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Application of physiological and biochemical assays as ecotoxicological tools for the detection and monitoring of pesticide pollution in earthworms and agroecosystem- A review

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ABSTRACT

The world population has grown exponentially over the last four or five decades and as a result food crisis with respect to the population had become a major problem for the governments of all the countries. As a result, it gradually became inevitable to increase the production of crops to meet the supply and demand ratio. Agriculture became much more advanced both in terms of technology and on field production to grow more genetically viable, disease and pest resistant variety and to grow them faster, application of chemical fertilizers drastically increased respectively with the addition of unmanaged application of pesticides resulting in advanced varieties of crops but more deteriorated soil quality, destruction of the entire ecology of beneficial and non-target soil organisms and also effecting the health of the food crops. Genotoxicity from food grains to humans has increased the prevalence of many dreadful diseases including cancer. Ecotoxicological research over the years has also developed and has helped us understanding the nature of the xenobiotic materials, specially pesticides, their mode of action, pattern of toxicity etc. Thus, researchers and different global organizations, specially Organization for Economic Cooperation and Development (OECD) has formulated different biochemical and analytical methods to study and determine the acute and chronic toxicity of pesticides and other xenobiotics.

1. Introduction

The synthetic pesticides machinery has been rolling since early 1940s when Dichloro diphenyl trichloroethane (DDT) was first introduced, bringing a novel paradigm in man's fight against pests and diseases (Rathore and Nollet, 2012). Unfortunately, this was the beginning of xenobiotic insult of pesticides to environment, non-target animals, man and human society. In order to increase agricultural output pesticides are always been major inputs in addition to seed, fertilizer and water in the modern agro-ecosystems which is reflected in the continuous growth of the global pesticide market especially in the tropical countries (Ecobichon, 2001).Increased food production is essential for mankind because human population is growing at an alarming rate and

is likely to cross the 9 billion mark by 2050 (UN, 2005). It cannot be denied that pesticides play an essential role in controlling harmful pests of crops. Inventions of high yielding varieties of crop, collective farming and specialization leading to the competent use of machinery prompted the farmers to use chemical pesticides. More and more potent pesticides were invented and the application rate continued to increase. The farmers got an immediate return with high yield. But continuous and indiscriminate use of all the kinds of pesticides since last few decades have caused serious damages to ecosystems (Reinecke and Reinecke, 2007) and concern for contamination of the environment gained tremendous importance in recent years (Zhu et al., 2004).Detection of insecticideresidue in fish (Tilak et al., 2003), milk products, vegetables (Lin and Shiau, 2005), food grains (Toteja et al., 2006), meat, groundwater and even in humanblood and breast milk (Strucinski et al., 2006; Furst, 2006; Damgaard *et al*.,2006) became priority research. Scientists throughout the world are engaged inevaluating damages caused by the pesticides (Hafez and Theimann, 2003).Increasing number of ecosystems are being contaminated by huge quantities of pesticides that are released daily into the environment (Sarkar, *et al*., 2006). Agricultural fields are the worst affected ecosystems. Ploughing, clearing, levelling, burning, watering etc. and application of synthetic fertilizer and pesticides make agricultural fields different from natural soil ecosystem. Some soil species cannot withstand such disturbances and may disappear either completely or partially from agricultural soil system and hamper natural equilibrium among organisms (Wallwork, 1970; Lasebikan, 1975; Aleinikova and Utrobina, 1975; Tadors and Saad, 1980).Scientists throughout the world are engaged in evaluating damages caused by the insecticides.Thus, the fate of insecticides in the soil and their effect on nontarget soil organisms are priority research in recent years (Hafez and Theimann, 2003).In this review, we will discuss about these different techniques and methodologies which can be used as ecotoxicological tools to detect and monitor pesticide pollution in soil organisms and agroecosystems.

2. Methodologies

2.1 Acute Toxicity

2.1.1 Lethal Dose (LD_{50})/ Lethal Concentration (LC_{50}) determination

Bioassays are made with age synchronized test specimensin the same small inert polythene boxes. In case of soil organisms, dried, finely ground soil (0.25 mm particle size) are usually laid in the experimental boxes as an experimental medium. Soil moisture of the test soil is maintained at 60–70 % level. Different levels of the pesticides based on their recommended agricultural doses (RAD) are administered into the test boxes with a micropipette (Bostrom and Lofs Holmin, 1982), but the actual doses used are presented in terms of mg / kg soil given in an experimental box. After the application of pesticides, the boxes are left undisturbed for about 30 minutes for

uniform spreading of the chemical in the medium. Each experiment is accompanied by a test box which received no pesticide and is treated as control. Then age synchronized test specimens are transferred into the control and treated test boxes. Finally, the experimental boxes are kept in an Environmental chamber at a constant temperature of 28 ± 0.5°C and 60-70 % moisture level. Mortality of the test specimensis recorded every 24 hours and dead specimens, if any, are removed from the experimental boxes (OECD, 1984).The total mortality of the test specimens obtained after 96 hours of exposure are subjected to probit analysis by EPA probit analysis program, version 1.5 (US EPA, 2006) to determine LC_{50} value and 95 % confidence limit of each insecticide.

2.1.2 Lethal Time (LT_{50}) determination

Bioassays to determine LT_{50} of the pesticide for test specimensare made in the same experimental boxes and experimental conditions as described above for bioassays to determine LC_{50} value. The treatment of a pesticide used for this bioassay is the RAD of that pesticide. The experimental boxes, after treatment of the pesticide, are left undisturbed for about 30 minutes for uniform spreading of the insecticide in the soil medium. Then age synchronized specimens are transferred into the boxes which are kept in an Environmental chamber at a constant temperature of 28 ± 0.5° C and 60-70 % moisture level. Observations were made every hour and the dead individuals were removed (OECD, 1984). The time taken to achieve 50% mortality was noted and expressed as LT_{50} of the insecticide.

