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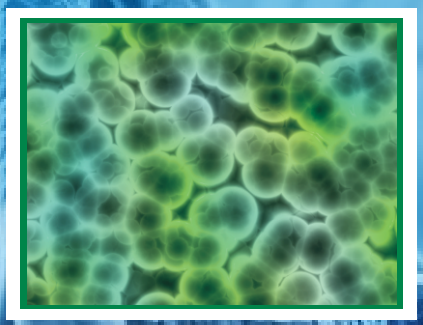

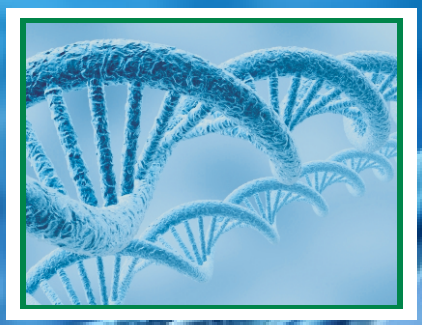

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National Environmental Science Academy
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Incharge Publication
Gian C. Kashyap
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Incarge- Accounts
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A STUDY OF FISH DISTRIBUTION IN BALAPUR POND OF PRAYAGRAJ (U.P.)

Ashok Kumar Verma

Department of Zoology
Government Post Graduate College, Saidabad Prayagraj-221508 (U.P.)

Research Paper

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ABSTRACT

The present survey is conducted to study the distribution of fishes naturally occurring in Balapur pond of Prayagraj. The survey was conducted during a period of one year from Jan 2018 to Dec 2018 and focussed mainly on distribution and diversity of fishes. A total of 12 species of fishes belonging to 11 genera, 7 families and 4 orders were identified as result of this survey. This was the first ever systematic survey on the fish diversity of this pond. Siluriformes were found most dominant order represented by 5 genera followed by Cypriniformes with 4 genera.

Keywords: Fish distribution, Systematic survey, Fish fauna, Conservation, Balapur pond.

INTRODUCTION

The term 'pond' refers to a relatively shallow body of water usually smaller than a lake, contained in an earthen basin retaining sewage or organic wastes. Hydrobiology is the study of life in water while limnology is the study of the physical, chemical, geological and biological aspects of all naturally occurring fresh water. Freshwater habitats such as lakes, ponds, dams, reservoirs are known as lentic (still) while running water such as rivers, mountain streams are known as lotic (flowing).

A large number of ponds and lakes are naturally occurring all over the globe. In India, a number of ponds, lakes and reservoirs are naturally found but they are not being utilized properly due to lack of insufficient study of their hydrobiology. One of the most important features of ponds is the presence of standing water, which naturally provides habitat for wetland biota including both plants as well as animals. A large number of micro-organisms and invertebrates feed on the decaying plants occur naturally.

Ichthyology is the study of fishes. These are cold-blooded, gill-bearing aquatic craniate vertebrates that include both the bony and the cartilaginous fishes but sometimes jawless fishes too. They belong to phylum: Chordata, subphylum: Vertebrata and super class: Pisces. The fishes are not only used as good source of food for mankind, having economic importance from medicinal point of view but also play a crucial role in the second tropic level of an aquatic ecosystem.

Prakash *et al* (2015a) performed the limnological Studies of Alwara Lake of Kaushambi (U.P.). Singh *et al* (2016) studied the hydrobiological conditions of Ganga River. Prakash *et al* (2015a and 2015b) and Verma (2016a) conducted the hydrobiological studies of Muntjibpur pond of Allahabad. Verma (2016b, 2016c, 2016d, 2017a, 2017b,

*Corresponding author: *akv.gdcz@gmail.com*

2018, 2019a and 2019b) and Verma et al (2016a and 2016b) conducted the study of fish biodiversity and physico-chemical factors in a fresh water body. The Balapur pond studied has rich biodiversity.

The present survey is undertaken as first systematic study to find out the distribution and diversity of fishes naturally occurring in Balapur pond of Prayagraj. This survey was conducted during a period of one year from Jan 2018 to Dec 2018.

STUDY AREA

Balapur pond is a natural pond, located on south side of the village. It is located in Koraon block and tahsil of Prayagraj district of Uttar Pradesh (image 1). The pond studied is approximately 62 KM away from District head quarter Prayagraj and 272 KM from State capital Lucknow. This village is surrounded by Janakpur in east, Paitiha in south west, Banshipur in north east and Murlipur in south. The month of March marks the beginning of summer and it lasts till June. Monsoon generally starts in the month of July and lasts till September. December to February is the winter season in and around this village. This pond (photo 1) is extended in more than two hectares and is surrounded by agricultural fields from three sides.

Image 1: Location of study area in Prayagraj.

MATERIALS AND METHODS

The pond was surveyed for fishes once in a month for the period of one year from January 2018 to December 2018. The fishes were caught and collected for present survey from Balapur pond by hand-nets, gill nets, cast nets, hooks, drag nets with the help of local people and fisherman.

Fishes were identified using the standard keys of Mishra (1959), Day (1989), Jhingran (1991), Jayaram (1999) and Srivastava (1998).

Photo 1: A view of Balapur pond in Prayagraj.

RESULTS AND DISCUSSION

All the three seasons namely summer, monsoon and winter show different seasonal fluctuation in various hydrobiological parameters in this pond studied. The water present in the said pond is useful for irrigation as well as fish culture. The water of this pond is although having some pollutants but is suitable for agricultural purposes also, as it is rich in organic humus, planktons and nutrients.

During the study period, a total of 12 species of fishes belonging to 4 orders, 7 families and 11 genera were recorded from the Balapur pond. The collected fish species including their zoological names, family and order are shown in the table given.

Fish fauna of the pond studied belong to 4 orders namely Siluriformes, Cypriniformes, Ophiocephaliformes and Clupeiformes. In present investigation Cyprinidae family was the most dominant group representing 5 species followed by Bagaridae family representing 2 species. The families Siluridae, Clariidae, Clupeidae, Saccobranchidae and Ophiocephalidae were represented by one species each. In this way, authors recorded 12 different species of fishes.

Table : Showing fishes reported from Balapur pond in the year 2018.

S.No.	Zoological name	Family	Order
1.	*Catla catla*	Cyprinidae	Cypriniformes
2.	*Labeo rohita*	Cyprinidae	Cypriniformes
3.	*Labeo calbasu*	Cyprinidae	Cypriniformes
4.	*Cyprinus carpio*	Cyprinidae	Cypriniformes
5.	*Cirrhinus mrigala*	Cyprinidae	Cypriniformes
6.	*Mystus seenghala*	Bagridae	Siluriformes
7.	*Rita rita*	Bagridae	Siluriformes
8.	*Wallago attu*	Siluridae	Siluriformes
9.	*Clarias batrachus*	Clariidae	Siluriformes
10.	*Heteropneustes fossilis*	Saccobranchidae	Siluriformes
11.	*Channa punctatus*	Ophiocephalidae	Ophiocephaliformes
12.	*Gudusia chapra*	Clupeidae	Clupeiformes

CONCLUSION

A total of 12 species of fishes belonging to 4 orders, 7 families and 11 genera were recorded from the Balapur pond during its first systematic survey conducted by author. Author strongly recommends a detailed study of this pond to understand its biodiversity and conservation status.

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Author is highly grateful to the Prof. Ashish Joshi, Principal, Government P.G. College, Saidabad-Prayagraj for providing necessary laboratory facilities. Author is also obliged to my senior colleague Dr Shri Prakash and my youngster Mr Prabhakar Singh, local people and Gram Pradhan for their co-operation during entire survey programme.

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THERAPEUTIC APPLICATIONS OF AMAZING SNAKE FRUIT (SALAK)

Upma Singh[1], Bina Rani[2], Ravi Sharma[3], Lakha Ram[4], Anju Sharma[5], Sukhraj Punar[5], and Raaz K Maheshwari[5]

[1]Department of Applied Chemistry, School of Vocational Studies & Applied Sciences, Greater Noida, Gautam Buddha University, Gautam Buddha Nagar, UP
[2]Department of Applied Sciences & Humanities, IIMT College of Engineering, Greater Noida, Gautam Buddha University, Gautam Buddha Nagar, UP
[3]Department of Chemistry, Jai NarainVyas University, Jodhpur, Rajasthan
[4]Department of Chemistry, JNMP Govt PG College, Phalodi, Jodhpur, Rajasthan
[5]Department of Chemistry, SBRM Govt PG College, Nagaur, Rajasthan

Review Article

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ABSTRACT

It turns out that there are so many fruits with good advantages to human health. And one among them pops out, is the snake fruit. Salak is not only a great fruit to include in your food for its flavor, but also for its nutrient content, which includes high levels of dietary fiber, protein, sugars, potassium, iron, calcium, phosphorous, vitamin C, and vitamin A, as well as various antioxidants and active ingredients. Salak's vitamins and minerals help our body to increase its self-sustainability. The Snake Fruit's antioxidants helps our body to recover from any possible damage and rejuvenate the broken cells. The quantitative analysis of several phenolic acid and flavonoids had shown that SPE (Snake fruit Peel Extract) contained chlorogenic acid. Chlorogenic acid is an ester of quinic acid and caffeic acid. Chlorogenic acid is an important biologically active dietary polyphenol and major compound found in coffee (Coffeaarabica).Consuming the Snake fruit regularly will prevent us from getting hemorrhoids. Despite its utilization, the phytochemical compound available in snake fruit, especially its peel have not been well documented. Present study envisioned to elucidate the phytochemical constituent of snake fruit peel and its anti-aging potency.

Keywords: Minerals, Antioxidants, Ageing, Vitamin A & C,Chlorogenic acid, SPE.

INTRODUCTION

Salak (*Salaccazalacca*) is a species of palm tree (family *Arecaceae*) native to Java and Sumatra in Indonesia. It is a very short-stemmed palm, with leaves up to 6 metres (20 ft) long. The fruits grow in clusters at the base of the palm, and are also known as snake fruit (Fig. 1) due to the reddish-brown scaly skin. They are about the size and shape of a ripe fig, with a distinct tip. For many people, eating something called snake fruit may not sound appealing, but salak is a very popular fruit in some parts of the world. With its growing popularity and availability around the world, it is important to understand the nutrient composition of salad, as well as some of its potential health benefits and proper ways to eat this unique fruit[(1)].

Salak is actually the name of a type of palm tree that is native to areas of Indonesia and parts of the South Pacific. These trees are cultivated for their fruits, which are found clustered near the base of the tree, and have a unique red,

*Corresponding author: *rkmgreenchem.jaipur@gmail.com*

scaly exterior, which earns it the nickname snake fruit[2]. The outside of the fruit is scaly like a snake and prickly like a cactus, but the inside is sweeter than honey, sour like a pineapple and incredibly juicy. Its flesh is slightly acidic, giving your tongue a citrus like tingle. The complex flavor wrapped into a lethal grenade looking package has a spectacular flavor.

Fig. 1,2,3: Raw & Peeled Snake Fruit.

Nutritional Value & Health Benifits of Snake Fruit

These fruits are only about the size of figs and may resemble a large garlic clove when peeled. Each fruit contains an inedible seed, but the flesh surrounding it is consumed for its sweet and often astringent flavor. This unusual flavor makes salak a popular ingredient in many different types of cultural dishes in the South Pacific and neighboring areas. There are more than 30 cultivars of this fruit, but most have a similar nutritional profile, albeit a slightly different taste and level of astringency[3]. Salak is not only a great fruit to include in your food for its flavor, but also for its nutrient content, which includes high levels of dietary fiber, protein, sugars, potassium, iron, calcium, phosphorous, vitamin C, and vitamin A, as well as various antioxidants and active ingredients . There are only 82 calories in a 100-gram serving of salak, which means that it has a low impact on your over all caloric intake. The nutrition content that snake fruit provides is the presence of a good amount of vitamin C. Consumption of 100 grams of snake fruit, provide 14% of daily value vitamin C needed. Snake fruit contains a good amount of iron contained. In another hand, if you got a lack of iron consumption, it will lead you to have chronic fatigue and even an anemia[4].

Hence, get yourself to eat this tasty fruit to prevent certain disease as well.From now on, say no to the presence of cholesterol. Since snake fruit has been containing no cholesterol inside, it is good for those who wants to make a preventing from getting certain diseases such as heart attack[5]. Surprisingly, snake fruit is having a good source of calcium which is like milk having a role in promoting bones and in strengthening the bones density as well[4]. The most important health benefits of salak include its ability to boost vision health, strengthen cognition, optimize digestion, increase energy, aid weight loss efforts and regulate blood sugar levels for diabetic patients. Thanks to the minerals and active ingredients in this fruit, such as potassium, beta-carotene, and pectin, this fruit is able to increase blood flow to the brain, which can boost cognition and improve your memory power, while also eliminating oxidative stress and lowering your risk of neurodegenerative diseases. Studies have found that salak is able to increase stamina and stimulate the metabolism, which can provide a solid boost to your energy levels[6]. The carbohydrates found in this fruit are also good for maintaining energy levels throughout the day. There is a high level ofdietary fiber in salak, which is ideal for increasing feelings of fullness and preventing unnecessary snacking and overeating. This also improves digestive efficiency and eliminates symptoms of constipation, bloating and cramping[5].

The potassium found in salak help it lower your blood pressure, as potassium is a vasodilator. This means that it can reduce the tension in blood vessels and arteries, which lowers overall strain on the cardiovascular system. Beta-carotene is one of the antioxidants found in salak, which has a direct link to vision health[7-10]. Having enough beta-carotene in your diet can lower your risk of macular degeneration and slow the progression of cataracts as you age. The high-fiber nature of this fruit makes it excellent for regulating blood sugar levels in the body, which is important for

diabetic patients. There is a decent amount of carbohydrates (simple sugars) in this fruit, but the fiber counteracts those effects and prevents the spike in blood sugar, easing the strain on the pancreas.

