrogen

Phytoestrogens have been categorized based on their chemical structures, which resemble estrogen. Currently, four different families of phenolic compounds produced by plants are considered phytoestrogens: the isoflavonoids, stilbenes, lignans and coumestans.

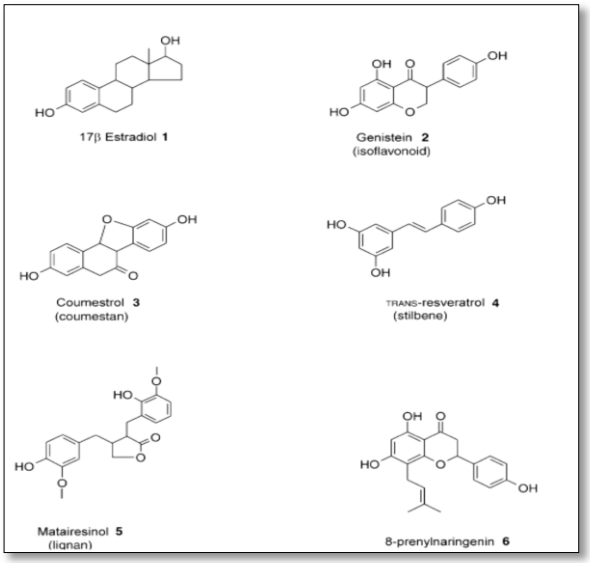
1. soflavonoids- The flavonoids are a large chemical class that are formed through the phenylpropanoid-acetate biodemial pathway via chalcone synthase and condensation reactions with malonyl CoA. The isoflavonoids are a subclass of flavonoids, where one phenolic ring has migrated from C-3 to C-2. The isoflavonoids from legumes, including genistein 2 and daidzein, are the most studied phytoestrogens. The major source of isoflavonoids in the diet is from soy-based foods. In Asia, the intake of soy can be as high as 30–50 g a day and plasma concentrations of genistein from

0.1–10 mM have been measured.[12] Soy is abundant in traditional Asian diets, resulting in isoflavone consumption as high as 50 mg/kg body weight/day[8]. In the US, consumption is estimated to be 1 - 3 mg/day for individuals.[8, 13, 14, 15] A vegetarian lifestyle or use of supplements can easily elevate phytoestrogen intake to levels at or above Asian levels.[8, 16, 17] This level of intake, even among Western Caucasians, is higher than for most synthetic endocrine disruptors, including Bisphenol A (BPA) which was recently estimate to be approximately 35 ng/kg per day.[18]

2. Stilbenes- Stilbenes, like the flavonoids, are produced through the phenylpropanoid-acetate pathway. The main dietary source of phytoestrogenic stilbenes is resveratrol 4 from red wine and peanuts. Although there are two isomers of resveratrol 4, cis and trans, only the trans form has been reported to be estrogenic.[19]

3. Lignans- The term lignan is used for a diverse class of phenylpropanoid dimers and oligomers. Secoisolariciresinol and matairesinol 5 are two lignan dimers that are notestrogenic by themselves, but are readily converted to the mammalian lignans, enterodiol and enterolactone, respectively, which are estrogenic. The conversion occurs by gut microflora and the mammalian lignans are readily absorbed. The phytolignans appear in high amounts in flaxseed, whole grain breads, vegetables, and tea. Fruits have low levels of these lignans with the exception of strawberries and cranberries.

4. Coumestans- Predominantly coumestrol 3 an 4 methoxycoumestrol show estrogenic activity The main dietary source of coumestrol, is legumes; however low levels have been reported in brussel sprouts and spinach.[20] Clover and soybean sprouts are reported to have the highest concentration, 28 and 7 mg/100 g dry wt., respectively; mature soybeans only have 0.12 mg/100 g dry wt.[20]