2.1.3 Residual toxicity determination

Residual toxicity bioassays are also carried out with the application of RAD of the pesticides tested. A total of 15 treatment boxes are used for each pesticide. Bioassays are done with age synchronized specimensin small inert polythene boxes each containingthe test medium. The test medium is dried, ground and sieved to get a particle size of 0.25 mm before laying in the experimental boxes. Moisture level of the test soil was maintained at 60-70 % level. Recommended agricultural dose of a pesticide is then applied into the test boxes. The boxes are kept in an Environmental chamber at a constant temperature of 28 ± 0.5°C (OECD, 1984). Three boxes are sampled at intervals of 7, 15, 30 and 45 days and age synchronized specimens are transferred into the boxes. Mortality was recorded after a period of 96 h (Chakravorty, 1990). Control sets were maintained for each pesticide.

2.1.4 Filter paper contact toxicity method

Acute toxicity test is performed following the method described in the OECD (1984) guideline for testing of chemicals no. 207. This is a simple screening test to identify the toxic potential of the chemical. The test vial is a petri dish (Wang et al., 2012) of 14cm diameter and 2cm height. Round filter paper (Whatman No. 1) is cut to the suitable size and placed in such a way that sides are lined with filter paper. 5ml test solution was pipetted into each vial in order to wet the filter paper. Blank tests were performed with

5ml of deionized water. Each treatment, consists of one earthworm per vial. Adult specimens,having a standardised wet weight, are selected for testing. Specimensare washed briefly with deionized water, and are kept on moist filter paper for 3h to devoid the gut content, after which it is rinsed again with deionized water, blotted on the filter paper and placed in a test vial. An earthworm is introduced per vial and the vial is covered with plastic film that had been punched with small holes using needles. Tests were done in the dark at 28±2°C for 48 h. After 48 hours the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part.

2.2Chronic Toxicity

The chronic toxicity bioassays are done with the main objectives of assessing the physiological and biochemical stress caused to soil organisms as a result of long-term exposure of the pesticides. The physiological stress assessment is done from the life cycle parameters, like biomass change, clitellum development and cocoon production and biochemical stress was assessed from the activities of certain enzymes and tissue nutrients of the test specimens. Different sub-lethal doses of pesticides are applied.

2.2.1. Life cycle bioassay

The same small and inert polythene boxes are used to carry out the bioassays with age synchronized test specimens as described in the acute toxicity experimental procedure. Standardised weight of dry, finely ground soil (0.25 mm particle size) is being put in the experimental boxes. Soil moisture of the test soil was maintained at 60–70 % level. Different sub-lethal doses of test pesticides are applied based onthe LC50 value of the respective pesticides. In each test box, standardised number of age-synchronized adult specimens is released. Before releasing, the specimens are washed with water, blotted dry on a filter paper and the total biomass is determined. Finally, the experimental boxes are kept in an environmental chamber at a constant temperature of 28 ± 0.5!. Food is provided for the test speimens. Additional food was given when all the food in the test boxes was exhausted. The test soil was checked by weighing the test containers at weekly intervals to determine the loss of moisture and replenished if needed (OECD, 2004).

i) Biomass change

On the 28th day, the weight of the test specimens are taken to determine the biomass change and are removed from the test boxes according to the protocols by OECD (2004).

ii) Reproduction

The rate of reproduction is evaluated to determine the reproductive success of the test specimens. For this purpose, careful examination of the test soil is done with a magnifying glass and the number of cocoons/ nymphs/larvae/eggs is counted every week for four weeks. The reproductive maturity is also studied. After counting, the cocoons/nymphs/ larvae/eggs are removed from the test boxes every week and the boxes were housed inside environmental chamber. The temperature and

moisture were maintained at 28 ± 0.5°C and at 60 – 70 % (OECD, 2004).

iii) Respiration

The adult specimens removed from the test boxes on the 28th day are used to determine the rate of evolution of carbon dioxide to assess the rate of respiration by alkali absorption technique (Aira et al., 2001). Earthworms are taken on moist filter paper in a Petri dish which is covered by an inverted glass beaker. 1N KOH is used to absorb carbon dioxide evolved. An experimental control, without the test organisms, was maintained simultaneously. Rate of carbon dioxide evolution is determined by titrating the 1N KOH used to absorb carbon dioxide evolved against 0.1 N HCl and wasexpressed in mg $Co_2/m^2/h$.

2.2.2. *Biochemical assays*

On the 28th day of the experiment, adult specimens are removed from the test boxes to determine acid and alkaline phosphatase and acetyl- cholinesterase of the earthworms.

i) Acid phosphatase (Walter and Schutt, 1974)

Standardisedamount of tissue is taken and homogenized in 5 ml normal saline and then centrifuged and 0.2 ml of supernatant is taken in a test tube to which 1 ml acid buffer is added thoroughly. A blank is prepared simultaneously by adding 0.2 ml of 0.7% saline to 1 ml of acid buffer. Both the tubes are incubated for about 30 mins at 37°C. At the end of the stipulated period 2 ml of 0.1 N NaoH is added to both the test tubes andmixed thoroughly. The amount of p nitrophenyl liberated in the tissue sample mixture is determined spectrophotometrically at 405 nm in a Systronix UV visible spectrophotometer after adjusting the absorbance of the blank.A standard curve is prepared in the same manner with known amount of paranitrophenyl and the values liberated were determined from the standard curve. The enzymatic activity was finally expressed in µg pnp/mg of protein/30 min after estimation of protein content of the samples.

ii) Alkaline phosphatase (Walter and Schutt, 1974**)**

Standardised amount of tissue is taken and homogenized in 5 ml normal saline and then centrifuged and 0.05 ml of supernatant is taken in a test tube to which 2 ml acid buffer is added thoroughly. A blank is prepared simultaneously by adding 0.05 ml of 0.7% saline to 1 ml of acid buffer. Both the tubes are incubated for about 30 mins at 37°C. At the end of the stipulated period 10 ml of 0.05 N NaoH is added to both the test tubes andmixed thoroughly. The amount of p nitrophenyl liberated in the tissue sample mixture is determined spectrophotometrically at 405 nm in a Systronix UV visible spectrophotometer after adjusting the absorbance of the blank.A standard curve is prepared in the same manner with known amount of paranitrophenyl and the values liberated were determined from the standard curve. The enzymatic activity was finally expressed in µg pnp/mg of protein/30 min after estimation of protein content of the samples.