It takes an important role in improving our body health. As a matter of fact, it promotes the protein formation, hormonal balance, and the cellular repairing process.it is known that snake fruit is also helping you to get the good amount of protein contained. With the great consumption of protein foods, they will help you to build muscle mass, supporting the neurological function, and also renewing the body cells[11].The pulp is edible. The fruit can be peeled by pinching the tip, which should cause the skin to slough off so it can be pulled away. The taste is usually sweet and acidic. Apart from their sweet and slightly acidic taste, Due to high fiber and antioxidant content, Salak is a sought-after diet for weight management diets. Since Salak consists of calcium and carbohydrates it provides necessary energy and stamina to the body while on diet. Its tea is a wonderful astringent that is beneficial in reducing weight[12].As mentioned before Salak is a nutrient dense fruit that helps in improving overall health of the body. Its rich nutrient profile consisting of vital vitamins and minerals that help to maintain proper body functions. Antioxidants help in fighting damage to the cells and tissues and prevent the body from harmful carcinogenic damage[13-16].

Chemical Constituents

Salak is a good source of nutrients, vitamins and minerals. Salak fruit consists of protein, beta-carotene, vitamin C, dietary fiber, iron, calcium, phosphorus and carbohydrates which are great for overall health. The beta-carotene in Salak is a powerful antioxidant and works well to prevent cardiovascular disease, strokes and even cancer[17-19]. The qualitative phytochemical screening was showed the presence of several important phytochemical in SPE, especially phenolic acid and polyphenolic compound. Both were commonly found normal human diet and ingested in large quantity compared with other phytochemical compound. Previous study showed similar result with current finding, where snakefruit peel was contained phenolic acid, flavonoid, and tanin[16]. Phenolic acid and flavonoid are considered as an important antioxidant compound. Flavonoids compounds are characterized by the presence of one or more phenol groups in their structure. This structure enabled phenolic acid and flavonoids to donate its hydrogen atoms, reducing the radical onto its neutral form. Flavonoid has huge impact in human health. *In vitro* studies showed flavonoid have wide range of biological activity antioxidant, antiinflammatory anti-microbial, antifungal, antiviral, and anticancer[20-28].

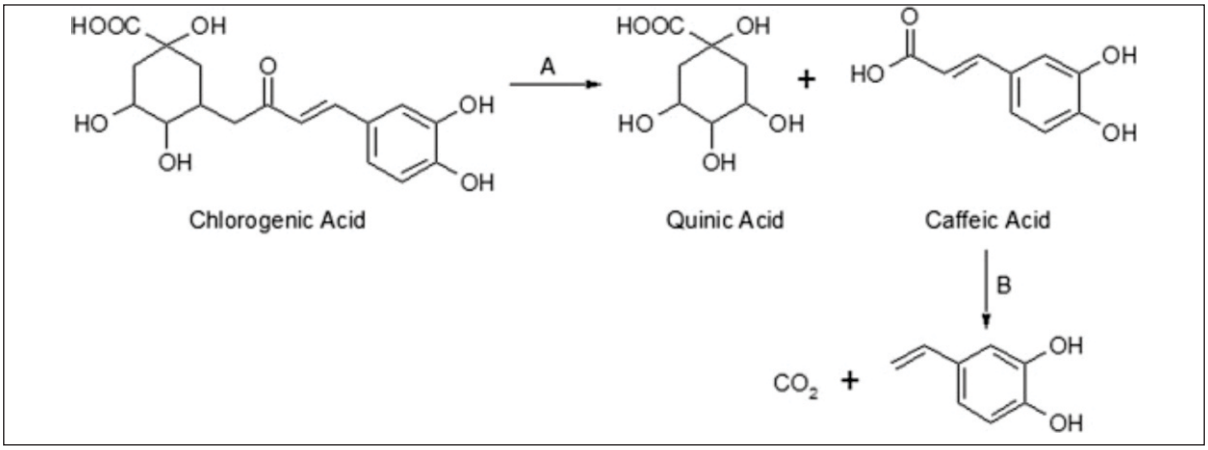

Fig. 2: Chlorogenic acid is an ester of quinic acid and caffeic acid.

Apparently, consumption of flavonoids was found to be related to the slowing down of aging process. This was based on the ability of antioxidants to trigger cell proliferation playing a close role in regenerating damaged tissue. Another flavonoid found in large amounts in tea, epicathecin, was able to protect fibroblast cells from ROS and subsequently apoptosis. The administration of plant extracts was also known to be able to strongly inhibit the degradation activity of elastase and also maintained the skin elastase. This also

supports the potential of flavonoid to play a role in maintaining skin elasticity due to its antioxidant activity. The quantitative analysis of several phenolic acid and flavonoids had shown that SPE (Snake fruit Peel Extract) contained chlorogenic acid. Chlorogenic acid (Fig. 2) is an ester of quinic acid and caffeic acid. Chlorogenic acid is an important biologically active dietary polyphenol and major compound found in coffee (Coffeaarabica).29-34

Numerous evidence has demonstrated that chlorogenic acid is known for many biological activities: including anti-carcinogenic, antiinflammatory, and antioxidant properties.Previous study showed the consumption of food rich in chlorogenic acid was correlated with low facial hyperpigmentation in Japanese middle-aged females.35-39The study speculated that polyphenols, including chlorogenic acids, may help protect human skin from photoaging and contribute to the decreased hyper-pigmentation of pigmented spots.Regular consumption of a portion of Salak in your diet helps to reduce the risk of hemorrhoid formation.40-45

CONCLUSION

Has anyone heard that Salak is also known as memory fruit. It has certain reasons, since Salaccazalacca contains lots of potassium and pectin. Hence, another health benefits of Salak is to increase the ability of our brain to save memory/remember. Not to mention, it is also increases the cognitive capability as well as improve the brain's memory capacity. Salak contains of vitamin A as well as beta-carotene. To keep the health of eye, consuming snake fruit regularly is another option other than carrot or tomato juice. Simply blend the flesh of Salak fruit and make it into juice. It has the same benefits with carrots and tomatoes juice. Hence, consuming Salaccazalacca regularly is very good for our eyes health.

Other good substances available inside the Salak are tannin, saponin, and flavonoid. Those are good substances for our digestive system. The health benefits of Salaccazalacca for our stomach is to avoid us form getting digestive disorder. However, the best way to eat Salak fruit is eat the fruit along with its soft whitish skin or epidermis, which is very helpful to prevent constipation. Salaccazalacca fruit consists lots of antioxidant, fiber, calcium, and carbohydrate. Hence, Salak fruit is very highly suggested in dietary program. Its calcium and carbohydrate provide us with enough energy while we're still on our diet.We can achieve the benefits of Salak fruit for diet program by take its fiber and antioxidant which are very good in rejuvenating damaged cells and postponing early aging process. The quantitative analysis of several phenolic acid and flavonoids had shown that SPE (Snake fruit Peel Extract) contained chlorogenic acid. Chlorogenic acid is an ester of quinic acid and caffeic acid. Chlorogenic acid is an important biologically active dietary polyphenol and major compound found in coffee (Coffeaarabica).Numerous evidence has demonstrated that chlorogenic acid is known for many biological activities: including anti-carcinogenic, antiinflammatory, and antioxidant properties.

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IMPACT OF ARSENIC ON CARBOHYDRATE METABOLISM OF A FRESH WATER CAT FISH, *MYSTUS VITTATUS*

Ashok Kumar Verma and Sadguru Prakash*

Department of Zoology Govt. PG College Saidabad, Prayagraj(U.P.)
*Department of Zoology, M.L.K. (P.G.) College, Balrampur (U.P.)

Research Paper

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ABSTRACT

The present investigation has been designed to study the effect of sublethal concentrations (10% and 30%) of heavy metal, arsenic on the total carbohydrate and glycogen content of liver and muscles of *Mystus vittatus* after exposure to 30 days. A significant reduction in total carbohydrate and glycogen content of liver and muscles in arsenic exposed fish, *Mystus vittatus* suggests that the tissue carbohydrate and glycogen might have undergone glucogenolysis, during the stressful situation in the intoxicated fishes. Thus the present study concludes that the carbohydrate metabolism of fish *Mystus vittatus* affected during arsenic exposure and reduces the nutritive value of fish.

Keywords: Arsenic, Carbohydrate, Sublethal, Balrampur, *Mystus vittatus*.

INTRODUCTION

Arsenic is an ubiquitous element, released into the aquatic environment from both natural and anthropogenic activities. It is considered to be a environmental pollutant and ecological dangers can arise if large amounts of arsenic are released into the environment at the time of metal smelting, chemical manufacturing, and agricultural activities. The drinking water containing more than 10 µg/L of arsenic is harmful to the body and chronic exposure to arsenic-contaminated water and food causes cancer. In the environment, arsenic is present in different forms (arsenite or arsenate form which are inter-converted through redox and methylation reactions) and their toxicity depends upon chemical form and oxidation states (Ananth *et al*, 2014). Arsenic exposure in the aquatic environment causes bioaccumulation in aquatic organisms and can lead to physiological and biochemical disorders (Han *et al*., 2019).

The environmental toxicant can induce physiological and biochemical changes in fish that lead to growth inhibition (Beyers *et al*. 1999). In aquatic environment, fish are usually regarded as organisms of choice for assessing the effects of environmental pollution on aquatic ecosystem, so the present study was undertaken to investigate the alterations in the levels of blood glucose level and glycogen content in tissues of *Mystus vittatus*, a fresh water cat fish exposed to sublethal concentrations of arsenic, a trace element widely detected in the aquatic environment due to natural effects and anthropogenic activities.

MATERIALS AND METHODS

The healthy *Mystus vittatus* ranging from 7.0-8.0 cm in length and weighting 8.0-9.0 gm were collected from ponds in and around Balrampur and washed with 1% solution of $KMnO_4$ for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Fish were acclimated to laboratory

*Corresponding author: *sadguruprakash@gmail.com*

conditions for 15 days at room temperature. The LC_{50} for arsenic trioxide for 96 hours was calculated using probit method (Finney, 1971) was 3.20 ppm. The LC_{50} values of arsenic for 24, 48, 72 and 96 hours were 4.71, 4.16, 3.68 and 3.25 ppm, respectively. Based on 96 LC_{50}, fishes were exposed to sublethal concentrations (10% and 30%) for treated and control period of 10, 20 and 30 days. A control group was maintained in an identical environment. The fishes were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. The fishes were sacrificed from both experimental and control groups on 10th, 20th and 30th days of exposure periods and subjected to analysis for biochemical changes. Total carbohydrate content and glycogen were estimated by the method of Roe (1961) and Carroll *et al*., (1956).

RESULTS AND DISCUSSION

The total carbohydrate and glycogen content in the different tissues of control and arsenic treated fish, *Mystus vittatus* , are presented in Table 1and 2.

Even though protein is an important source of energy in fish but stress conditions causes rapid depletion of stored carbohydrate, primarily liver and muscle glycogen. Clarke (1975) had also noticed that carbohydrate is considered to be the first among the organic nutrients degraded in response to stress conditions imposed on animals. Total carbohydrate and glycogen of tested tissues of *Mystus vittatus* showed that they decreased with increasing concentration of arsenic and exposure duration.

Total carbohydrate and glycogen in *Mystus vittatus* was decreased with increasing concentration of arsenic and exposure. The results showed hyperglycemia and a reduction in glycogen content of tissues of arsenic exposed fishes. The present finding also supported by other scientists who had noticed that glycogen content of muscle and liver tissues was declined with increasing concentration and duration of industrial effluent exposure (Shaff, 1981; Oikari and Nakari, 1985). Marked glycogenolysis found in the present study, resulting from chronic exposure to arsenic may be due to a stress-induced increase in circulating catecholamines (McLeay and Brown, 1975; Prakash and Verma, 2018).

Total carbohydrate and glycogen content in liver and muscles tissue of arsenic exposed *Mystus vittatus* were decreased significantly which facing the utilization of excess energy needed to cope with stress under arsenic exposure. Anoxia or hypoxia increases carbohydrate consumption and thereby induces a sort of respiratory stress on organisms even at a sublethal level resulting in additional expenditure of energy. The increased glycogenolysis indicated a general disturbance in carbohydrate metabolism, which might have an adverse effect on the life of exposed animals (Dhavale *et al*., 1988).Thus, the nutritive value of the fish could be altered during the arsenic exposure.

Table 1: Effects of sublethal concentrations of arsenic on total carbohydrate level (mg/g) in liver and muscles of Mystus vittatus at different period of exposure (N=6).

Tissues	Group	Exposure periods in days			F. value
		10	20	30	
Liver	Control	21.56±0.19	22.05±0.18	22.21±0.20	0.039NS
	10%	15.43±0.21	12.32±0.12	10.09±0.31	37.58*
	30%	13.48±o.24	10.13±0.24	7.14±0.28	42.38*
Muscles	Control	7.23±0.26	7.28±0.21	7.32±0.31	0.031NS
	10%	5.86±0.21	4.98±0.31	4.05±0.12	29.17*
	30%	4.54±0.14	3.59±0.25	3.02±0.31	33.16*

NS= Non Significant; *=Significant at 5% level of F test ($p<0.05$)

Table 2: Effects of sublethal concentrations of arsenic on glycogen (mg/g) content in liver and muscles of *Mystus vittatus* at different period of exposure (N=6).