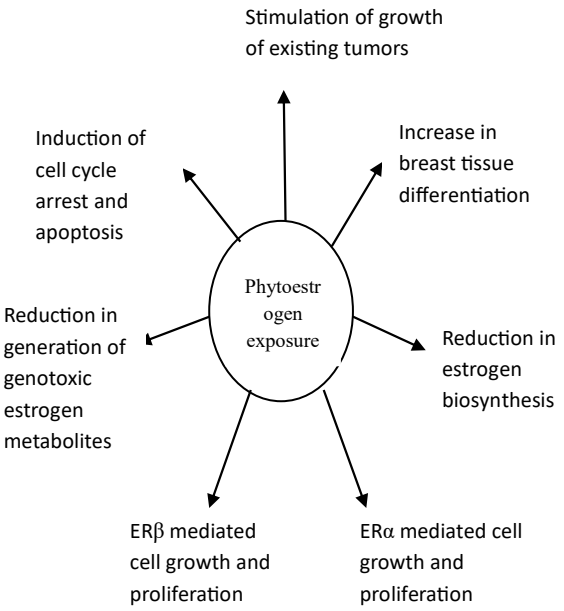


Structure of estradiol and some phytoestrogens

Mechanism of action

Phytoestrogens are able to interact with enzymes and receptors, and because of their stable structure and low molecular weight they can pass through cell membranes[21]. These interactions allow them to bind to ERs, induce specific estrogen-responsive gene products, stimulate ER-positive breast cancer cell growth, interfere with steroid hormone metabolism or action[21] and alter ER structure and affect transcription. Some genomic mechanisms of action include estrogenic and antiestrogenic effects on ERs, while other effects may not involve direct interaction with ERs.[22] Nongenomic effects that do not involve ERs include: induction of cancer cell differentiation, inhibition of tyrosine kinase and DNA

topoisomerase activities, suppression of angiogenesis and antioxidant effects of phytoestrogens. The different activities and the bioavailability of phytoestrogens vary depending on such factors as the form of administration, dosage, individual metabolism and the ingestion of other pharmacological substances. Target tissue, concentration dependency, number and type of ER, and the presence or absence of endogenous estrogens also influence the effect of phytoestrogens.[23] The relative estrogenic potencies of various phytoestogens have been quantified by studies on various in vitro models which involved receptor binding studies,[24, 25] and cell proliferation assays using oestrogen sensitive human cell lines derived from three different female oestrogen-sensitive tissues, including breast (MCF-7/BOS and T47D), endometrial (ECC-1) and ovarian (BG-1) cells.[26] The overall trend emerging from this overview is that phytoestrogens are less potent oestrogens than E_2. Furthermore, the data shown for most phytoestrogens that have been investigated indicate a higher binding preference for the ERβ than for the ERα. Only for a few like ferutinine, kievitone and for psoralidin indicate a higher binding preference for the ERα. It is also of interest to note the possible role of G protein coupled oestrogen receptors (GPERs), since many of the phytoestrogens including the soy isoflavone, genistein and possibly equol activate GPERs.[27] In cell lines of thyroid, ovarian, endometrial and breastcancers, stimulation of GPERs with oestrogens including genistein, activates a signalling pathway that promotes proliferation, although inhibition of proliferation has also been reported. In particular, genistein has been reported to stimulate growth of MCF-7 cells through a GPER-dependent mechanism .[27]



Avenues of phytoestrogen action upon exposure (Mense*et. al.*, 2008)

Effect on human health

Beneficial effects

1. Lowering of cardiovascular diseases- The similarity of phytoestrogens to oestrogens (which can decrease cholesterol levels) and the lower cardiovascular disease mortality rates in populations consuming soy suggested that phytoestrogens are protective against cardiovascular disease. Indeed, there is a considerable body of evidence to indicate that the intake of soy can have beneficial effects on low-density lipoprotein (LDL) and total cholesterol levels, but this requires that the isoflavones be consumed intact in soy protein. There is some evidence that flaxseed-fibre also has a beneficial effect on cholesterol. Several mechanisms of action reported to explain the hypocholesterolemic

1. effects of phytoestrogens include: increased bile acid secretion, which aids removal of low density lipoprotein (LDL); affected hepatic metabolism coupled with increased removal of LDL by hepatocytes; and enhanced thyroid function.[28] LDL shows increased oxidative resistance when isoflavones are incorporated into LDL cholesterol. Additional mechanisms of action have been suggested to explain the effects on plasma lipid concentrations including: action on ERs, reduction of endogenous cholesterol synthesis, and increased activity of cholesterol receptors.[23]