iii) Acetylcholinesterase (Ellman *et. al*., 1961)

Standardized portions from the surviving

specimensare separated to estimate theacetylcholinesterase activity. They are homogenized in 10% (w/v) 0.1 M, phosphate buffer,pH 7.5 using a homogenizer. The homogenate is centrifuged at 10,000 g for 10 min and thesupernatant was further centrifuged at 10,000 g for 10 minutes at 4° C (Remi cold centrifuge).The resultant supernatants are stored in ice and used for Acetylcholine esterase assay.Kinetic measurements are performed with acetylthiocholine iodide as the substrate. Reactions are performed in 300 µl of 0.1 M Phophate buffer, pH-8 containing-

a. 20 µl of 0.01 M DTNB (5,5'-dithio-(2-nitrobenzoic acid))
b. 20 µl 0f 0.075 M substrate
c. 10 µl tissue extracts

Contents are thoroughly mixed and absorbance was measured at 412 nm in systronics UV-Vis spectrophotometer. Substrates are continued to be added before adding of the substrate till a stable reading was recorded. After addition of the substrate, the change in absorbance is recorded for a period of 10 min at an interval of 2 min. Change in absorbance per minute is thus determined. The enzymatic activity is finally expressed in nmoles/min/mg of protein after estimation of protein content of the samples.

2.2.3. Digestive enzyme and tissue nutrient bioassay

Separate bioassays are made to determine the effects of the sublethal doses of the pesticides on the digestive enzyme cellulase, alpha-amylase, total tissue carbohydrate and proteins under laboratory conditions in natural garden soil. Ten specimens of earthworms were kept inside inert polyethylene boxes each with 192 cm2 area and containing 250g of sieved garden soil. Distilled water was added to maintain 60-70% moisture. In each of the test boxes a small Petri-dish was placed, so that the soil surface and the brim of the Petri-dish are at the same uniform level, with finely cut cashew leaf litter as food for the earthworms during the entire experimental period. The experiment is set following the procedure of open choice experiment as described by Maityand Joy, (1999). The food is contaminated with pesticide in the treatmentboxes. The whole set up is kept inside an environmental chamber and thetemperature (28±0.5°C) and humidity (67%) is maintained. The determinationof cellulase activity,alpha-amylase, total tissue carbohydrate and protein is performedon 3rd, 7th, 15th and 30th day from the setting of the experiment. The testspecimens were kept in starvation before setting of the experiment.

i) Determination of specific activity of cellulase (Sadasivam and Manickam, 2010**)**

Standardized quantity of tissue from test specimen are homogenized in 5ml of normal solution and then centrifuged to get the supernatant with enzyme extract. 0.45ml of 1% carboxymethyl cellulose and 0.05ml of enzyme extract are mixed together and incubated at 55! for 15 minutes and 0.5ml of Dinitrosalicylic Acid Reagent is mixed immediately after removing the mixture from water bath. Then the mixture is heated in boiling water for 5 minutes. While the tubes are worm, 1ml of potassium sodium tartrate is added and let to

cool to room temperature. 5ml of distilled water is added to make the volume up to 5ml. The absorbance is measured at 540 nm. The specific activity of the enzyme is expressed as mg glucose released per minute per mg protein.

ii) Determination of specific activity of á-amylase (Sadasivam and Manickam, 1992)

Standardized quantity of tissue from test specimen are homogenized in 5- 10ml of ice cold 10mM calcium chloride solution and then centrifuged to get the supernatant with enzyme extract. 1ml of starch solution and 1ml of enzyme extract are mixed together and incubated at 27°C for 15 minutes and 2 ml of Dinitrosalicylic Acid reagent is added immediately after taking out the mixture out of the water bath. Then the solution is heated in boiling water bath for 5 minutes and while the test tubes are warm 1ml of sodium potassium tartrate solution is added and cooled under running tap water. The final volume is made to 10ml by adding distilled water. The absorbance of the solution is measured at 560 nm. A unit of á-amylase is expressed as mg maltose produced during 5 minutes of incubation with 1% starch.

iii) Determination of total tissue carbohydrate by anthrone method (Sadasivam and Manickam, 1992)

Standardized quantity of tissue from test specimen are hydrolysed in test tube for 3 hourswith 5 ml of 2.5 N-HCl and cooled to room temperature. The mixture is neutralized with sodium carbonate. The final volume is made up according to the weight of the tissue then centrifuged to get the supernatant with enzyme extract.1ml aliquot is collected and 4ml anthrone reagent is added. The mixture is then heated for 8 minutes in water bath and cooled rapidly. The colour of the solution will go from green to dark green, absorbance of which is measured at 630 nm. The amount of carbohydrate present in the standardized quantity of tissue is expressed in percentage mg of glucose divided by volume of sample.

iv) Determination of total tissue protein (Lowry *et. al*., 1951)

The blue colour developed by the reduction of the phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry's method.0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard (Bovine Serum Albumin) are pipetted out into a series of test tubes. 0.5 ml of the sample extract is taken in another test tube. The volume is made up to 1 ml with distilled water. A tube with 1 ml of distilled water served as the blank. 5 ml of alkaline copper sulphate solution was added to each of the test tube, mixed well and allowed to stand for 10 minutes. Then 0.5 ml of diluted (1:2) Folin-Ciocalteau reagent was added to each of the test tube, vortexed and incubated at room temperature preferably in dark for 30 mins. The intensity of blue colour developed was measured spectrophotometrically at 660nm.

2.2.3 Determination of genotoxicty

i) Coelomocyte count method

For the study of toxicity posed by the pesticides on the test specimens in DNA level, free coelomocyte cells of the coelomic fluid are used. Coelomocytes are collected from the coelomic cavity along with coelomic fluid by a non-invasive extrusion technique described by Eyambe et al., (1991). Extracted coelomic fluid in the extrusion medium is then centrifuged in 150 X g at 4°C and cells are collected from pellets and used for all assays. The extrusion medium prepared of 5.0%ethanol and 95% saline supplemented with 2·5 mg/ml EDTA and 10mg/ml of the mucolytic agent guaiacol glycerol ether, and adjusted to pH 7.3 with 1N NaOH.