Tissues	Group	Exposure periods in days			F. value
		10	20	30	
Liver	Control	32.43±0.21	32.76±0.24	32.12±0.31	0.051^{NS}
	10%	28.21±0.22	25.04±0.31	22.12±0.23	29.52*
	30%	24.31±0.24	21.85±0.35	19.71±0.24	35.22*
Muscles	Control	11.25±0.22	11.53±0.21	11.42±0.19	0.031^{NS}
	10%	9.89±0.11	8.32±0.21	7.16±0.14	25.12*
	30%	8.12±0.23	6.71±0.22	5.29±0.25	43.36*

NS= Non Significant; *=Significant at 5% level of F test ($p<0.05$)

CONCLUSION

Glycogen is immediate source of energy which gets converted into glucose by glycogenolysis to overcome the stress by pollutants. The present study obviously indicates that low concentration of arsenic is toxic to fishes and decreases the glycogen of different tissues to cope up with the stress.

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MOST ABUNDANT SPECIES OF GRASSHOPPER i.e. *ACRIDA EXALTATA* (ORTHOPTERA: ACRIDIDAE) ON GREEN ROOF OF INDIA

Mohd. Kamil Usmani, Md. Humayoon Akhtar and Mohd Ali

Section of Entomology, Department of Zoology
Aligarh Muslim University Aligarh 202002, Uttar Pradesh, India.

Review Article

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ABSTRACT

Acrida is genus of grasshopper in the family Acrididae. The genus contains around 40 species, which are found in Africa, Europe, Asia, North America, Hawaii, and Australia. Insects of this genus are omnivorous, and are a well-known pest of many agriculture crops. Grasshoppers are brown to greyish-green jumping insects that are moderately long and have prominent heads and large compound eyes. The front pair of wings are narrow, leathery, and thickened. The hind pair are thin, broadly triangular, transparent, or sometimes brightly coloured. A pair of hard, horny, heavy, large, with jagged inner edges and dark coloured triangular structures found one either side. The two mandibles move in horizontal motion and crush food between them.

Keywords: Grasshopper,Acrididae, Pest, India.

INTRODUCTION

The grasshopper is a flying animal belonging to order Orthoptera and Class Insecta. About 11,000 species exist. They are herbivorous and commonly seen in autumn; a few appear in summer and spring. During mating the male grasshopper deposits sperms into the female's vagina, which finds its way to the eggs through canals known as micropyles. An adult grasshopper goes through the stages egg, nymph and adult, and has a lifespan of approximately one year.

This is the initial stage of a grasshopper's life cycle. The mother grasshopper lays fertilised eggs in midsummer, and they remain 1 or 2 inches under the sand or in leaf litter. She sprinkles them with a sticky semisolid substance that sets to form an egg pod. Each egg pod contains 50 to 150 eggs, depending on the species. Normally female grasshopper can lay up to 25 pods. The eggs remain underneath for about 10 months in autumn and winter before hatching into nymphs during springs or in the initial days of summer. This is the second stage of the grasshopper's life and the initial stage during which a young grasshopper sees the outside world. Nymphs look like adult grasshopper, called molts, apart from the fact that they are wingless and lack reproductive organs. They undergo five sub stages known as instar before fully developing into adult grasshoppers; each instar is characterised by shedding of the cuticle skin and gradual growth of wings. In order to survive, nymphs start to feed on succulent and soft plant foliage barely one day after hatching from the egg. This stage lasts for about five to six weeks before the young nymphs mature to adult grasshoppers.

Molting takes place during the nymph stage. The locust sheds its exoskeleton before maturing into an adult. While the exoskeleton covers the nymph's body, providing it with protection against external injuries, it inhibits its growth

*Corresponding author: *usmanikamil94@gmail.com, alimalla76@gmail.com*

because of its rigidity and inability to give room for expansion. The nymph has to shed it in order to achieve growth. It undergoes five to six molts in which it changes its structure and form before reaching adulthood. This is a fully grown grasshopper. It takes about one month before the wings are fully developed. The mature grasshopper is more mobile than the nymph, a characteristic that helps them to hunt and flee from predators. The reproductive organs are fully grown, so the female can lay eggs until they are 1 or 2 weeks old, to allow them to gain enough weight before they start laying eggs. Once she starts laying eggs, the female continues to lay eggs at interval of three to four days until she dies. Adult grasshoppers live for about two months, depending on the weather.

The climate of India comprises a wide range of weather conditions across a vast geographic scale and varied topography, making generalisation difficult. Based on the Koppen system, India hosts six major climatic subtypes, ranging from arid desert in the west, alpine tundra and glaciers in the north, and humid tropical regions supporting rainforests in the southwest and the island territories. Many regions have starkly different microclimates. India is home to an extraordinary variety of climatic regions, ranging from tropical in the south to temperate and alpine in the Himalayan north, where elevated regions receive sustained winter snowfall. The nation has four seasons: winter (January and February), summer (March to May), a monsoon (rainy) season (June – September), and post- monsoon period (October- December).

In most of India summer is very hot. It begins in April and continues till the beginning of October, when the monsoon rains start to fall. The heat peaks in June with temperatures in the northern plains and the west reach 45° C and more. The monsoons hit the country during this period too, beginning 1st of June when they are supposed to find the Kerala coast, moving further inland from day to day. Moisture laden trade winds sweep the country bringing heavy rains and thunderstorms; sometimes these monsoon rains can be very heavy, causing flooding and damage, especially along the big Rivers of India, Brahmaputra and Ganges. The plains in the north and even the barren countryside of Rajasthan have a cold wave every year in December- January. Minimum temperatures could dip below 5°C but maximum temperatures usually do not fall lower than 12°C. In the Northern high altitude areas of the northern mountains it snows through the winter and even summer months are only mildly warm.

MATERIALS AND METHODS

Grasshoppers were collected by the ordinary aerial insect net and through hand picking as well. It is preserved by killing, pinning, and drying. Grasshopper can be killed by freezing or with chemicals. The easiest and safe technique is to place insects into a freezer for several hours. They can also be killed by exposing them to a small amount of toxic fumigant such as ethyl acetate. Toxicants are usually used in conjunction with a specially prepared killing jar. The killing jar has a layer of plaster of paris poured in the bottom of the jar. Once ethyl acetate is poured onto dried plaster of Paris, the chemical is absorbed by the plaster, and the jar will produce toxic fumes for several days.

Usually it is not desirable to kill nymph because they lack characters needed for identification. Also, due to their soft body they do not preserve well in a dry state. They are best placed in alcohol to prevent excessive distortion. To mount adult grasshoppers on a pin, insert a pin into the dorsal surface, with the point protruding from the ventral surface. The preferred location for pinning is usually the posterior area of prothorax, and to the right of the midline. The grasshopper is pushed up on the pin so that not only the end, but a small amount of the shaft is protruding. This gives ample room to pick up the dead grasshopper without touching the insect body. Below the grasshopper body, collection data are provided via a label. This is accomplished by writing or printing data on stiff paper, and cutting the label to a small rectangle. A pinning block often is used to align the insect body and label(s) to standard heights. Data that should be included on the label include the date of collection, place of collection, and collector's name. Ecology data such as habitat or host plant may also be included. Pins vary in size and quality. It is highly desirable to use rustproof insect pins. Insect pins are longer and sharper than standard pins, allowing attachment of labels and easy mounting. To fully appreciate the beauty and to assist in identification, spread at least one forewing and hind wing. The usual procedure is to spread the left forewing perpendicular to the grasshopper body. Similarly, the leading edge of the hind wing is spread perpendicular; this results in full extension of the remainder of the hind wing, though if it is done the spread wing may aid identification.

To properly spread the grasshopper wings, some support is needed to keep the wings elevated and flat. A spreading board is usually used to provide wing support. A spreading board consisting of Styrofoam or another suitable pinning surface should have a strip of similar material glued on part of the board, so that one surface is higher than the other. Thus, the lower pinning board is used to support the grasshopper body on its pin, and the elevated portion is used to support the wing. Strips of the paper and pins are used to hold the wing in place. Whether or not the grasshopper wings are spread, grasshoppers must be dried to aid preservation. Drying can be accomplished by placing the pinned insect, often with its wings spread, in an oven at low temperature until the subject is dry and stiff. Once dried, the wings, antennae, and legs cannot be moved without breaking, so it is important to get the body parts aligned before drying.

It requires nothing more than a tight box with pinning material in the bottom. However, it is imperative that the box be tight, or carpet beetles and cockroaches will gain access and devour the pinned insects. To help prevent damage to specimens, place the moth balls or moth crystals in the box with the specimens. This will kill any insects that gain access, particularly ants, cockroaches, and book lice.

RESULTS

Acrida exaltata are general feeders on grasses and weeds and often move to cultivated crops. Their populations vary from year to year. Crops damage is likely to be greatest in years when dry weather accompanies high populations. Drought conditions reduce natural vegetation, forcing grasshoppers to move to cultivated crops. The two mandibles move in horizontal motion and crush food between them.

Family Acrididae shows maximum diversity, comprising 8,000 species, of these 136 species and 28 genera are endemic (Chandra and Gupta, 2013). Members of the family Acrididae cause considerable damage to agricultural crops, pastures and forests and are well reputed for their destructiveness all over the world (Joshi *et al*., 1999). The primary diet for grasshoppers are grasses and forbs (Behmer&Joern, 1994). It is primarily graminivorous, feeding on several common grasses and sedges (Mulkern, 1967).

Acrida exaltata were recorded from Kashmir throughout the extreme winter in the month of December, January and February (Azim and reshi, 2008) while reported throughout the year except in December (Khan and Aziz, 1973). Population of the species start declining fron July onwards and resurgence occurred in March-April in West Bengal (Shushanta and Halder, 1998). Akhtar *et al*., (2012) recorded the species from rice ecosystem of uttar Pradesh; Chitra, *et al*., (2000) from rice field of Coimbatore, Usmani *et al*., (2012) from pulses and paddy fields in Bihar and Jharkhand, Kandibane *et al*., (2004) described from irrigated fields of rice ecosystem of Tamil Nadu. Kumar and Usmani (2014) also reported the species from the desert of Rajasthan.

TAXONOMIC ACCOUNT

Acrida Exaltata (Walker, 1859)

Truxalis exaltata Walker, 1859. *Ann. Nat. Hist.* (3): 222

Tryxalis brevicolis bolivar, 1893. FeuilleJeunes nat. 23: 162. Syn.By Drish and Uvarov, 1953. *Tijdschr. v. Entomologie*. 96: 232.

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Acrida lugubrisastigmata Prasad, 1956. Proc. Nation. Acad. Sci. India. B-26 (1): 22.Syn.By drish, 1961. Eos.37: 398.

Acrida Exaltata (Walker); Nayeem and Usmani, 2012. *Munis Entomology & Zoology*.71: 404.

Diagnostic characters: head conically ascending, fastigium of vertec wide, protruding, considerably concave with parabolic apex and apparent carinula of vertex; pronotum with lateral carinae prominent, not lined internally with black, prozona shorter than metazona; pronotum and head of about equal length, transverse sulcus of pronotum placed near middle of disc; tegmina fully developed with pointed apex, a little produced beyond the hind knees; wings yellowish, hyaline, slightly shorter than tegmina; hind tibiae straight with two rows of spines.

Distribution India: Andaman & Nicobar Island, Andhra Pradesh, Assam, Bihar, Chhattisgarh, Delhi Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir. Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Orissa, Punjab. Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, and West Bengal. **Elsewhere:** Afghanistan, Bangladesh, Iran, Pakistan, Saudi Arabia, South Arabia, South East Tibet, Sri Lanka, Yemen and West Aden.

DISCUSSION

Population of the grasshoppers are greatly influenced by the climate and availability of the host plant and damage caused is directly proportional to their population. Pest status of *Acrida exaltata* has been reviewed in detail by numerous workers from different state of the nation whereas its population has been recorded from each and every state of the India. Damage to cereal crop is generally concentrated near field margin and is caused when hatching grasshoppers move out of egg beds into filed edges. Damage to cereal includes leaf notching and stripping but is most costly when stems are severed just below the head of maturing or mature crops.

Emphasizing the overriding priority of maintaining high economic growth rates and biodiversity assessment, investigation of species is of prime importance to raise living atandards and sustainable development.

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BIOADSORBENTS MEDIATED DEFLUORIDATION OF GROUNDWATER IN NAGAUR CITY OF RAJASTHAN

Harsukh Chharang[1], Anju Sharma[1], Ravi Sharma[2], Lakha Ram[3], Raaz K Maheshwari[*1]

[1]Department of Chemistry, SBRM Govt PG College, Nagaur, Rajasthan
[2]Department of Chemistry, Jai Narain Vyas University, Jodhpur, Rajasthan
[3]Department of Chemistry, JNMP Govt PG College, Phalodi, Jodhpur, Rajasthan

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ABSTRACT

Water samples of 12 locations of Nagaur city of Rajasthan, were chemically analyzed for determining fluoride ion concentrations. High fluoride containing localities were identified on the basis of fluoride levels of water samples and also on prevalence rate of dental, skeletal and non-skeletal fluorosis of the study area. As per the desirable and MPL (Maximum Permissible Limit) for fluoride in drinking water, determined by WHO, the groundwater of studied sites is unfit for drinking purposes. Due to the higher fluoride level in drinking water, several cases of dental and skeletal fluorosis have appeared at alarming rate in this region. There is an instant need to take ameliorative steps in this region to prevent the population from fluorosis. The evaluation of various defluoridation methods on the basis of social and economical structure of India reveals that the clay pot chip, activated alumina adsorption, and Nalgonda techniques are the most promising. Water samples containing high fluoride levels were defluoridated with economically cheaper materials prepared from plant by products. These materials were found successful in decreasing fluoride ion concentration to a permissible limit (0.5 to 1.5ppm) without disturbing potable water quality standards.

Keywords: Defluoridation, SpectrophotometerAC, PJC, CLC, GAC.