2. Cancer- There is a large body of epidemiological studies showing people who consume high amounts of isoflavonoids in their diets have lower rates of several cancers including breast, prostate and colon cancer. A high plasma concentration of the mammalian lignan, enterlactone, is correlated with a reduced risk of breast cancer.[29] The protective effect of phyto-estrogens on cancer may be due to their role in lowering circulating levels of unconjugated sex hormones. Estrogens mainly circulate as inactive conjugates of sex hormone binding globulin (SHBG) or albumin. Dietary supplementation with soy isoflavonoids or lignans was shown to increase the levels of SHBG.[30] More recent studies on prostate cancer and stated the findings that support the hypothesis that soy consumption prevents prostate cancer, yet more studies are needed.[1]

3. Bone density and Osteoporosis- Estrogen plays an important role in maintaining bone density by regulating the formation and resorption of bone.[31] Estradiol levels are lowered in postmenopausal women leading to loss of calcium from bone to blood. Most of the studies suggest that phytoestrogens are somewhat effective in maintaining bone mineral density (BMD) in postmenopausal women.[32]

4. Cognitive abilities- There is a decline of cognitive abilities in post menopausal women. A few studies have examined the effect of phytoestrogens on cognitive function A ten-week study demonstrated that diets high in soy increased student's long term and short-term memory. A follow-up study of the cognitive functions of postme-nopausal women taking soy isoflavonoids or a placebo showed increase in recall of pictures, sustained attention, and ability to plan tasks.[33] It has been suggested that phytoestrogens act as estrogen agonists and may increase spine density and synapse formation in the hippocampus of adults.[35] In addition, phytoestrogens may interact with the transcription of neurotrophin genes.[35]

Adverse effects

1. Disruption of ovulatory cycle- The hypothalamus and pituitary respond to estrogen by producing gonadotropins, FSH, and LH, which control ovulation. Increased estrogensignaling in these tissues would most likely result in the negative feedback action of estrogen and reduce ovulation. Estrogensignaling in the ovary is important for controlling gene expression necessary for follicle growth and the expression of FSH receptors and LH receptors that respond to gonadotropin signaling from the hypo-thalamus and pituitary. Very high levels of estrogen are produced in the ovary and it is not clear if excess estrogen would disrupt processes in this tissue. Phytoestrogens found in natural diet cause reduced fertility. The first observation made in the 1940s on ewes grazing on clover rich fields in

1. Australia suffered from high rates of infertility, spontaneous abortion, and reproductive abnormalities. The high levels of phytoestrogens in the clover contributed to those effects[35]. A second example was a study on cheetahs in a zoological population who were being fed a soybased diet and suffered from infertility. The high level of phytoestrogens in this diet was the cause and replacement with a non-soy–based diet returned their fertility.[36] These studies demonstrate that in high enough levels, phytoestrogens can override the natural cyclicity of animals, most likely due to anovulation. A symposium on soy and prevention of disease reviewed 7 studies with intakes of 32–200 mg of isoflavones.[37] The findings showed a decrease in midcycle gonadotropins, trends for increased cycle length, and lower estradiol, progesterone, and serum hormone binding globulin (SHBG) following soy intake. It was reported by the physicians at SUNY Downstate Medical Center in a 2008 clinical case report where 3 women (aged 35–56 y) were treated for abnormal uterine bleeding, endometrial pathology, and dysmenorrhea. In all 3 cases, symptoms improved after soy was withdrawn from their diet, suggesting that high intake of soy isoflavones can compromise female reproductive health.[38] A very interesting study found suppressed luteal estrogen levels following increased soy intake of 32 mg isoflavones/d for 7 months, only in women of Asian descent,[39] suggesting that ethnicity should stand out as a factor regarding the potential human health effects of soy isoflavones.

2. Behaviour- Phyto-600-fed males performed significantly better than Phyto-free-fed females; and surprisingly, males displayed an opposite pattern (i.e., males switched to the Phyto-free diet significantly outperformed males fed the Phyto-600 diet lifelong.[40] The findings described above suggest that dietary isoflavones may sex-reverse the typical sexually dimorphic expression of visual spatial memory performance. Specifically, tasks requiring reference memory appear to be affected more than working memory, possibly due to the abundance of ER-beta in the frontal cortical area, where approximately 50-fold higher concentrations of isoflavones have been observed in Phyto-600 versus Phyto-free-fed animals.[40,41] Other behaviors may be affected as well including social, aggressive, and anxiety-related behaviors. Increased aggression and circulating testosterone levels have been reported in male Syrian hamsters maintained for 5 weeks on the soy-rich Purina 5001 diet compared to control animals fed a soy-free diet.42 Research has revealed that isoflavone intake can suppress female sex behavior in rats. Administration of a commercially prepared phytoestrogen supplement to adult female rats, at a dose that results in serum levels between those seen in Western and Asian (human) adults, attenuated lordosis to the same degree as the SERM tamoxifen.[43,44]