According to Muangpra and Gooneratne (2011), coelomic fluid is smeared on a set of three glass slides from each concentration. After drying the fluid, the coelomocytes are fixed with a methanolic fixative solution for differential staining of cellular components (Wright Rapid Stain). From three slides of each applied fungicide concentration, a gross of 3000 coelomocytes (1000 cells x 3 slides) are observed and tallied by a compound microscope (Olympus, CH20i) at 100X magnification in oil immersion to determine the micronuclei and binucleate cells frequency.

ii) Comet Assay

Single cell gel electrophoresis or comet assay is done to assess the level of DNA damaged in coelomocytes of the test specimens exposed tosub lethal doses of the pesticides. A standard protocol prepared by Bajpayee et al. (2019), is followed to prepare the conventional slides with isolated coelomocytes from the test specimens in 1% low melting point agarose gel (1% LMPA) on the base layer of 1% normal melting agarose gel (1% NMA). After proper cell lysis in lysing solution, slides are kept in electrophoretic chamber in alkaline electrophoresis buffer and incubated for 20 minutes. Then power supply turned on to 24 volts and adjusted the current to 300 milliamperes and the gels are electrophoresed for 30 minutes. Slides are then neutralized in neutralization buffer and stained with EtBr, and covering with coverslips viewed under fluorescence microscope to capture the images of the damaged nucleuses. The images are analysed by ImageJ software.

2.3 Molecular Toxicity Assessment

2.3.1. Mass Spectrometry (MS)

Risk assessment of chemical effects in the environment requires the understanding of the fate and behaviour of anthropogenic chemicals in natural and technical systems. The exposure data obtained by environmental chemists are in turn used to evaluate the significance of toxicological effects in organisms, as studied by environmental toxicologists. Mass spectrometry-based techniques are frequently applied to monitor the exposure or investigate the effects of chemicals, particularly their mechanism of action.

Interactions between chemicals and living organisms are governed by toxicokinetic and toxicodynamic processes (Ashauer *et al*., 2011),

and contemporary studies in both fields rely heavily on mass spectrometry (Groh and Suter, 2014). Toxicokinetics describes uptake, biotransformation, distribution, and excretion of a chemical by an organism, also referred to as absorption, distribution, metabolism, and excretion (ADME) processes (Kirla et al., 2016; Mottaz *et al*, 2017). Toxicodynamics looks at the actions of a chemical or its metabolite, carried out at the target sites where toxicity becomes manifested (Groh *et al*., 2015). Such actions could include, for example, DNA adduct formation (Madureira *et al*., 2014), oxidation of membrane lipids (Pillai et al., 2014), or binding to a nuclear receptor, which in turn could trigger gene or protein expression changes and metabolite alterations (Oliveira *et al*, 2016; Mottaz *et al*, 2017; Nestler *et al*., 2012a; Nestler *et al*., 2012b; Hidasi *et al*., 2017).

Information on effective internal organismal or tissue concentrations of chemicals and their transformation products is important for toxicokinetic modeling (Kirla *et al*., 2016) and can be obtained with the same approaches as applied to environmental compartments (Ammann *et al*., 2014; Ammann and Suter, 2016; Hidasi *et al*., 2017). Taking samples at different time points, performing depuration experiments, or carrying out non-targeted or targeted metabolite screening allows constructing time-resolved profiles of chemical uptake, biotransformation, and excretion (Kirla *et al*., 2016; Mottaz *et al*, 2017; Di Paolo *et al*., 2015; Kirla *et al*., 2018). MS has also been instrumental in obtaining information that sheds light on the internal distribution of chemicals. This can be done by measuring chemical contents in the dissected body parts or by using MALDI imaging to decipher chemical location on tissue sections (Kirla *et al*., 2016; Kirla *et al*., 2018). With regard to toxicodynamics, MS can provide data on gene expression and cellular signaling cascades, for example through looking at proteins (proteomics) and metabolites (metabolomics) (Sturla *et al*., 2014). The study of metabolomics is rapidly establishing itself in ecotoxicological research (Viant *et al*., 2017), also because it's analytical pipelines are often similar to untargeted environmental chemical analysis (Ammann and Suter, 2016; Viant and Sommer, 2013).

2.3.2. Nanotoxicology

One subfield of toxicology which has received a lot of attention in recent years is nanotoxicology. Research focused on elucidation of fate and effects of nanoparticles in humans and the environment would not have been possible without the MS advances in place (Sigg *et al*., 2014). Inductively coupled plasma MS (ICP-MS), has been routinely applied to study metal-based nanoparticles, with dedicated separation methods such as ultra filtration, used to distinguish between ionic and nanoparticulate forms (Groh *et al*., 2015; Yue *et al*., 2017).

2.3.3. Ecotoxicological risk assessment using DNA chips and cellular reporters

i) Microarray and 'in vivo' testing for risk assessment

Gene expression profiling is a unique way of characterizing the response and adaptation of an organism to changes in the external

environment. The gene profile can be characterized by (eco) toxicogenomics techniques, which can therefore be considered as a technology with high added value for risk assessment purposes (Robbens *et al*., 2007).

Currently, several strategies can be applied to investigate differential gene expression in these – genomically poorly characterized – species, such as heterologous hybridization (Lettieri, 2006; Renn *et al*., 2004) and suppressive subtraction hybridization (SSH) PCR (Moens *et al*., 2006). The recent construction of array platforms based on SSH PCR for several ecotoxicologically relevant organisms has proven to be generally applicable and has promoted the application of microarray gene expression profiling in ecotoxicology– that is, in ecotoxicogenomics (Robbens *et al*., 2007). In ecotoxicology, following exposure to a chemical, gene expression profiling using microarrays is used to address two complementary tasks: to provide information about the mechanism of action of the toxicant and to form a 'phenotypic signature' for the identification of toxic products (Moens et al., 2006). Both types of information can contribute to ecological risk assessment by identifying biomarkers of effect and biomarkers of exposure, and thus lead to being able to assess the effect upon a cell or organism and to monitor the presence of a toxicant (Robbens *et al*., 2007). Microarrays provide a snapshot view of genomic activity; a first factor involves the fact that gene expression data are meaningful only in the context of a detailed description of the conditions under which they were generated, including the state of the experimental organisms, possible perturbations and confounding factors, in addition to the microarray platform and experimental design and processing used. Therefore, an important task is to define the standards for microarray data, to facilitate the effective management, integration, interpretation and sharing of (eco) toxicogenomic data (Ankley *et al*., 2006; Boverhof and Zacharewski, 2006; Pognan, 2007). Keeping this complexity in mind, a minimum standard for information associated with microarray experiments has been defined, known as 'Minimum Information About a Microarray Experiment' (MIAME), ensuring the interpretability of the experimental microarray results and their potential independent verification (Brazma *et al*., 2001). The strategy of ecotoxicogenomics is often to compare gene expression patterns in two samples – one control and one exposed to the toxicants under study (Snell *et al*., 2003). Controls provide an implicit reference collection of 'normal' gene expression levels under a given set of conditions and variables (Robbens *et al*., 2007).