INTRODUCTION

With rising population and dwindling groundwater resources, the groundwater quality of Rajasthan is deteriorating day by day. As per WHO report, 20 per cent of the fluoride-affected villages in the whole world are in India. Out of 33,211 fluoride-affected villages in the country. Rajasthan has 16,560 villages, which is more than 51 per cent1. From these figures we can draw an inference that nearly 10% of fluoride-affected habitation in the world is in the Rajasthan alone. The high concentration of fluoride in drinking water leads to destruction of enamel of teeth and causes a number of conditions referred to collectively as fluorosis. This disease is slow and progressively crippling malady. At low concentration (<1.0 ppm), fluoride prevents tooth decay, but it has been medically proved that, high fluoride intake by individuals from water, food, air and medicines results in fluorosis. Fluorosis not only affects older persons, but there are ample evidences that even newborn baby and children of younger age have also been its victims. It not only affects the body of a person but also renders them socially and culturally crippled. There is a need to develop a well thought out strategy to attack this problem, which requires an urgent attention from both medical as well as of social workers. Considerable work has been done all over the world on treatment of fluorosis. Unfortunately the results indicated that the effects of fluorosis are irreversible in children.

Numerous people have conducted surveys on the problem

*Corresponding author: **gcacs60@gmail.com; rkmgreenchem.jaipur@gmail.com*

of fluoridation and treatment option available for defluoridation processes, but, however, a safe efficient and cost effective defluoridation technique/process needs to be developed in order to prevent the occurrence of fluorosis. Nagaur district of Rajasthan has been known for excessive concentration of F (Fluoride) in groundwater. Due to the lack of surface water resources and semi-arid climatic conditions, increase in groundwater fed irrigated agriculture and erection of a number of groundwater abstraction structure for public water supply, the measure groundwater resources of the district are in the heavy stress. In the villages of Nagaur district, the effects of high F-concentration are severe. Presence of excess F in groundwater has drawn attention of the society due to its pathophysiological impact on human body. F-content in al the villages of Nagaur district has been found in the range of 1-62ppm. Over exploitation of water resources in the Didwana block has resulted in the depletion of groundwater table, salination of aquifers and deterioration in chemical quality of groundwater at an alarming rate. Therefore, study area is recommended to adopt adequate measures for conservation and judicious management of groundwater resources.

Drinking water with excessive concentration of fluoride causes fluorosis which progresses gradually and becomes a crippling malady in the long run. It affects young, old, poor, rich, rural, and urban population2. It has attained a very alarming dimension. There being no perennial surface source for drinking water, the state is dependent chiefly on groundwater and its level is deeper year-by-year due to over exploitation. As the water table is receding more and more water sources are becoming prone to higher fluoride concentration. The pattern and prevalence of fluorosis in human population are determined by a number of epidemiological factors like water chemistry, demographic and nutritional profile of the community and high mean annual temperature of the area. The high concentration of fluoride in drinking water leads to destruction of enamel of teeth and causes a number of conditions referred to collectively as fluorosis3. At low concentration (<1.0 ppm), fluoride prevents tooth decay, but it has been medically proved that, high fluoride intake by individuals from water, food, air and medicines results in fluorosis. Nagaur district of Rajasthan has been known for excessive concentration of F in groundwater. Due to the lack of surface water resources and semi-arid climatic conditions, increase in groundwater fed irrigated agriculture and construction of a number of groundwater abstraction structure for public water supply, the meagre groundwater resources of the district are in the heavy stress. In the villages of Nagaur district, the effects of high F-concentration are severe. Presence of excess F in groundwater has drawn attention of the society due to its pathophysiological impact on human body4.

Fluoride could be found in a number of minerals, of which fluorspar, cryolite, topaz and fluorapatite are the most common5. Many epidemiological studies of possible adverse effects of the long-term ingestion of fluoride via drinking water have clearly indicated that fluoride primarily produces effects on skeletal tissues (teeth and bones)6. Skeletal fluorosis observed when drinking water contains 3-6mg/L, crippling skeletal fluorosis develops where drinking water contains over 10 mg/L. In India totally 19 states have been reported as fluoride prone areas but severe problem occurred in the states of AP7, TN8, Rajasthan9 and MP10. Fluorosis is an irreversible disease and there is no cure

The presence of fluoride in groundwater is human made. Over exploitation of groundwater in the last 20 years, is the chief reason for the spread of fluorosis in Rajasthan. With the coming of diesel pump sets things have changed. Farmers have started to dig deeper into the earth's crust and are literally extracting poison. Rajasthan is a classic case of falling water tables and increasing incidence of fluoride in water. Thus it appears that the situation vis-a-vis fluoride is on the rise despite substantial efforts by the Government and NGO's action plans in the field. The situation is grim and warrants a holistic approach to ameliorate the situation and a concerted action accordingly. It is, in fact, one of the most bones seeking acute toxin of notable chemical qualities and physiological properties as well. The main occurrence of fluorine in rocks is in the form of fluoride bearing minerals. The arid climate with high evaporations and insignificant natural recharge might have accelerated the strengthening of fluoride concentration in the groundwater of this area. In the vast geographical expanse and varied geographical set-up in Rajasthan the cause of fluoridation of groundwater are many. Some natural, some human-made Over exploitation of water resources in the Nagaur has resulted in the

depletion of groundwater table, salination of aquifers and deterioration in chemical quality of groundwater at an alarming rate. Therefore, study area is recommended to adopt adequate measures for conservation and judicious management of groundwater resources.

Geography of Nagaur District

Nagaur District is district of the state of Rajasthan in western India. Nagaur city is the district headquarters. Nagaur district is bounded by Bikaner District to the northwest side, Churu District to the north side, Jaipur District to the east side, Ajmer District to the southeast side, Sikar District to the northeast side, Pali District to the south side, and Jodhpur District to the southwest and west side.

Latitude of Nagaur city: 26°25' & 27°40' **North**
Longitude of Nagaur city : 73°.10' & 75°.15' East
Nagaur is situated amidst seven districts namely Sikar, Jaipur, Ajmer, Bikaner, Churu, Pali, Jodhpur. Nagaur is the fifth largest district in Rajasthan with a vast terrain spreading over 17,718 sqkm. Its geographical spread is a good combine of plain, hills, sand mounds & as such it is a part of the great Indian Thar Desert.

Experimental Details

Several methods are in vogue to mitigate fluoride from drinking water11. These includes Nalgonda technique, activated charcoal technique, bone char technique, capacitative deionization technique, CEDI technique, reverse osmosis technique and ion exchange technique9, but the literature survey clearly indicates the material used in the present studies not reported any where. Aforementioned techniques for defluoridation of drinking water are costly and non-applicable on mass scale. The present manuscript present the use and applicability of adsorbent carbon materials prepared from dry fruits of various plant materials and the obtained results were compared with commercially available granulated activated Carbon (GAC) purchased from Ranbaxy Laboratories Lit, India.

Area wise survey was conducted on the residents of the Nagaur district along with the registered medical practitioner to standardize the readings. The Jackson index of dental fluorosis was employed. In this survey, people are broadly divided into three categories depending on the age groups between 5 to 15, 15 to 25 and above 25 years of age. In each group total 10 persons were examined and prepared statistical report. After conformation of fluorosis presence totally 20 sample were analyzed for fluoride ion concentration by SPAND'S method12.

Sample Collection

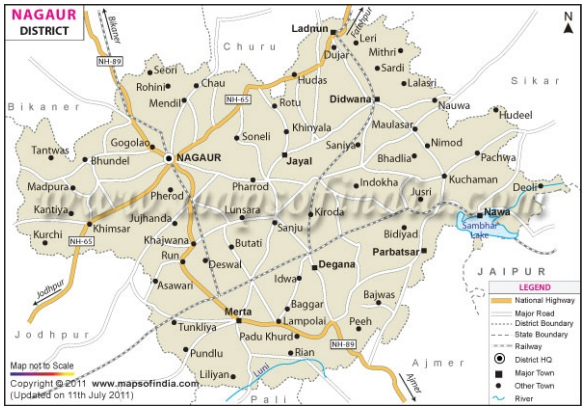
Water samples were collected from all the existing 16 sources of drinking water in the study area for investigation and chemical examination. For the present investigation, separate samples are collected for chemical and biological analysis from the source. The bottles for sample collection have been thoroughly cleaned by rinsing with 8N HNO3 (nitric acid) solution, followed by repeated washing with double distilled water. They are further rinsed with sample water before collection. Physicochemical analysis was done using standard procedure12.

Material Preparation

Defluoridating materials were prepared from dry fruits, collected from plants Enterolobium saman (ESC), Acasia Arabica (AAC), Prosopis juliflora (PLC) belonging to family Mimosideae and Citrus lemon (CLC) belonging to family Rutaceae. These materials are available as agricultural wastes and carbonized at 400 to 500°C in muffle furnace. The prepared carbons were chemically treated with 0.5M HNO3 solution and then washed with distilled water and finally sieved in to 75µm particle size.

Defluoridation Method

0.5g of adsorbent was mixed with 100ml of each water sample and stirred at 120 rounds/minute speed on Remi shaker for 30 minutes. Solution was filtered through Whatman no. 42 filter paper and the filtrate was examined for further fluoride ion concentration on UV visible spectrometer (model no. Elico UV-2600). Experimental conditions were obtained with the above prepared carbon adsorbents in batch mode study as 45



minutes agitation time, 4g/L adsorbent concentration; optimum pH is 7-8. The same conditions were applied in defluoridation of drinking water samples in batch mode study.

RESULTS AND DISCUSSION

Groundwater is the only source of potable water for majority of people in the study area. However, the inhabitants here are averse to drink bore well water or water from public water system. They say that water drawn from deep depths is not tasty, hence their preference to open well water or hand pump water. A survey of residents of the selected localities in the study area on the impact of water used for drinking on health of users revealed that, most of residents suffer from dental discolouration, early tooth decay and bone deformations. The practicing physicians of the study area also confirmed our observations. Symptoms of dental fluorosis in female population were more common than the male population. However, this anomaly was observed to be more prevalent between 5 and 15 years of age group, which decreases with increase in age. The concentration of fluoride in all samples of study area has varied from 1.4 to 4.5mh/L, 1.57 to 4.21mg/L and 1.06 to 3.5mg/L in hand pumps, bore well and open well water samples respectively. As per chemical analysis the study area was broadly classified into five categories depending upon the concentration of fluoride ion. 8 water samples from bore well, 7 samples from hand pump, 5 samples from open well water was fallen within the range of 1.5 to 4.5mg/L concentration of fluoride ion, but only 1 from bore well, 2 from open well water samples having less than 1.5mg/L concentration.

Defluoridation Studies of Potable water Samples

Water samples collected from various locations indicate that samples 1 to 5 of hand pump water, samples 6 to 12 of bore well and samples 13 to 15 of open well water contain excess of fluoride beyond permissible World Health Organization limit 1.5mh/L6. Hence the defluoridation studies were carried out on these particular samples employing prepared bio adorbents from ESC, AAC, PLC and CLC. The results were compared with those of GAC. In order to reduce the fluoride content below the permissible limit, optimum condition reported in previous have been adopted. For water samples, which contain fluoride range between 3.0 and 4.0mg/L, the dose of adsorbent is 4.5g/L and for those water samples, which contain fluoride, ranging from 1.5 to 3.0mgL, the dose adsorbent is 4.0 gL. The optimum contact time is 45 minutes with constant stirring at 200 rpm speed. The concentrations of fluoride ion in these samples after defluoridation have also been reported. A comparative study of the results of some physicochemical analysis of water from bore well, hand pump and open well before defluoridation and after defluoridation, indicate that water quality parameters like pH, EC, TDS, PO_4^{3}-, SO_4^{2}-, Cl-, K+, etc. values were increased slightly but negligible in many water samples when the adsorbents, ESC, AAC, PJC, CLC and GAC are used for the defluoridation process. Among the adsorbents, ESC, AAC and PJC decrease the fluoride content in potable water samples to a considerable extent without affecting the permissible limits of other water quality parameters. The order if adsorption capacity in the mitigation of these adsorbents is ESC>AAC>PJC>CLC>GAC.

Aquifers Help Pump Out Fluorosis: An Innovative Study

Tribal children with stained teeth, some with skeletal deformities, walked past a yellow-painted hand-pump. They filled water in plastic bottles from a white water-tank on a sunny morning while going to their school in Bahadra (district Dhar; MP) village, they have been doing this since a long time and a s a result, the residents of this village have been suffering from fluorosis for the last two decades. But now, thanks to an amazing innovation. Children are now aware that drinking water from a yellow hand-pump is not safe.

It's natural innovation of using shallow aquifers, with locally sustainable technology, community participation and successful convergence of efforts of independent organisations and state public health engineering department (PHED). This has created awareness and transformed the lives of tribal inhabitants of Bahadra and a number of other villages of Dhar district where fluorosis has already taken considerable section of rural population under its grip. Like in Bahadra, which has six hand-pumps, the problem of fluorosis emerged in the villages of Dhar when the hand-pump technique was introduced as a solution to the scarcity in the drought prone district. Each of the 1,452 villages have five to six hand-pumps and studies have pointed out that water in 61 per cent of the total hand-pumps in the district are highly contaminated with fluoride content above permissible (safe) limit of 1.5ppm (Parts Per Million or grams per

litre). Complete dependency on hand-pump for fetching has led to emergence of fluorosis and signs of fluorosis to varying degrees are visible in many villages in the district.

Bahadra village is now a silver lining as about 67 tribal families here now has access to safe drinking water – thanks to the community's efforts for innovation that has addressed major issues of fluorosis. “Now, we know about fluorosis. We don't drink water from hand-pumps which are painted yellow,” stated a villager, who was used to fetch water from the village hand-pumps for many years, without knowing that she was bringing fluorosis to her family. “Now, she has a water tap connection and get safe water.” She said.