3. Cancer- Phytoestrogens at low dose have been reported to stimulate breast cancer as they are considered as endocrine disruptors. The effect of phytoestrogens depends on age at exposure and hormonal environment. There have been reports of genistein increasing tumour cross sectional area, elevated tumour multiplicity, elevated percentage of proliferative cells in tumours and increased the weight of estrogen dependent mammary tumours.[45,46] US National Toxicology Program conducted a study in non-ovariectomized animals administered genistein at doses ranging

0.3–44 mg/kg b.wt daily (NTP, 2008). This study provided some evidence of carcinogenic activity of genistein in female rats based on an increased incidence of mammary gland adenoma or adenocarcinoma.

Discussion

The great diversity of phytoestrogens makes it difficult to make general conclusions about their health effects, since different members of this class may have different activities, pharmacokinetic properties and metabolic fate. Clinical trials that study phytoestrogens are often performed with a variety of botanical formulations and thus cannot be compared directly. Non-estrogenic compounds present in phytoestrogen- rich plant sources used in clinical research may interact with phytoestrogens and either potentiate or interfere with their activity and bioavailability. In addition, some phytoestrogens may act as estrogen agonists or antagonists depending on their structure and concentration. Much research is required to clearly define the pharmacological effect of dietary phytoestrogens. Thus, some dietary phytoestrogens may decrease menopausal symptoms without increasing the risk of breast cancer. This is just one of many opportunities that exist in the study of the endocrine effects of food components and in defining their pharmacological use. Adverse as well as beneficial effects of phytoe-strogens have been reported in case of breast cancer. They do not act by a single mechanism to achieve their effects. It still remains a riddle whether phytoestrogens are beneficial or harmful. Some factors such as age, health status, presence or absence of specific gut microflora are worth mentioning for the population under study.

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Chapter 13

Release of Urushiol from Mango Peel Due to Infestation by Fruit Borer Autocharis albizonalis Leads to Contact Dermatitis

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Release of Urushiol from Mango peel due to Infestation by Fruit Borer*Autoch arisalbizonalis* leads to Contact Dermatitis

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Abstract

Urushiol is an organic oily compound found in the plant of family Anacardiaceae such as mango (*Mangifera indica*). It causes an allergic skin rash in human, known as dermatitis. Mango skin, bark and leaves contain these watery yellow allergic substance. Urushiol oil secretions are a unique adaptation used for plant defense. Usually in a particular mango growing area, three or four major pests occur. Amongst them mango fruit borers (*Autocharis albizonalis*) are of prime importance along with mango hopper. The larva of borer is known as red banded caterpillar which bores into the young fruit and eats fleshes and seeds. The first instar larva of borer damages the young fruit as a pin hole injury on the skin above the distal part of the fruit. Due to infestation a necrotic zone develops around the point of entry hole. At this time some liquid is released from the skin which contains urushiol.The survey on the infestation of fruit borers were conducted in different mango growing districts of West Bengal, namely Malda, Murshidabad, Nadia and North 24 parganas. The survey period was fixed depending upon the local farmer's report in the month of March- May from 2015 to 2016. Detail observations on seasonal incidence of fruit borers were determined regularly and several meteorological parameters like temperature, relative humidity and rainfall were also recorded. From the data it is revealed that the degree of infestation is positively correlated with the temperature and humidity of that area. It is found that the occurrences of fruit borer are much higher in recent times with average increase in temperature. Therefore more fruits are affected and secrete urushiol which may leads to allergic reaction when it comes into contact with the skin of human.