Although the growing list of reports on the application of microarrays in ecotoxicogenomics is proof of the great opportunities for ERA for chemicals, standardization alone will not suffice for a proper implementation of new technologies in ERA. The application of this technology is still in its infancy (Moens *et al*., 2006) and most data are of an exploratory or research nature. Before full implementation in ERA, effort must

be undertaken to overcome several considerations, inherent not only to gene expression data, but also to the use of many other biomarkers in ERA (Forbes *et al*., 2006).

ii) Cellular Reporters in ecotoxicology

A cellular reporter can be defined as a cell that contains a promoter and regulatory sequence in control of a reporter gene. Following interaction with a chemical compound or through a more complex signaling chain, activation of the promoter by the transcriptional regulator leads to the upregulated expression of the reporter gene, yielding an output signal that can be detected, calibrated and interpreted. Light-based and fluorescence-based reporters are the most frequently used reporters (Denison *et al*., 2004). However, many other types of reporters have been used in various studies (Haruyama, 2006; De Coen *et al*., 2004). Cellular reporters can be ranked as general, semispecific or specific (Harms, 2006; Sorensen, 2006). These different types of cellular reporters are used according to the toxicological questions to be answered. General cellular reporters often contain a constitutive or general promoter and produce a decreasing reporter signal as a result of a general toxic stress. Semispecific and specific cellular reporters produce a chemical-specific signal. Semispecific reporters are responsive to a group of related stress-inducing compounds. Well-known examples are the chemical-activated luciferase (CALUX)-based bioreporter, such as dioxin-responsive CALUX (DR-CALUX1), endocrine-responsive CALUX (ER-CALUX1) and thyroid-responsive CALUX (TRCALUX1). DR-CALUX1 responds by emitting light to all chemicals [e.g. dioxin and polychlorinated biphenyls (PCB)] that are able to activate the aryl hydrocarbon receptor (Scippo *et al*., 2004; Pliskova *et al*., 2005). Amore recent version of this reporter uses the green fluorescent protein as a reporter–chemically activated fluorescence expression (CAFLUX) (Denison *et al*., 2004). A dual reporter system has already been developed with different luciferase mutants; however, so far it has not been used in an ERA environment or for monitoring purposes (Branchini, 2007). It is clear that this step will be essential for further validation of cellular reporters for risk assessment purposes.

3. Conclusion

In conclusion the above discussed techniques play very important functional roles in both environmental chemistry and toxicology. These techniques are used in the (i) measurement of chemicals in environmental compartments and biota, (ii) identification of unknown chemicals potentially causing adverse effects in the environment, which and (iii) providence of insights into the internal toxicokinetic processes and molecular mechanisms of toxicity. Thus these methodologies have become essential in both fundamental and applied research in ecotoxicology, supporting risk assessment and management of chemicals.

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Innovative Practices of Medicinal Uses of Cultivated Garden Plants by the Peoples of Purba Medinipur District, West Bengal.

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ABSTRACT

Long being inhabitant of the district of Purba Medinipur, West Bengal, it has the scope of study and to know about the different uses of some newly introduced cultivated garden plants as medicinal uses. The medicinal uses are the new information and thus here being presented as new data as well as additional medicinal uses of these plants and plant parts as provided by some gardeners and nursery men. Of course, many of these plants are well known for their medicinal properties and uses as recorded in different literatures. All these plants are mostly the garden annuals or some are perennials. The local inhabitants or the people are considering these plants mostly for external uses for the remedial measures of their different ailments or diseases which are more commonly related to minor cut, fresh cut, injury, bleeding, pains, wounds, headache and inflammation or swelling, etc. It is, further, seen that in most cases the leaves are used as remedial purposes. The additional medicinal uses are provided for 13 plants and these are *Aerva javanica, Agave sisalana, Ageratum haustonianum, Ayapana triplinervis, Barleria lupulina, Calendula officinalis, Catharanthus roseus, Celosia argentea, Coleus hadiensis, Eryngium foetidum, Stachytarpheta jamaicensis, Tagetes erecta and Talinum portulacifolium*.Photographs are provided to facilitate the identity of these plants . Moreover, a brief description along with habit and habitat conditions are mentioned.