The turnaround came with the tireless effort of Vasudha Vikas Sansthan (VVS), an NGO, which launched a three year programme for creating newer sources of potable water for the village Bahadra with the help of Water Aid, an independent organisation which enables the world's poorest people to get access to safe drinking water. State PHED and the administration too extended their help while the village panchayat and community joined in the efforts. Subsequently, a major awareness campaign was launched at the village level during which the villagers were told about the harmful effects of using fluoride contaminated water, citing increased incidents of dental fluorosis in children and some instances of skeletal fluorosis that caused numerous deformities. “Creating awareness itself was a major task as people were used to fetching drinking water from the hand-pumps and there was no other source of safe drinking water. Finally, an old well abandoned by the Bahadra villagers came to their rescue, ” stated director of VVS. Subsequently, this well was cleaned up and a tank was established for to use it for drinking water purposes as fluoride content in water is adulterated only when it's drawn from deeper sub-surface sources. The well has been equipped with such structures through which piped water is distributed to different areas of the village.

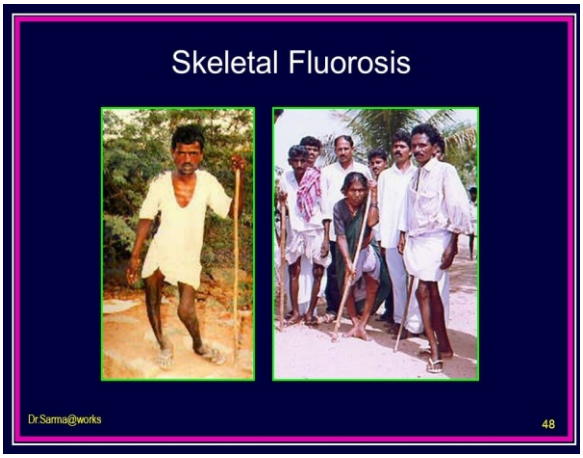
War Against Fluorosis

- MP - PHE (Public Health Engineering) has given colour code to all hand pumps, with fluoride contaminated as red, blue for safe, and red as non-functional. Safe drinking water sources have been painted as white and blue ij entire district.
- Fluoride contamination problem persists in all the three blocks of Dhar district. Fluoride contamination has been reported from nearly 30 out of 50 districts in MP.
- MP government, Water Aid and ‘Vasudha’ have signed a MoU (Memorandum of Understanding) and also entered into an agreement of fluoride mitigation through capacity building in the villages.
- At few villages, the gram panchayat itself has taken up the responsibility of operation and maintenance of newly created safe drinking water sources while at most of the places local communities have come together to make the facility sustainable.

The local tribal community too played their part by forming a water service committee from among them for the maintenance and operation, realising that sustaining the availability of safe drinking water was the key to combat fluorosis and better health. Now, all the households contribute Rs 20 each every month to meet the expenses towards cost of pump operator's remuneration and electricity charges. “It's a unique approach of using shallow aquifers with sanitary protection to ensure availability of safe water in areas with high fluoride contamination in groundwater,” stated programme officer of Water Aid, which provided technical assistance, training and other assistance to 'VVS' and local communities to develop and run their own safe drinking water projects.

Bahadra’s success motivated people in other villages to think on the same lines. As of now, 12 villages around Bahadra have developed safe drinking water sources using the same model wherein the local tribal community is taking care of operation and

maintenance while 'VVS' is now engaged in a similar capacity. The state government has identified 197 high-fluoride affected villages for implementing a river-based scheme bit it could take a couple of years for the project to start functioning. The PHED has already constructed tanks in many villages under the river-based water supply plan and painted them blue.



THREE APPROACHES VIZ. HEALTH EDUCATION, TREATMENT OF THE DISEASE, PREVENTIVE MEASURES ARE SUGGESTED

(I) HEALTH EDUCATION:

Creating awareness about the fluoride and fluorosis: the main area of interest will be

(a) Creating disease awareness: Creating awareness about the disease should be in the form of graphic presentation of the final consequences of the disease to the extent possible.

If required, live presentations of the patients who are suffering from the severe form of the disease in areas where the gravity of the problem has not reached to that extent. It may be of use to demonstrate the most severe extent of the disease and to motivate them to use the preventive or therapeutic measures.

(b) Creating awareness about the sources of the fluoride: The creation of awareness will help in implementing the need based preventive measures in the affected community.

(ii) Treatment Of The Disease:

Vitamin C and D, salts of Ca, Mg or Al were prescribed in an attempt to reverse these effects. Published results were, however, inconclusive and largely negative. Recent studies conducted in Rajasthan under Raj DST sponsored studies indicated that fluorosis could be reversed, at least in children by a therapeutic regimen (Nutritional prophylaxis), which is cheap and easily available. The choice of the reported therapy was logical. The presence of calcium in gut directly affects the absorption of fluoride ions and also improve serum levels as observed by Teotia et al (1995). Vitamin D3 in low doses enhances Ca absorption and retention without causing hypercalcemia and thus directly affects the absorption of fluoride ions. It also inhibits the excessive release of parathyroid hormone thereby preventing excessive activation of osteoblasts thus preventing hyperosteoidosis and osteopenia. Ascorbic acid controls collagen formation,maintains the teeth structure and bone formation. The structures are adversely affected by higher fluoride intake.

(iii) Preventive Measures

(a) Providing Defluoridaled Water for Drinking Purpose:

- Methods of defluoridation recommended so far are aimed at bringing the fluoride levels to the WHO standards.
- Desirable characteristics of defluoridation process, cost effectiveness, easy to handle, (operation by rural population) - the major sufferer - independent of input fluoride

concentration alkanity, pH, temperature, no effect on taste of water, no addition of other undesirable substances (e.g. Al salts) to treat water, all these parameters must be considered for adopting any of the defluoridation process/technique.

(b) Changing the Dietary Habits:

Defluoridation of drinking water alone shall not bring the fluoride level to safe limit. It would be necessary to overcome the toxic effects of the remaining fluoride ingested through other sources. This can be done by effecting minor changes in the diet and dietary habits of the population compatible with their social system and available resources. The main aim must be to

- Restrict use of fluoride rich food
- Avoiding use of fluoride rich cosmetics

Use of food rich in Calcium, Vitamin C and antioxidants

In people with exposure and those with clinical and sub clinical symptoms, the only available measure as of today is eliminating the intake of fluorides. No chemical till date is capable of extracting fluoride absorbed in the body. In patients with disease symptoms, the following interventions should be practiced:

(a) Reduce as much as possible the fluoride through water and food.

(b) Practice consumption of diet rich in calcium, vitamin C (ascorbic acid), vitamin E and anti-oxidants.

A properly designed nutritional regimen can beneficially interfere with the toxic effects of fluoride. Vitamin C, vitamin E and anti-oxidants, which are beneficial and are not very expensive, can be produced in rural areas without much investment.

- **Calcium:** Milk, Curd, Yoghurt, Green leafy vegetables, Jaggery, Drumstick, Sesameseeds
- **Vitamin C:** Aanola, Lemon, Orange, Tomato, Sprouted cereals/pulses and Dhania leaves
- **Vitamin E:** Vegetable oil, Nuts, White grain cereals, Green vegetables and Dried beans
- **Anti-oxidants:** Garlic, Ginger, Carrot, White onion, Papaya, Pumpkin and Green leafy vegetables.

All the above items have antagonistic effect; thereby play the prophylactic role in preventing fluorosis.

(c) **Rain Water Harvesting:** (Alternative water source)

Fluoride affects the people and the animals as well. Therefore it is desirable that the animals should also be provided with fluoride free water for maintaining their longevity. Defluoridation of drinking water for animals will be too costly and not feasible and therefore the only solution of this problem is water harvesting. The water harvesting technology should be aimed not only to provide fluoride free water to human beings but also to animals. Rainwater storage can be a major source of fluoride free drinking water for the animals.

These three-pronged attacks can prove to be a blessing for the population especially for the younger generation living in fluoride rich areas having no choice except to drink the water contaminated with fluoride and suffer the inevitable consequences including permanent deformities.

Commonly Used Domestic Defluoridation Processes

Advantages and disadvantages of various commonly used processes available for defluoridation:-

(1) Nalgonda Process:

It looks a cumbersome technique not suitable for use by less educated population - the section that needs it the most. The process can be used only for water having a fluoride content of <10ppm & turbidity <1500ppm. There is a high residual Al content in output water. It is reported that the residual Al ranges from 2.01 ppm to 6.80 ppm. It is relevant to note that Al is a neurotoxin and concentration as low as 0.80 ppm of Al in drinking water is reported to have caused Alzheimer's disease. The ISO 10500 for drinking water sets an absolute max limit of 0.2 ppm for Al, which is well below the minimum reported in the output water, generated by this process. Also the taste of output water is generally not acceptable.

2) Activated Alumina Process:

Reactivation of filter material is cumbersome and it can be done only with the help of trained persons generally not available in most of our villages. This process also results in high residual Al in output water ranging from 0.16 ppm to 0.45 ppm.

3) KRASS Process:

This process differ from the known processes in its simplicity, cost effectiveness and only traces of residual Al in outlet water. There is no limit on fluoride concentration in input water. Temperature, pH, alkalinity and TDS of input water do not affect this process. It is a practical approach especially for our rural population. The importance of the process is a defluoridation process, which is easy to use by illiterate villagers, requires minimal involvement of technical personnel. In this process, once the filters are laid, the only expenditure is in terms of recharging with alum. This process have verified by CSIR and PHED of Rajasthan. The large scale, field installation KRASS's plants is under process.

4) Other process:

Processes like electro-dialysis, ion exchange and reverse-osmosis require special equipment, power, especially trained person to operate and require maintenance and are expensive.The triumph in the fluoride and fluorosis mitigation achieved through persistent and consistent effects spanning over a period of 2 decades by scientists. From a variety of disciplines including the water sector and biomedical sciences across the country have made the proud. It is no small an achievement. It is the desire of every Indian that let the success achieved in the fluoride and fluorosis front be a model for those involved in dealing with other contaminants like nitrate, salinity, arsenic, heavy metals and pesticides, to be pursue a path, resulting in unquestionable victory. Several years of fundamental research on fluoride action on animal and human body tissues at the cellular and molecular levels generated a wealth of information and knowledge. The path breaking discoveries emerged through unconventional approach adopted for multidisciplinary investigations of both soft and hard tissues were a revelation. The true disease characteristics emerged, ted to early diagnostic procedures for the disease. Differential diagnoses of fluorosis from other disease with overlapping clinical manifestations become necessary. Simple tests with focus on assessing that poison levels in body fluids provided meaningful information. No sooner the disease could be correctly diagnosed at very early stages, it become necessary to eliminate / reduce the poison levels in the body fluids, leading to disappearance of health complaints that are non-responding to medication. What are the health complaints which are referred to? First and foremost gastrointestinal discomfort with pain, constipation, nausea and loss of appetite should never be dismissed as casual. Low haemoglobin content (< 9 gm/ dl) should alert. Anaemia, depression, fatigue and muscle weakness are health complaints overlooked. It is equally important to be aware of excess thirst (polydipsia) and tendency to urinate more frequently (polyurea) can also be due to fluoride interfering with hormonal production which has adverse effects on kidney function. If any of the above health complaints is confirmed to be closed by fluoride poisoning require no medication except for practice of interventions.

Withdrawal of the source (S) of fluoride entry to the body results in arrest of the progression of the disease and health complaints referred to above shall cease to exist. However, if speedy recovery is the aim, yet another intervention i.e. diet enriched by essential nutrients along with micro minerals (Zn, Cu, Mg and Se) besides vitamin C and E, and other antioxidants need to be promoted through fruits and vegetables. An enriched diet insures repair and maintenance of the damaged parts of the body, rise in haemoglobin and total recovery of the disease. It is a new era for practice of integrated disease management. A patient of fluorosis if diagnosed correctly and early by the consulting physician and if the source of fluoride entry is through drinking water, then the responsibility lies on the Water Supply Agency to guide/ advise and direct the patient as to now to obtain 'safe water' for consumption. The intervention procedure dealing with enriched diet with nutrients and antioxidants shall be dealt with by the consulting physician. Monitoring the patient for complete recovery and assessing the impact of the practice of the interventions shall be of the responsibility of the hospital or the physician dealing with the patient. The Water Supply Agency (ies), till date have never practiced nor is aware how a patient is going to look-upon to the Public Health Engineer(s) for quality water for getting rid of the disease after obtaining the diagnosis of the disease from a physician. The present practice of indiscriminate supply of defluoridated water to the community leaving in an endemic area may not relieve those who are afflicted with fluorosis, as the fluoride entry to the body may be much more from the sources other than drinking water.

CONCLUSION

The result of the study indicates that the area under study is fully affected with endemic fluorosis, and the

concentration of fluoride ion in all water sources varies from place to place. All of these results may arise due to the nature of rock and soil formation. Especially higher concentrations were observed in bore well and hand pump water. The low cost adsorbents ESC, AAC, and PJC remove fluoride content from potable water to a larger extent compared to the other adsorbent GAC. Hence the adsorbents ESC, AAC and PJC can be used for the defluoridation of potable water at household level. Finally the results also suggest that the area fully contaminated with fluoride is not suitable for drinking purpose and proper care must be taken by the people.

According to nationwide study of New Delhi based FRRDF (Fluorosis Research and Rural Development Foundation), "the occurrence of fluorosis can vary among different locations having almost the same fluoride concentrations in drinking water and can be affected by factors such as climate, individual susceptibility and biological response". The study concluded that "Poor nutrition also plays an important role in aggravating endemic fluorosis", thus explaining why poor people are often the worst affected.