Keywords: Urushiol, Fruit borer, Dermatitis, mango, Pest, Necrotic zone

Introduction

Mango is the most pre eminent among the subtropical fruits of India in view of the acreage, production and admiration among the people. Mango is the national fruit of India and known as the 'King of fruits'. It is one of the oldest cultivated fruit crops, having been grown in India for at least 4000 years. It is now widely spread all through the tropics and subtropics. Mango has some useful nutrients, vitamins, fibres and other essential elements which are inevitable for body growth. The peel of mangoes is responsible for the allergy in most occasions. The urushiol chemical is responsible for triggering the allergic reaction. Urushiol is an organic oily compound found in the plant of family Anacardiaceae such as mango (Mangifera indica).[1] It causes an

allergic skin rash in human, known as dermatitis or mango itch. When people get urushiol on their skin, it causes an allergic contact dermatitis. This is a T cell-mediated immune response, also called delayed hypersensitivity, in which the body's immune system recognizes as foreign, and attacks, the complex of urushiol-derivatives with skin proteins.[2] Mango skin, bark and leaves contain these watery yellow allergic substance. Urushiol oil secretions are a unique adaptation used for plant defense. Mango is attacked by more than 400 pests in the world.[3] Of these, about two dozens of insect pests severely injure different parts of mango tree.[4] Usually in a particular mango growing area, three or four major pests occur. Amongst them mango midges and fruit borers (*Autocharis albizonalis*) are of prime importance along with mango hoppers. Mango fruit borer, *Deanolis* (=*Noorda*) *albizonalis*(Hampson), though marked as minor pest but is assumed as serious pest in Orissa Andhr Pradesh and Karnataka.[5] reported that the fruit borer, *Autocharis albizonalis* recently appeared in serious proportion in the major mango growing areas of the West Bengal, particularly in the districts of Malda, Murshidabad, Nadia & Hooghly causing 10-52% damage of fruits from pin head stage to full maturity.[6] They observed that the insect passed through five larval instars in 11-13 days & the incubation, pre-pupal and pupal period lasted for 2-3 days, 5-6 days & 9-11 days respectively. The adult moths have wings of a shining bluish fawn colour with a well marked darker border and a narrow, dark streak across the end of the forewing cell.[7] The larva is quite distinctive; its body is covered with alternating red and white bands with a black collar on the first segment. The larva of borer is known as red banded caterpillar (RBMC) which bores into the young fruit and eats fleshes and seeds. RBMC larvae feed on mango fruit in all stages of development. First and second instar caterpillars feed just beneath the skin surface and tunnelling towards the seed. The first sign of infestation is the presence of a sap stain containing urushiol running from the entry hole and collecting on the drip point at the fruit apex. In the present survey, a comparative investigation based on sample fruits collected from various growers of principle districts of Malda, Mushidabad, Nadia and Midnapur. The occurrence of the fruit borer in the grown up fruits was observed. The infestation and the degree of damage by the borer were recorded along with the different abiotic factors of that area.

Methodology

The field survey was conducted during fruiting period of mango cv Himsagar from March to May, 2015 and 2016 at selected orchards in the districts of Malda, Mushidabad, Nadia and Midnapur in West Bengal. The survey period was fixed depending upon the local farmer's report in the month of March- May from 2015 to 2016. To study the biology and damage pattern of fruit borer, an orchard at Gayeshpur, Nadia, West Bengal was selected which located at 23^0 N latitude and 89^0 e longitude at an elevation of 9.75 metres from mean sea level. The soil of the experimental field was typically Gangetic alluvial having clay loam texture, neutral in reaction and moderate in fertility. Slit and clay fractions together exceeded 60% of mineral particles of the soil, signifying its good water holding capacity. Here the number of replication was three and the number of random samples taken per replication was two hundred fruits. Numbers of fruits infested by fruit borer from each replication were counted by visualizing the symptom of

infestation. The observation was taken at an interval of ten days. During this observation several meteorological parameters like average temperature, average relative humidity and rainfall were recorded. The correlation coefficient was calculated between the data of average percentage of infestation with average temperature, relative humidity and rainfall respectively.

Result and Discussion

Table 1: Location wise average infestation (means of three replications) by fruit borer during 2015-16

Month and Year	Malda	Murshidabad	Midnapur	Nadia
March,2015	36.32	33.19	36.33	32.0
April,2015	40.08	35.71	35.0	28.99
May,2015	44.77	38.83	41.89	35.15
June,2015	34.51	31.59	32.34	28.74
Total	155.68	139.32	145.56	124.88
Location wise avg infestation	38.92	34.83	36.39	31.22
Annual avg infestation	**35.34**			
March,2016	36.11	33.70	34.78	27.21
April,2016	35.36	35.06	35.37	31.0
May,2016	41.20	37.33	38.0	33.46
June,2016	33.01	30.87	30.17	26.89
Total	145.68	136.96	138.32	118.56
Location wise avg infestation	36.42	34.24	34.58	29.64
Annual avg infestation	**33.72**			