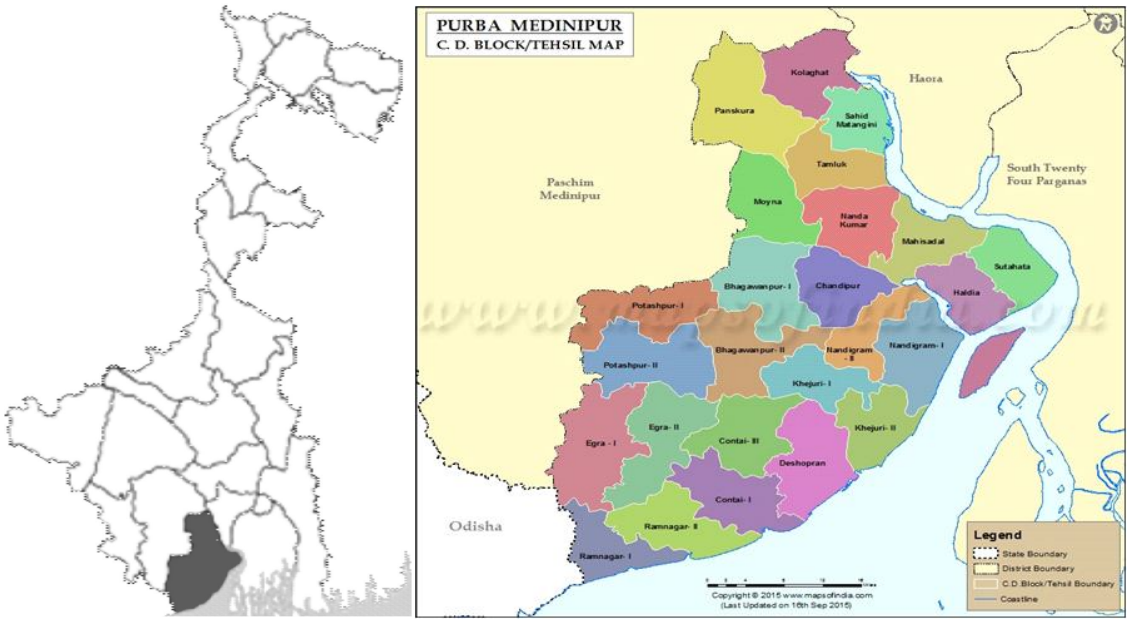
Introduction : The indigenous plants have the history of their therapeutic uses for human being as well as for the veterinary medicines due to their properties of chemical constituents having active physiological effects against different ailments. The inventory of these medicinal uses are from the time immemorial due to the effectiveness of herbal drugs to the patients who used the local therapy as satisfied with such treatment and get the remedy and relief without any bad effects. Thus the trials and errors are going on to select the suitable herbal drugs to prevent diseases and to gain healthy life span.

Now a days, besides the known medicinal plants people have the innovative practices to get more and more herbal plants for their daily uses for satisfactory healthcare. Local peoples by their trial and error are with the aim to evaluate the properties of some of the plants which are not indigenous to our country and rather introduced

and under cultivation in gardens. All these plants are available as being regularly cultivated in gardens. Thus the easy availability and evaluation both have taken up by the human being. Moreover, the local people have already made the cultivation at least without any harm and bad effects to use or apply these plants. On the other hand the plants have the beneficial effects or remedial effects as being used.The men of Purba Medinipur district, West Bengal are mostly engaged in cultivation of major crop as rice and then the vegetables and betle vine. The gardening is mostly done as a commercial basis for the production of flowers and foliages and to sell them in a commercial basis. So the nurseries are developed to raise newly cultivated plants in their gardens. Thus with the availability of these exotic plants the men have the scope to study and to use these garden plants in various ways. The acquired knowledge is, therefore, transmitted amongst the local people and gradually the men are using these plants.Most of these garden plants are exotic and many of them are used as external application as their remedial measure or treatment. A few plants are taken for internal uses. So, new inventory or the additional information of medicinal uses of some cultivated garden plants are here provided that have been known from the local people of Purba Medinipur district, West Bengal.

Objective: This study is undertaken with an idea to add some additional information about the garden plants, most of them are exotic, particularly for any medicinal purpose besides their commercial values as regular trades in the markets. This work was initiated as being known to use some plants and plant parts by the nursery men while working in the field suffering from cold and cough and particularly the head-ache and with certain cut or injury with bleeding. Thus with the visit and quiry in many of the gardens for nearly four years many of the information are gathered and aimed here to make documentation of these uses as the innovative practices of local peoples to use exotic garden plants in Purba Medinipur district, West Bengal.Besides the present documentation a details of the previous information of all these plants were also incorporated to compare the present uses and practices with that of the previous ones.

Study Area : Purba Medinipur district, West Bengal is located at the south end of the state covering an area of 4736 sq. km. situated at 87.77 East longitude and 21.93 North latitude. The district is highly populated with 1100 per sq.km. and most of the areas are with the cultivated area besides a few industrial area and small township. Besides the major cultivation crops as rice and other vegetables, there are some specific pockets where the people are with the major practice of gardening and nursery. The study area is confined to work in different major localities like Contai , Tamluk,Haldia, Chandipur, Panskura, Mayna, etc. of Purba Medinipur district, West Bengal.Many times studies had been done in the localities of Panskura and Mayna,and then Tamluk areas where there are more nurseries for regular cultivations of various garden plants throughout year. However, maximum information are



gathered from Chandipur.

The studied areas can be found in Map below.

Field Survey: No specific and regular routine survey was conducted but during the collection trip and visits to different locations of the different blocks and some private gardens and nurseries, the uses of these exotic garden annuals/perennials were known and recorded from 2016 – 2019.The informationfor medicinal uses were gathered as provided by the local people and mostly by the owners of thesegardens and nurseries, as presented in Table A. Some of these plants are very common in our residential localities of Haldia, Tamluk, Contai, Chandipur, Panskura, Mayna, etc. and the plants are also available as grown by the villagers and gardeners. Many of the plants as *Ayapana triplinervis, Aerva javanica, Calendula officinalis, Catharanthus roseus, Celosia argentea, Coleus hadiensis, Eryngium foetidum, Stachytarpheta jamaicensis* and *Tagetes erecta* are very common in these gardens.

Materials and Methods: All total 13 exotic garden plants have been studied and the information of their respective uses are hereby presented. The identity of each plant is confirmed by study of life specimen matching with different books and literatures as Bose *et al*.(1991), Anonymous (2004), Santhosha and Kar (2017), Chadha (2009), Heywood (1978), Staples and Harbst (2005) and Bose *et al*. (2006-2009).For each plant the presently accepted correct botanical name, synonyms/basionyms, if present, and the respective family names are provided.The local names in Bengali, with a few of Hindi and English are given. The native place and the distribution and occurrence are also provided for each plant. A short description, specially the vegetative features, a few floral

TABLE - A

INFORMATION GATHERED ON MEDICINAL USES FROM PERSONS OF THEIR RESPECTIVE LOCALITIES

Names of plants	Locality	Information provided by (Names of persons)
Aerva javanica *Tagetes erecta*	Contai	Mr. Kanai Ghorai
Agave sisalana *Barleria lupulina* *Eryngium foetidum* *Stachyterpheta jamaicensis* *Talinum portulacifolium*	Chandipur	Mr. Nani Gopal Maiti
Ayapana triplinervis *Catharanthus roseus* *Celosia argentea*	Haldia	Mr. Sudam Pradhan
Ageratum haustonianum *Celosia argentea* *Coleus hadiensis*	Tamluk	Mr. Gourhari Bhoumik
Calendula officinalis *Tagetes erecta* *Catharanthus roseus*	Panskura	Mr. Tapan Kumar Jana Mr. Kalyan Maiti Mr. Saroj Kumar Maiti
Calendula officinalis *Tagetes erecta*	Mayna	Mr. Adhir Chandra Sahoo

characters and the mode of propagation are given. All these information have represented in table format. Photographs are provided to facilitate the identity of the plants.