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NITRIC OXIDE: A MASTER PLAYER IN PLANTS

Rachana Singh

Department of Botany
University of Allahabad, Prayagraj- 211002, India

Review Article

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ABSTRACT

Nitric oxide (NO) is a gaseous reactive oxygen species (ROS) that regulates each step of plant life. It acts as signalling molecule during abiotic and biotic stress. However, the knowledge of NO production and signal transduction remains largely unclear. Therefore in this review we will try to uncover the current knowledge of NO production and its action in different physiological responses.

Keywords: Nitric oxide, abiotic stress, metal, proteins, sulfhydryl group, free radicals.

INTRODUCTION

The world's population is expected to grow 10 billion by mid of the century i.e. 2050, boosting agricultural demand by almost 50 percent compared to 2013. Income growth in low- and middle-income countries would hasten a dietary transition in relation to cereals towards higher consumption of meat, fruits and vegetables, which will add enormous pressure on natural resources. Similarly, much remains to be done to fulfil the vision of Food and Agriculture Organization (FAO, 2017): to create 'a world free of hunger and malnutrition and one in which food and agriculture contribute to improving the living standards of all, especially the poorest, in an economically, socially and environmentally sustainable manner'.

On the other hand, to survive under adverse environmental condition, plants respond at the cellular, molecular and physiological level by interfering with ionomics, genomics, transcriptomics and proteomics level which involves a multifaceted network following perception and transmission of stress signals, which consequently initiate a plethora of responses (Nakashima *et al*., 2017). A deep understanding of the mechanism reinforcing the plants for stress adaptation might provide novel opportunities to develop crops with an increased ability to stand against environmental fluctuations that eventually lead to enhanced productivity. Among plant responses to abiotic stresses, the common factor is the generation of redox active molecules i.e. reactive oxygen species and reactive nitrogen species (ROS and RNS) (Mittler 2002). Though, excessive amount of ROS and RNS can be fatal to cell. Among these redox active molecules, nitric oxide (NO) is the chief one, which is present in the environment since the time ~2.7 billion years ago when molecular oxygen (O_2) was introduced into our atmosphere by O_2-evolving photosynthetic organisms. During the last few decades, NO emerged as a signalling molecule in plants, since then it became associated with a large number of phenomena. Being a ROS or free radical, NO jobs as a gasotransmitter-diffusible multitasked messenger, which easily can cross the membrane without any carrier. It was first defined in mammals, where it plays various functions ranging from blood vessel relaxation, neurotransmission, participation in the fertilization process and immune defense responses (Zhou and Zhu, 2009). In plants, both NO and other ROS found associated in mediating signalling responses in tip growing cells, where they are involved in regulating polarity and growth (Wudick and Feijo, 2014). NO may act

*Corresponding author: *rachanaranjansingh@gmail.com*

as a positive as well as a negative regulator during stress. According to Mur *et al*. (2013) this two fold effect of NO either as a promoteror as an inhibitor is predominantly depend on its concentration and production. Once generated, NO can react with other redox-related molecules and potentially regulate protein function (S-nitrosylation) via distinct mechanisms. During the past decade, NO function and S-nitrosylation have emerged as major regulatory mechanisms for abiotic stress signalling. Therefore, in this review we focus on the current state-of-the-art regarding the roleof NO and S-nitrosylation in abiotic stress responses in plants.

Table 1: Depicting the nitric oxide (NO) induced effects on plants under abiotic stresses.

Stressor	Plant	NO induced response	Reference
Drought/osmotic stress	*Nicotiana tabacum, Pisum sativum*	Involving in ABA signalling, stomatal closure induction of ABA synthesis, LEA expression.	Gould *et al.* (2003), Leshem and Haramaty (1996)
Heavy metal toxicity (Al)	*Hibiscus moscheutos,*	Increased the root elongation; reduced the NOS activity (Reduced NO level).	Tian *et al*. (2007),
Cd	*Triticum aestivum*	Increased antioxidants activities; reduced the ROS and indices levels; Improved PSII photochemistry.	Kaya *et al.* (2020)
Herbicide	*Scenedesmus obliquus, Chlamydomonas-reinhardtii*	Promoted the activity of antioxidant enzymes.	Mallick *et al*. (2000), Sakihama *et al.* (2002)
High temperature	*Medicago sativa, N. tabacum*	Increased tolerance of seedlings; rapid NO release.	Leshem *et al*. (1998),
Low temperature	*S. obliquus*	Decline the ROS level.	Gould *et al*. (2003) Mallick *et al*. (2000)
Mechanical injury	*Arabidopsis thaliana, Taxus brevifolia*	NO burst result in cell death.	Garces *et al*. (2001), Pedroso *et al*. (2000)
Nurient deficiency	*S. obliquus*	----------	Mallick *et al*. (2000)
Salinity/salt	*N. tabacum, Maize, Ocimum basilicum*	Increased osmotic tolerance; induce expression of Na^+/H^+antiporter gene. Improved the morphological parameters.	Gould *et al*. (2003), Zhang *et al*. (2006), Gohari *et al.* (2020)
UV-B radiation	*A. thaliana, Paulownia tomentosa*	Induced the expression of CHS gene. Inhibited pollen germination and tube growth (exogenous NO).	Mackerness *et al*. (2001), He *et al*. (2007)
Iron deficiency	*A. thaliana,*	Upregulation of the expression of iron uptake-related genes.	Koen *et al*. (2012),
Iron deficiency and salt stress	*Capsicum annuum*	Decreased G6PDH and NADP-ICDH activity, decreased fruit ripening.	Muñoz-Vargas *et al*. (2020)

NO role under abiotic stressed conditions

Since past decades, a number of articles regarding endogenous NO production/ reduction in response to different abiotic stresses have been addressed (Rodriguez-Serrano *et al*., 2009; Besson-Bard *et al*., 2009; De Michele *et al*., 2009) Table 1. In many of these studies, the assessment of exact location and quantity of NO in plants with the timing and

2009), whereas upon long-term Cd exposure (50 μM), NO induced senescence process (Rodriguez-Serrano *et al*., 2009).

Drought is another highly studied stress that interferes with crop productivity but the role of NO under stress is unclear (Gould *et. al*., 2003; Zhang *et al.*, 2007). During drought stress, stomata closure, an essential process controlled by abscissic acid (ABA) where NO helps ABA but during dehydration involvement of NO is not necessary. This suggests that NO is involved in regulation of stomatal closure in turgid leaves which occurs in response to environmental fluctuations (Neill *et al*., 2008; Wilson *et al*., 2009). NO also plays significant role under herbicides, high temperatures, ozone, UV-B, salinity, mechanical damage (Neill *et al*., 2008; Gohri *et al*., 2020; Kaya *et al*., 2020). However, more studies are needed to define NO signalling especially with transcriptomic analysis (Besson-Bard *et al*., 2009).

NO have also been reported to arbitrate hormone-regulated (ABA, salicylic acid (SA), ethylene, auxins or DELLAs) processes in plants, and a cross-talk in NO and hormones may also involve secondary messengers like Ca or kinases, under varying environmental conditions (Simontacchi *et al*., 2013). The equilibrium between ROS and NO is important to decide the fate of the cell especially under abiotic stressed conditions in context of ROS generation and antioxidant defence system of plants (Rodriguez-Serrano *et al*., 2009). Future studies are focuses on NO-dependent protein regulation, mainly through nitration and S-nitrosylation (Vandelle and Delledonne, 2011).

Biological effects of NO

To understand the framework of different events of plant system, the study of chemical biology of NO with different biological molecules is necessary (Wink and Mitchell, 1998). Being a paramegnetic molecule NO has an unpaired π* electron and easily can diffuse across the membranes. The nitrosonium anion (NO^+) formed after oxidation contributes in nitrosation reactions when added to thiol, amine, or hydroxyl aromatic group. When second electron is added to 2p-π orbital of NO nitrosyl anion (NO^-) is formed. According to Hughes (1999), interconversion of NO, NO^+ and NO^- prevails in cellular conditions and by reacting with free radicals, metals, oxygen molecule and thiols, NO regulates plant responses.

Reactions of NO with free radicals

NO reaction with superoxide anion ($O_2^{\bullet -}$), is diffusion limited because NO is not too fast toreact (Henry and Guissani,1999). This reaction between reactive nitrogen and oxygen species leads to the formation of a toxic powerful oxidant peroxynitrite ($ONOO^-$), which reacts majorly with macromolecules (Wink and Mitchell, 1998). NO is a strong inhibitor of lipid peroxidation and scavenges peroxyl radical ($LOO^\bullet$) (Hogg and Kalyanaraman, 1999). Lipid peroxidation is a toxic component formed due to oxidative injuries, when antioxidant defence system of plants surrenders under abiotic stresses, while NO accumulation under these conditions shows its protective behaviour (Patel *et al*., 1999). Formation of NO^- derived species and nitrolipids (nitro fatty acids) have been reported by Fazzari *et al*. (2014), which suggests their role as signal transduction mediator.

Reactions of NO with metals

NO after reacting with transition metals forms coordination complexes like with Fe it forms nitrosyl iron complex (a NO^+carrier). Through Fenton's reaction toxic iron catalyzesthe formation of hydroxyl radical ($HO^\bullet$. According to Jasid *et al*. (2008), NO have the ability to protect plants from oxidative injuries by inhibiting Fenton chemistry binding ferrous iron (by directly reacting with Fe, NO forms nitrosyl Fe in presence of sGC and catalase). NO after reacting with iron-heme componentof sGC enzyme, increases its activity and leads to the production of cGMP, which triggers different responses in animals (Neill *et al*., 2008).

Reactions of NO with tyrosine in protein

Nitration is the process of addition of nitro group (NO_2^+) tyrosine residues in proteins, which interferes tyrosine phosphorylation, a general process to control the enzymatic activities. The nitration reaction is mainly influenced by RNS and CO_2 concentration, in different plant cells (Santos *et al*., 2000). Camejo et al. (2013) have reported that in salt stressed pea plant protein nitration increases with NO increase, where PSI, PSII, cytochrome $b_{6/f}$ and ATP synthase complex are the major tyrosine nitration sites in chloroplast (Galetskiy *et al*., 2011). Nitration targets were identified in sunflower hypocotyls (Chaki *et al*., 2009) and in *Arabidopsis* under hypersensitive (Cecconi *et al*., 2009) as well as non-stressed (Lozano-Juste *et al*., 2011) condition. When nitroproteomic analysis was

done in salt stressed root and leaves of citrus plants, defence/ disease related proteins were the majorly affected group in roots while photosynthesis related proteins were chiefly affected group in leaves (Tanou *et al*., 2012), where 86 and 88% proteins underwent tyrosine nitration, respectively.

Reactions of NO with sulfhydryl groups

Due to its strong electrophilic nature, NO^+ reacts with most of biological –SH (Gaston 1999), resulting the formation of S-nitrosothiols (SNO). Generally, nitrosoglutathione (GSNO) and SNO are NO^+ carrier and reservoirs in biological system. GSNO with the help of GSNOR activity transform into oxidized glutathione (GSSG) and ammonium, but the higher concentration of NO inhibits GSNOR activity via S-nitrosylation reactions and therefore, excessive NO inhibits GSNO degradation with probable reduction in nitrate uptake rate (Frungillo *et al*., 2014). The reaction between NO^+ and cysteinyl sulfhydryl moieties(S-nitrosylation), is an important signalling event which involves in various plant physiological processes. In addition to NO levels, protein nitrosylation have also been linked with GSNO accumulation, as in case of low GSNOR/reduced thioredoxinh5 (TRXh5) activities (Kneeshaw *et al*., 2014). TRXh5 activity in plants reveals a strong protein-SNO reductase activity, which is the basis for salicilic acid-dependent plant immune signalling (Kneeshaw *et al*., 2014).

Concluding remarks

Nitric oxide defends plants from oxidative injuries, by maintaining photosynthetic capacity, antioxidant defence systemand other major metabolic processes; either by interacting with plant hormones or other molecules/ messengers. Although, NO-mediated responses to abiotic stresses have been well documented; however, the multiple signalling pathways implied in morphological and physiological stress responses needs further studies which could help to develop strategies to improve the plant growth and yield under adverse conditions that also will help to combat human nutrition problems.

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GAMETOGENIC CHANGES IN FRESHWATER BIVALVE: LAMELLEDINS CORRIANUS IN MONSOON DUE TO REMOVAL OF CEREBRAL GANGLIA AND INJECTION OF ITS EXTRACT

N.G. Shinde[1*], D.M. Gaikwad[2], A.N. Vedpathak[3] and S.R. Bhupalwar[4]
Department of Zoology
[1]K.J. Somaiya College, Kopargaon, Dist. Ahmednagar, Maharashtra
[2]Rajashri Shahu College, Pathri, Tal. Fulambri, Dist. Aurangabad, Maharashtra
[3]Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra
[4]LBS College, Dharmabad, Nanded, Maharashtra

Research Paper

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ABSTRACT

In freshwater bivalve molluscs *Lamellidens corrianus* there are seasonal changes in the reproductive behavior. Present investigation deals with the alteration in gametogenic activity due to removal of Cerebral Ganglia (CG) and injection of Cerebral Ganglionic extract during monsoon season. These molluscs are bottom dweller and filter feeder, in which CG plays a key role in gametogenic activity. Based on earlier report and present study in the region, it is concluded that gametogenesis occur in complete monsoon which include pre and post monsoon. In monsoon season effect of CG removal (ablation) and its extract injection was observed which revealed that, removal of CG accelerated the growth of gametes and injection of their extract gradually increases the growth. In this season lipid globules from the follicle of lumen in experimental group considerably diminished in quantity showing enhanced maturation of gamets as compared to the control. It is likely that the cerebral ganglia in *Lamellidens corrianus* may produce inhibitory principle since removal of cerebral ganglia even in immature gonads showed rapid development of gametes leading to lysis before they are released.