The primary phase of infestation appeared when the fruits are young and marble size. It was found that due to small size of the fruit the larvae migrate to other fruits in the tree. During the survey period, it was observed that infestation started on the first week of March and peak in the month of May. From table 1.it is observed that annual average infestations of all surveyed districts are 35.34% in 2015 and 33.72% in 2016. It is recorded that the Annual infestation is highest in case of Malda districts during both season. In the month of May the infestation were 44.77%, 38.83%, 41.89%, 35.15% in Malda, Murshidabad, Midnapur and Nadia respectively during 2015 and 41.20%, 37.33%, 38% and 33.46% during 2016.The lowest infestation were recorded in Nadia districts at an average of 31.22% and 29.64% in 2015 and 2016 respectively.

Table 2. Incidence of fruit borer in relation to major abiotic factors of environment during 2015-16

Season	Avg infestation (%)	Avg temp (^{O}C)	Avg RH (%)	Avg rainfall (mm)
2015	35.34	33.35	76.4	16.23
2016	33.72	33.09	75.34	3.43
r		+1	+1.2	+ 0.9

It is observed from table 2 that the average percent infestation of fruit borer are positively correlated with temperature (r = +1), humidity (+ 12) and average rainfall (+0.9) during the period of investigation Regarding the effect of abiotic factors, it is evident that the activity of the pest species are positively correlated with temperature, humidity and rainfall, having profound influence of humidity (r =1.2).

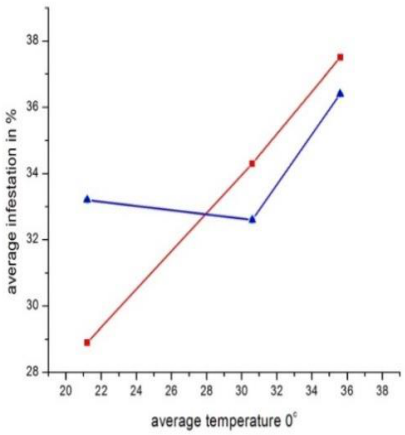

Fig 1. Average infestation of fruit borer in relation to temperature during 2015-16

From fig 1 it is found that the infestation of fruit borer is gradually increased with the rise of temperature. It is evident that the peak infestation is found when the temperature is at the highest level. It is already stated that the first instar larva bores into the young fruits in the month of

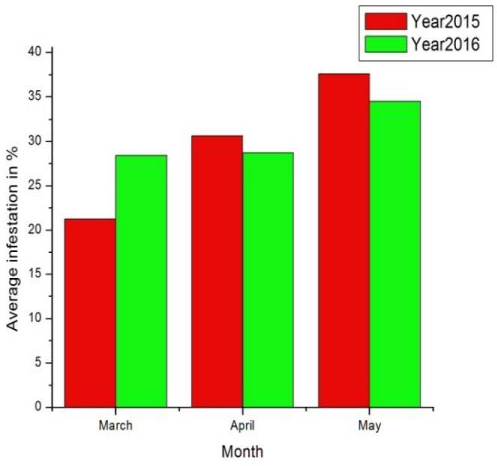

Fig 2. Month wise infestation by fruit borer during 2015-2016

March when the temperature is low compare to April and May. In the month of May borer population is rises and they infested more fruits. The highest degree of infestation is found in the month of May during the surveyed period.

Conclusion

It is observed that the insect passing through five distinct larval instars and the first instar larvae bore into the fruit flesh at the apex. The first two instars feed on the mango flesh, with later instars feeding on the seed. If competition occurs between larvae in the same fruit, some individuals may leave that fruit to search the more food. It is reported that this is mainly due to the fact that marble sized fruit provide insufficient food for development therefore larvae need to move through several small fruit to get the same amount of food as a large fruit.[8] From the data it is revealed that the degree of infestation is positively correlated with the temperature, humidity and rainfall of that area. The average highest infestation was found in the month of May during 2015-16 in West Bengal. It is reported that the occurrences of fruit borer are much higher in last ten years in our country. It is found that the abundance of RBMC is correlated with the changing

of climatic conditions of that area. It is concluded that if favourable climatic condition arises, the insect population rises and affected several mango fruits in that particular area. Therefore more fruits are affected and secrete urushiol which may leads to allergic reaction when it comes into contact with the skin of human.

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