The information of medicinal uses were gathered through oral interview about the plants used for the treatment of various diseases and ailments. The preparation of medicines,systems of uses, doses, duration and other ingredients as well as applications were thoroughly known from some men and gardeners and recorded (Table A).Under observation the uses, particularly for medicinal purposes, are discussed in brief with proper references, without going through the chemical constituents and properties.

The present observations as additional information to medical uses are provided in each case as new inventory to these plants.

Observation and Results:The results of studied plants are stated below arranged in alphabetical sequence. The plant parts and their respective uses are provided along with the previous records or information of medicinal uses as mentioned in some recent literatures or books related to these plant species.

Table-1

1. Names of plants with family, local names and native country	Identifying characters of plants
Aerva javanica (Burm. *f*.) Juss. ex Schultes, Syst. Veg. ed. 15,5:565.1819. Basionym *:Irsenia javanica* Burm.*f*., Fl. Ind.217.1768 Synonym: *Aerva tomentosa* Forsk. Family :Amaranthaceae**(Pl. I,Fig. 1)** Local names: *Lal gand, Lal bishallakarani* in Bengali. Native country: South-East Asia	Perennial herb, 60-80 cm. Stems and branches terete, purple-red. Leaves simple, oblong to oblong-spathulate, 2-3 cm long and 1-1.5 cm wide, acute to acuminate, entire, hairy. Flowers small, aggregated in spike, dull white while blooming in November to March. Grows as garden escape and hedge.

Table-2

2. Names of plants with family, local names and native country	Identifying characters of plants
Agave sisalana Perrine in U.S. Senate Docum. 300:36.1836. Synonym: *Agave rigida* Mill.var. *sisalana*Englm. Family : Agavaceae.**(Pl. I,Fig. 2)** Local names: *Kiya, Keya,Tiya* in Bengali and sisal in English. Native country: West Panama, Caribbean Islands and Venezuela.	Plant shrub, almost stemless, having many rosette leaves. Leaves spiral, linear-lanceolate, 50-70 cm long and 10-15 cm wide, spinous at apex and almost entire along margin, very strong and thick, somewhat concave, bluish-green in colour. Flowers profuse in various fasciculate branches, white, usually not forming fruits but forming numerous propagules with parthenocarpic growth as sterile pentaploid (2n = 150). Flowering is less often found, blooming during November to March whenever present in case of very aged plants.

Table-3

3. Names of plants with family, local names and native country	Identifying characters of plants
Ageratum haustonianum P.Mill., Gard. Dict. Ed. 8.1768. Synonym: *A. mexicanum* Sims. Family : Compositae (Asteraceae).**(Pl. I,Fig. 3)** Local names: *Uchunti, Dochunti, Kukursoka in Bengali.* *Native country:* South-East Mexico to Central America.	Annual, much branched herb, usually 30-50 cm high, pubescent. Stems and branches terete, more compactly pubescent or hairy. Leaves opposite, simple, ovate-cordate, 5-7 cm long and 4-6 cm wide, acute at apex and serrate along margin. Flowers in homogamous capitula; florets blue with more exserted stigma. The flowers appear during October to March. Grows as garden escape, hedge.

Table-4

4. Names of plants with family, local names and native country	Identifying characters of plants
Ayapana triplinervis (Vahl) R.M. King & H. Robinson in Phytologia 20:212.1970. Basionym: *Eupatorium triplinerve* Vahl, Symb. Bot. 3:97.1794. Synonym: *E.ayapana* Vent. Family : Compositae (Asteraceae). **(Pl. I,Fig. 4)** Local names: *Ayapan in* Bengaliand Ayapana in English. *Native country:* Brazil.	Perennial, branched, decumbent herb, often forming suckers. Stems and branches often purple-green. Leaves opposite, simple, lanceolate, 8-12 cm long and 1.5-2 cm wide, acute, entire, prominently 3-veined, greenish-purple, or copper-green in colour. Flowers in homogamous capitulum (head) , borne in lax, branched terminal and axillary peduncles; florets many, purple or pinkish. Flowering during August to February. Grown as hedge plant.

Table-5

5. Names of plants with family, local names and native country	Identifying characters of plants
Barleria lupulina Lindl., Edwards's Bot. Reg. 18:t.1483.1832. Family : Acanthaceae.**(Pl. I,Fig. 5)** Local names: *Bishalyakarani, Kanta bishalyakarani, Ban-bajra-danti* in Bengali. *Native country:*Tropical Africa and Malagasy.	Plant perennial branched shrub, 1 to 1.5 m high. Stems and branches tetrangular, slightly swollen at nodes. Leaves opposite decussate, simple, linear-oblong, 10-13cm long and 1-1.5cm wide, acute at apex, entire, glabrous on both the surfaces, copper-green with reddish mid-vein, glaucous. Flowers yellow, borne in both axillary and terminal cymes, thyrsoid, with spines and compact green bracts and bracteoles. Flowering is mostly from March to August. Grows as garden plants.