Keywords: Cerebral ganglia ablation, cerebral ganglia injection, Freshwater bivalve, *Lamellidens corrianus*, Monsoon season.

INTRODUCTION

In mollusca, interactions with the environment are handled by the nervous system (including the sense organs) and the muscular system (the muscles that making the foot and those that attach the animal to its shell). Both systems, of course, have been of different forms in different group of animals. Basically there are two extreme types of neural organization (with many intermediate forms). The chord system, in which the neural tree taper sand branches in smooth lines; and the ganglia system, which is characterized by knots of nerve cells called ganglia, bound together by nervous tissue consisting of slender elongations of the cells. The nervous and endocrine systems coordinate the activities of the various organs and the tissues in the body that the animals function as individuals. Majority of the neurons with granular activity, are known to be necessary for the transmission of transient impulses, with their highly localized production of chemicals such as neurohumors / neurohormones, which are rapidly destroyed. Neurons have similar properties throughout the animal kingdom, although their morphology and arrangement may vary.

Evidence for the occurrence of a wide variety of neurotransmitters in different tissues of lamellibranchs

*Corresponding author: *dr.nitinshinde2012@gmail.com*

including the nerve ganglia has been discussed from the functional point of view (Leak and Walker, 1980). Under the light microscope, neurons are characterized by the presence of abundant secretary materials in their perikarya. This material is seen also in the axons which oftenly end blindly adjacent to muscular spaces rather than innervating their target structures directly. These blindly ending terminals serve a storage release function and in the more advanced groups of animals such as crustaceans such compact structures are termed as neurohemal organ by Knowles and Carlisle (1956). The neurosecretory cells (NSCs) with their combination of neuronal and glandular capabilities are perfectly suited to translate a neuronal input into the hormonal output best suited to long-term process. In this capacity, the NSCs may produce hormones, which act directly upon the peripheral target or it may exert its effect indirectly by influencing the activity of other, non-neural, endocrine organs. In this later case, the NSCs organ may act via the production of blood- born hormone. Knowles and Bern (1966) stated that the significance of NSCs as connecting link between nervous and endocrine systems and neurosecretory neurons "participate either directly or indirectly in endocrine control and form all or part of endocrine organ". Hormones are consequently well suited to exert their effects over extended period of time, and the endocrine system controlling-term process within the body such as the coordinated growth of organs or the maintains of appropriate metabolite concentration in the blood and tissues.

From a functional point of view, Lubet (1955, 1956) was the first to suggest that a relationship might exist between the neurosecretory cells in the cerebropleural and visceral ganglia and reproduction in two marine mussels, Mytilus edulis and Chamys varia. During the period of gamete maturation, he found that cerebral neurosecretory material accumulates within the perikaryon and is discharged just prior to the extrusion of gametes. This pattern of cell synthesis and release is repeated before each subsequent gamete evacuation. Surgical removal of the cerebral ganglia has little effect on maturation of the gonocytes, but accelerates their discharge. Lubet thus concluded that the cerebroplural ganglia in some way inhibited spawning until a few days before the beginning of the reproductive period, at which time this inhibition was removed coincident with the discharge of neurosecretory substances, the transport of this material maybe via axons directly to a target site, thus insuring stimulation of the correct structure at the appropriate time. The mussel then becomes sensitive to the correct environmental stimuli with completion of the spawning process. Very little work on involvement of neurosecretion in reproduction and energy metabolism is reported in case of freshwater species (Kulkarni, 1987). To extend the knowledge in this field, the present work has been undertaken on the freshwater species using the bivalve, Lamellidens corrianus (Lamark).

MATERIALS AND METHODS

The freshwater bivalve molluscs, Lamellidens corrianus (Lea) were collected from Jayakwadi backwaters (Nathsagar) at Paithan, 45 km. away from Aurangabad. After brought to the laboratory, the shells of the bivalves were brushed and washed with fresh reservoir water so as to remove the fouling algal biomass and mud. The animals of 80-85 mm shell length were selected for experiment and they were acclimatized for 24 h. at laboratory condition in fresh aerated reservoir water (with renewal of water at the interval of 12-13 h.) and stocking capacity was given during this period and no food was given to the bivalves during laboratory acclimatization and subsequent experimentation. After 24 h., reservoir water was once again renewed and aeration was given. Each aquarium contained 15 liter well aerated reservoir water, and experiment was run for 12 days. The water from each aquarium was changed at an approximate interval of 12-13 h. throughout experimental period. After a lapse of 1hour animals extended their organs (foot, mantle, siphons) to maximum and soon surgical operations were done. A rubber cork kept between two shells and with lamp light focuses on cerebral ganglia and with the help of needle bilateral cerebral ganglia were removed. For injection of ganglionic extracts, cerebral ganglionic extract was prepared in ice cold distilled water, 10 ganglia in 1ml cold distilled water were centrifuged and the supernatant (0.2 ml/animal i.e. equivalent to 2 ganglia/animal) was injected into the foot (muscular region) of normal control bivalves. In sham operated control animals were injected by 0.2ml cold distilled water. The result for control and sham operated groups were similar and hence a comparison was made

between extract injected to normal control group of animals only.

5 animals from each group during monsoon seasons were also fixed in Bouins Hollande on 7th, and 12th day for histological study of gonad. The animals were soaked carefully with the help of filter paper and flesh of the animals was fixed in Bouins Hollande fixative for 24 hrs. The fixative was renewed for next 24 hr. to facilities better fixation of the tissues. During experimental period gonad tissue were then removed and processed for preparation of paraffin blocks. Dehydration of gonadal tissue and ganglia was done through serial grades of ethyl alcohol and tertiary but/anol respectively while xylene was replaced by toluene during the process of dehydration. The tissues were embedded in paraffin wax at 58˚C-60˚C and the sections of gonad were cut at 6.0 to 7.0 µm thickness using Spence-rotary-microtome. The gonads were stained with Mallary's Triple as well Gomories Chrome Alum stain, (as shown by Illanes and Lubet, 1980). All the sections were observed under the research binocular microscope and wherever necessary, measurements were made before microphotography.

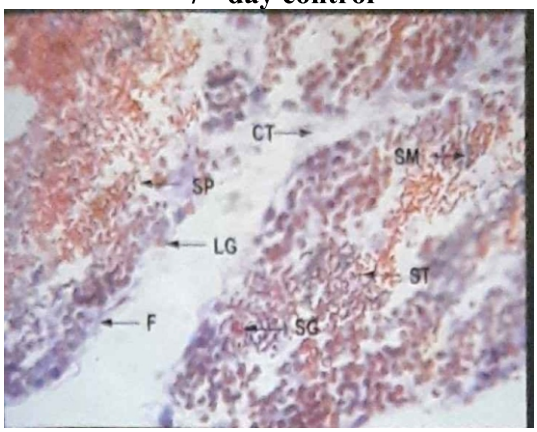
RESULTS

The details of the histological structure of *Lamellidens corrianus* about male and female gonads are given in the fig. 1 and 2. Measurements of the developing oocytes from female gonads in of different groups on 7th and 12th day during monsoon seasons are given in Table 1. During monsoon seasons, on 7th day the previtellogenic oocyte shows the diameter of 48.728±4.462 µm, in control group, which is somewhat smaller as compare to CG ablated group (85.437±7.74 µm) and CG extract injected group (80.905±7.45 µm). As compare to the vitellogenic oocyte on 7th day in monsoon season, it shows a diameter of 195.116 ±9.48 µm in CG ablated group, which is larger than oocyte diameter of 7th control (154.982±12.249 µm) and 12th day CG extract injected group (137.21±11.24 µm). On 12th day of experiment the as far as the size of previtellogenic oocyte are concern CG extract injected group has the larger diameter 91.688±21.219 as compare to CG ablated group (79.897±11.293) and Control group (52.818±16.273). About the vitellogenic oocyte the diameter again larger in CG ablated group i.e. 194.829±14.288 and it is somewhat smaller in CG extract injected group (187.281±17.109) and Control group (173.829±12.814).

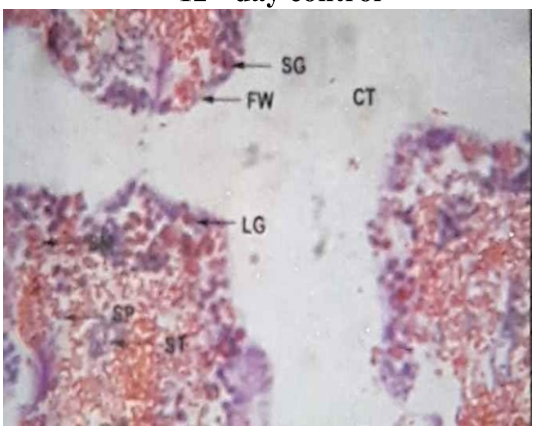
Table 1: Measurements of the developing oocytes from control, ablation of cerebral ganglia and injection of ganglionic extracts in Lamellidens corrianus during monsoon season (All the values are um).

Experimental Period		Previtellogenic oocite range	Vitellogenic oocite range
7th day	Control	48.728±4.462	154.962±12.249
	Ablation of cerebral ganglia	85.437±7.74	195.116±9.48
	Injection of cerebral ganglionic extracts	80.905±7.45	137.21±11.24
12th day	control	52.818±16.273	173.829±12.814
	Ablation of cerebral ganglia	79.897±11.293	194.829±14.288
	Injection of cerebral ganglionic extracts	91.688±21.219	187.281±17.109

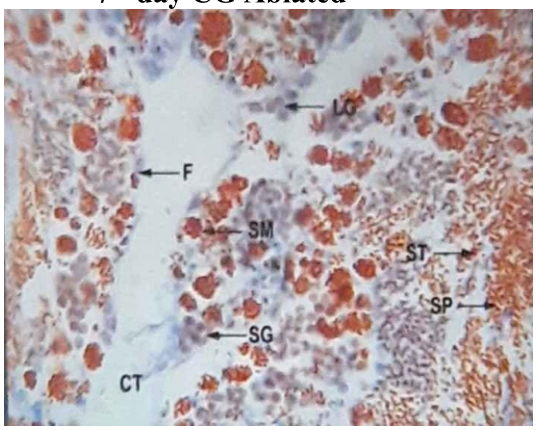
7th day control



12th day control



7th day CG Ablated



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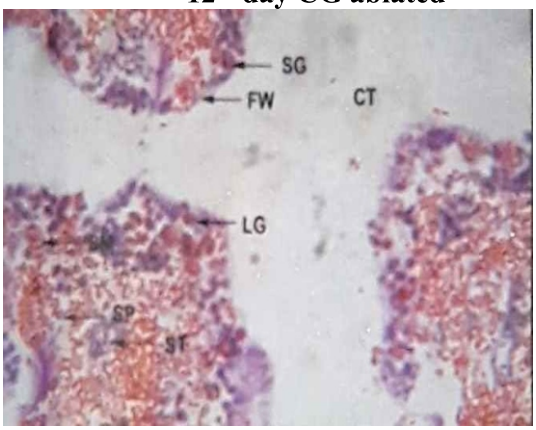
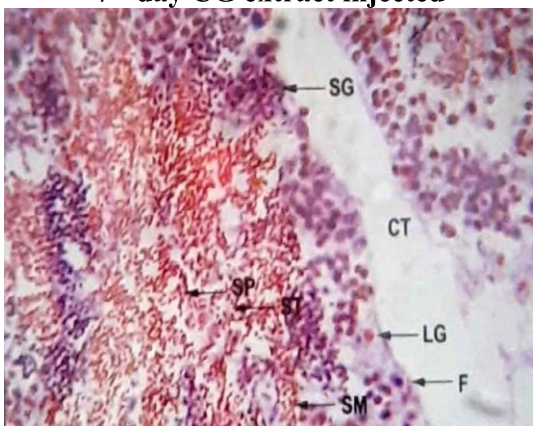


7th day CG extract injected



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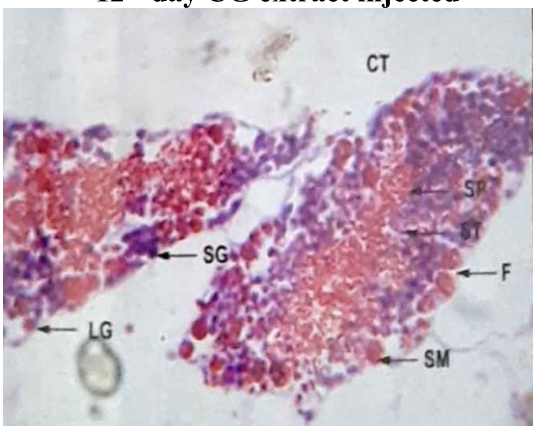
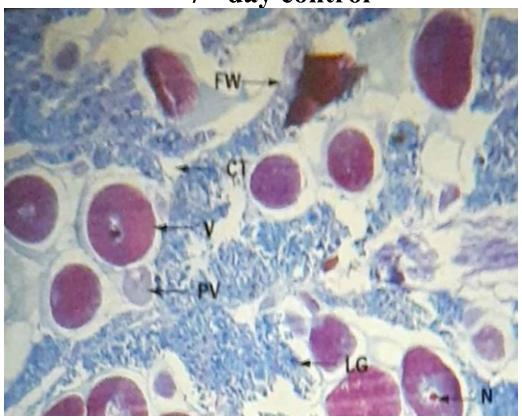


Histological changes in male gonad on 7th and 12th day due to ablation of cerebral ganglia and injection of their extracts in Lamellidens corrianus during monsoon season

F =Follicle **FW = Follicular wall** **LG =Lipid globule**
SG = Spermatogonia **SM =Sperm morulae** **ST=Spermatid**
SP=Sperms **CT = Connective tissue**

7th day control



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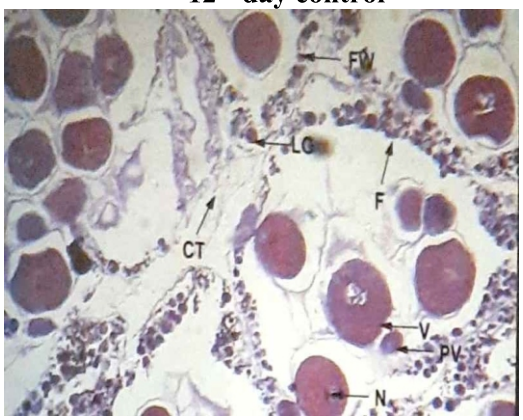
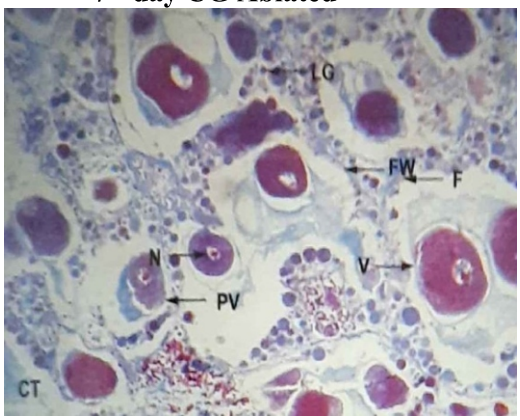
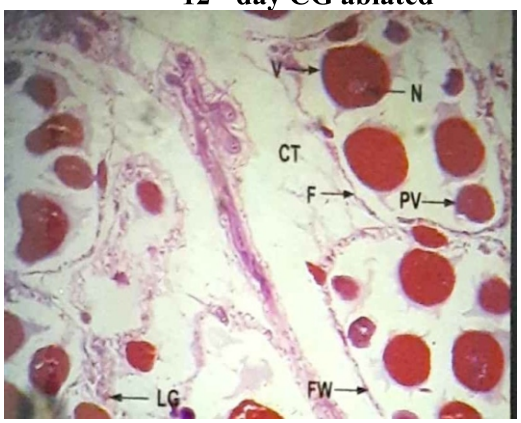


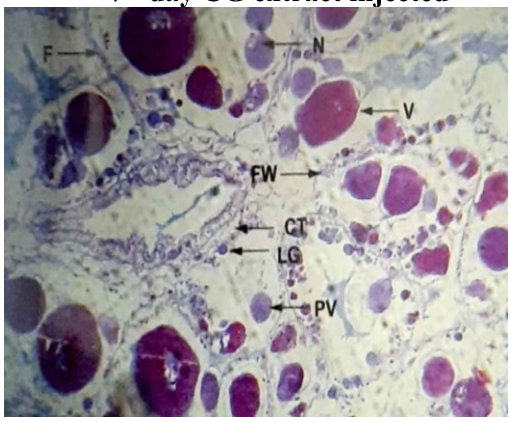
7th day CG Ablated



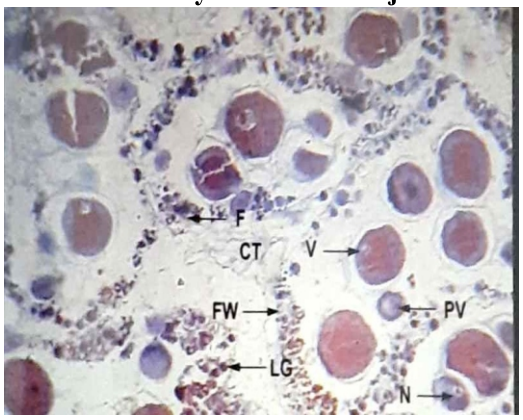
12th day CG ablated



7th day CG extract injected



12th day CG extract injected



Histological changes in female gonad on 7th and 12th day due to ablation of cerebral ganglia and injection of their extracts in Lamellidens corrianus during monsoon season

F =Follicle **FW = Follicular wall** **LG =Lipid globule**
N = Nucleus **PV =Previtellogenic oocytes** **V=Vitellogenic oocytes**
CT = Connective tissue

Bivalve reproduction has been extensively studied. Much of the literature is concerned with gonad development and breeding periods. From marine species this aspect has been nicely reviewed by Sastry (1979 and Andrewes (1979), and for freshwater species better description is given by Purchon (1977) and Mackie (1984). Reproduction in both the marine and freshwater species is cyclical, and it may be annual, semi-annual or continuous. In reproductive cycle the gonad development, spawning and fertilization are the three major phases. These phases function continuously in coordination with seasonal environmental changes and produce the pattern characteristics of a species. Sastry (1970a) stated that the reproductive cycle of a species is a genetically controlled response to the environment and the pattern is apparently determined through the co-ordination of successive reproductive events with changes in the external environment. The author further stated that in species occurring in several climatic zones, the reproductive cycle may vary in relation to local environment as a phenotypic response of a single genotype or it could be directly genetic, or both. Studies carried out by several workers indicate that a reproductive response is produced through an interaction of environmental factors-especially temperature, salinity, light and food and endogenous factors within an organism. After attaining a certain physiological state, an organism exposed to the required environmental prerequisites begins gonad growth and gametogenesis. The life cycle of an animal is usually so timed by some environmental variable or variables that the young are produced at a period favorable for their survival (Giese, 1959). It is likely that neuroendocrine activity plays a significant role in coordinating the physiological processes, the reproductive events within the organism to produce a reproductive response relative to changes in the external environment (Lubet, 1959, 1965; Sastry, 1970a, 1975). The gonads of Indian freshwater bivalves may be active throughout the year or the activity is restricted to a certain period. Lomte and Nagabhushanam (1969) reported that the freshwater bivalve Parreysia corrugate showed restricted activity to certain period of the annual cycle. Nagabhushanam and Lohagaonkar (1978) found that Lamellidens corrianus breeds throughout the year but with definite peaks, as reported for Lamellidens marginalis by Ghosh and Chose (1972). Patil and Bal (1967) studied seasonal gonadal change in unisexual freshwater mussel, Parreysia favidens from Mula river, Kirkee at Pune. The authors recorded the commencement of spawning from March, which continued up to October indicating a prolonged breeding period. The authors correlated the filling of lipid globules in the follicles with development and maturation of gametes. There may be one or two spawning seasons in freshwater bivalves. Nagabhushanam and Lohagaonkar (1978) reported that in Lamellidens corrianus intense spawning occurs during September to December, i.e. the period of post-monsoon to early winter. In the present study spawning occurs during post monsoon and during winter, spawning occurs at a reduced rate in other months as well. In the present study, Lamellidens corrianus from Jayakwadi backwaters also revealed gametogenesis occurs in both male and female follicles during pre-monsoon and maturation of gametes found during post-monsoon. However, based on the literature and on the histological sections of the gonad of Lamellidens corrianus, the period of gametogenes is starts with rise in temperature and pre-monsoon but at a slow rate since the food availability are low and the energy demand for maintenances of physiological activities rise. The activity of gametogenesis resumed vigorous in monsoon at the time the food availability increases. Thus, in Lamellidens corrianus environmental factors synchronies the gonadal development at an early stage in its progress of maturity so that spawning can be coordinated to achieve synchronous release of gametes.

Demonstration of the role of neurosecretion in the reproduction of bivlaves has been difficult with standard surgical procedures. There are different interpretations regarding the role of NSCs from cerebral ganglia in giving spawning reaction. Antheunisse (1963), who worked with freshwater animal Dreissina polymorpha, suggested that premature spawning might be a function of the intensity of the operative shock when removing the ganglia. The author concluded that, in spite of a parallelism between the neurosecretion and reproductive cycle in this species; a direct causative relationship did not operate. Umiji (1969) found that ablation has no effect on follicle development or on the intra follicular connective tissue. Removal of cerebral ganglia in March, just before spawning of Katelysia opima, results in release of gametes; females are much more responsive than males and immediately release gametes (Nagabhushanam and Mane, 1973).

Similarly, ablation of cerebral ganglia in oyster, Crassostrea gryphoides in July at the time of mature gonads also releases gametes (Mane and Nagabhushanam, 1976). In Perna viridis a large number of mussels operated in the late June and early July at the time of mature gonads release gametes after cerebralectomy, in contrast to those operated during previous and next period (Mane, 1986). The author further stated that comparing the spawning reaction in cerebralectomized and visceralectomized mussels, visceralectomized mussels do not show accelerated spawning as cerebralectomized ones. Although cerebral ganglia appear to secrete neurosecretory substances during gametogenesis, it is impossible to decide whether the mechanism is nervous or hormonal in nature. If the activities of neurohormones are important, removal of an internal inhibition, such as the neurosecretory product of the cerebral ganglia, may allow the animal to become receptive to exter nal stimuli, which then induce the release of gametes. Clark and Olive (1973) gave an excellent account on endocrine control of maturation and reproduction in the polychaetes. They stated that decerebration is known to have a profound effect on the developing coelomic oocytes but different responses have been found in different species. In most nereids prostomial ablation causes the accelerated growth of coelomic oocytes but in Perinereis cultrifera they tended to degenerate after this operation. The products of this accelerated growth also vary between species. Possible role of a brain hormone in the oogenesis of Poecilobdella viridis has been given nicely by Kulkarni and Nagabhushanam (1980). The effects of brain removal on the oogenesis stages were found to be profound during the non reproductive period; the authors found that the brain removal causes adverse effect upon ovarian development when the leeches are operated in November. Their experiments indicated that the brain neurosecretory profile is the site of formation of gonadotropic hormones which is necessary for activation and initiation of oogenesis. The details regarding the role of cerebral ganglia in maturation of gametes in bivalves are also available with organ culture techniques. Mathieu and Lubet (1980) stated that in Mytilus edulis the achievement of gametogenesis in adult mussel, during the annual reproductive cycle dependents upon the action of different neuroendocrine factors: a mitotic factor allowing the multiplication of oogonia and spermatogonia, a meiotic factor triggering the first meiotic division in males, a previtellogenic factor leading oocytes to villogenesis and a vitellogenic factor acting on vitellogenesis. The authors concluded that the factors are neither sexualized nor specific; they are released by cerebropleural ganglia and less by visceral ganglia. In accordance with the earlier findings of Lubet with organ culture technique it was further reported by Lubet and Mathieu (1982) that the achievement of gametogenesis in adult Mytilus edulis, Crassostrea gigas and Ostrea edulis during the annual reproductive cycle depends upon the action of the neuroendocrine factors: a mitotic factor triggering the gonial mitosis, a meiotic factor acting on the first division of meiosis in males, a previtellogenic factor leading oocytes to vitellogenesis and a vitellogenic factor causing vitellogenesis. In case of annelids also it has been noted by Clark and Olive (1973) that an isolated parapodium of a male Nereis diversicolor cultured in liquid medium showed an accelerated spermatogenesis when a source of cerebral ganglia hormone is introduced into the cultured medium. The measurement made under present study on the normal enhanced growth of vitellogenic oocytes due to decerebration requires special mention. In monsoon, the lipid globules were more in control group and quantity diminished due to ablation of cerebral ganglia. In monsoon the ablation of cerebral ganglia produced normal mature gametes. Exactly similar effect was noticed on the male follicles. The cerebral ganglia of freshwater bivalves, like Lamellidens corrianus might also produce inhibitory principle since, removal of cerebral ganglia even in immature gonads showed rapid development of gametes leading to lysis before being released. However, based on the reports on reproductive cycles in freshwater bivalves from Maharashtra State and the histological details from the present study it can be said that the active gametogenesis takes place in monsoon and the gametes are shed from post-monsoon (September) onwards. After fertilization of gametes, the embryos are retained in the body for further development. In Lamellidens corrianus inner demibranch served this function and the glochidia larvae are released in bunch which have been called swarms. In the present study removal of cerebral ganglia produces significant changes in the development of sex products in different seasons. In this season i.e. monsoon, the removal of cerebral ganglia accelerated the growth of gametes and

injection of their extracts gradually increases the growth. In monsoon, the lipid globules from follicles lumen considerably diminished in quantity showing enhanced maturation of gametes as compared to the control. In addition, removal of cerebral ganglia showed reappearance of lipid globules, more in number than normal one, in the lumen of the follicle revealing nourishment of the gametes for the rapid development. The lipid globules in Lamellidens corrianus necessary for enhanced vitellogenesis may probably produce vitellogenine as in other invertebrates and this aspect requires further research work. In monsoon the removal of cerebral ganglia produced normal mature gametes, which are ready to release. This has been confirmed by the measurements of the developing oocytes till their ripening from normal animals and also from those of ablated and injection of ganglionic extracts to animals. It is likely that the cerebral ganglia in Lamellidens corrianus may produce inhibitory principle since removal of cerebral ganglia even in immature gonads showed rapid development of gametes leading to lysis before they are released (N.G. Shinde 2008). Comparing these two groups, it can be stated that even after removal of cerebral ganglia sufficient concentration of pre-secreted hormone might be left to affect the gonads for enhancement of gametes growth.

In general, the present study of ablation of cerebral ganglia and giving injection of their extracts to ablated animals revealed that the cerebral ganglia play an important role, mostly inhibitory one, in the regulation of gonad development and spawning. These aspects include the regulation by neurohormones/neurohumors of normal physiological functioning and homeostasis. It is tentatively suggested that the cerebral ganglia in bivalve molluscs, including Lamellidens corrianus, elaborate some principles including neurohormones, neurotransmitters and other unknown factors which trigger the metabolic demands of animal during different reproductive phases. Various aspects involved in such mechanisms require separate evaluation. Experiments on bivalve molluscs using exogenous sources of neurotransmitters and various sex steroids can also give details in endogenous control via central ganglia of reproductive physiology. In the present study removal of cerebral ganglia caused significant changes in the aspects of reproductive processes of Lamellidens corrianus, including the swarming behavior, in monsoon season.

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