Table-6

6. Names of plants with family, local names and native country	Identifying characters of plants
Calendula officinalis L. , Sp.Pl. ed. 1:921.1753.**(Pl. I,Fig. 6)** Family : Compositae (Asteraceae). Local names: *Calendula* in Bengali, *Zergul* in Hindi, Scotch Marigold, Pot Marigold or even Marigold in English. *Native country:* Mediterranean region.	Plant a small annual herb, usually 50 cm. high, finely hairy. Stems terete. Leaves simple, somewhat rosette, spathulate, 10-16 cm long and 3-6 cm wide, obtuse at apex, entire along margin, dull green in colour, juicy. Flowers borne in heterogamous capitula (head); involucral bracts many; ray florets many, variously coloured from yellowish to orange, saffron or even light reddish. The plant flowers during January, February as garden annual and is propagated by seeds (cypselar fruits).

Table-7

7. Names of plants with family, local names and native country	Identifying characters of plants
Catharanthus roseus (L.) G.Don, Gen, Syst. 4:95.1837. Basionym: *Vinca rosea* L. , Syst. Ed. 10:944.1759. Synonym: *Lochnera rosea* (L.) Reichb.**(Pl. II,Fig.1)** Family Apocynaceae. Local names: *Nayantara* and *chirabasanta* in Bengali, *Sadabahar* in Hindi, *Nityakalyani* in Sanskrit, Periwinkle, Old maid, Cayenne jasmine in English. *Native country:* Malagasy.	Plant an erect branched undershrub or herb, attaining to 60 cm. high. Stems and branches tetrangular, glabrous. Leaves opposite-decussate, simple, oblong, 4-7 cm long and 2-2.5 cm wide, obtuse to acute at apex, entire, glabrous and glossy-green. Flowers usually in pair, axillary cyme, with 2-2.5 cm long corolla tube and 1.5-2 cm long twisted and then spreading corolla lobes (salver-shaped), mostly pinkish, to variously pinkish red and white coloured. Fruits a pair of follicle, narrow, 3-4 cm. long, few-seeded.Flowering is throughout the year.

Table-8

8. Names of plants with family, local names and native country	Identifying characters of plants
Celosia argentea L. , SP.Pl.ed.1:205.1753. Family : Amaranthaceae.**(Pl. II,Fig. 2)** Local names: *Morog phul, Mayur sikha* in Bengali, Foxtail Amaranth in English. N*ative country:* Tropical Africa (Staples and Herbst, 2005).	Erect branched annual herb, to 1 m. high or sometimes even more longer. Stems and branches terete-striate. Leaves alternate, simple, lanceolate to ovate-lanceolate, very variable in size, to 15 cm long and 10 cm wide, acute, entire. Flowers numerous, borne in both terminal and axillary spikes; light pink to reddish coloured, with scaly perianth. The plant flowers during winter but may be continued to April.Plants of both tetraploid(2n=36) and octaploid(2n=72). Grown in garden.

Table-9

9. Names of plants with family, local names and native country	Identifying characters of plants
Coleus hadiensis (Forssk.) A.J.Paton, Phyto-Keys 129:54.2019. Basionym: *Ocimum hadiensis* Forssk Fl. Aegypt-Arab.109.1775. Synonym: *Coleus barbatus* Benth.; *C. forskohlii* Briq.; *Plectranthus barbatus* (Benth.) Spreng.**(Pl. II,Fig. 3)** Family : Labiatae (Lamiaceae). Local names: *Coleus, Pathar chur* in Bengali. N*ative country:* Western Asia, Arabia.	Plant a perennial, aromatic, decumbent, branched herb, villous throughout. Roots tuberous, thick, found in fascicles, 10-15 cm long and 1.5-2 cm thick, fusiform or straight, aromatic, fleshy. Stems and branches tetrangular. Leaves opposite-decussate, simple, cordate, 8-12 cm long and 5-8 cm wide, acute, crenate, very thick, fleshy, villous. Flowers bluish, irregular, bilabiate, borne in verticillaster in a terminal inflorescence rachis. Flowering August to April. Grown as garden plant.

Table-10

10. Names of plants with family, local names and native country	Identifying characters of plants
Eryngium foetidum L. , Sp.Pl.ed.1:232.1753. Family : Umbelliferae (Apiaceae**).(Pl.II,Fig. 4)** Local names: *Bilayeti dhaney, Bilati dhaneypata*in Bengali and Eryngo, Culantro, Saw Leaf Herb, Thorny coriander in English. N*ative country:* Tropical South America and Caribbean Islands. Now cultivated in Asia including India (Devi *et al*., 2016) and also cultivated in kitchen garden of West Bengal (Santhosha and Kar, 2017).	Plant more or less dichotomously branched, decumbent, aromatic herb, often biennial, perennial. Stems and branches terete, glabrous. Leaves radical ones rosette, few, oblong, 12-18 cm long and 1.5-2.5 cm wide, acute, serrate-dentate and spinescent, somewhat soft, light green; cauline leaves ovate-lanceolate to oblong-lanceolate, smaller, to 3 cm long and 1 cm wide. Flowers in small sessile axillary heads, many, very small, whitish green. Fruits mericarp, small. Plant flowers during August to December.Grown in kitchen garden.

Table-11

11.Names of plants with family, local names and native country	Identifying characters of plants
Stachytarpheta jamaicensis (L.)Vahl., Enum.1:206.1804. Basionym: *Verbena jamaicensis* L., Sp.Pl.ed. 1:19.1753. Synonym: *Stachytarpheta indica auct.non* Vahl; *S. urticaefolia* (Salisb.) Sims.**(Pl. II,Fig. 5)** Family : Verbenaceae. Local names: *Jarbas, Jarbo* and *Jalageli* in Bengali (Bangladesh) and *Kalobhramar* in Bengali (Purba Medinipur), Devil's coach whip, Blue make weed, Brazilian Tea in English. N*ative country:* Trinidad and Tobago (Singh, 2006)	Perennial branched undershrub, attaining usually to 1 m or so. Young branches tetrangular. Leaves opposite, simple, ovate, 3-4 cm long and 2.5-3.5 cm wide, acute, serrate, rugose with depressed veins, deep green, petiolate. Flowers many, borne in 20-30 cm long slender inflorescence rachis as spike of both terminal and axillary; bluish, irregular, somewhat bilabiate. Plant flowers during October to April. Grown as garden escape.

Table-12

13. Names of plants with family, local names and native country	Identifying characters of plants
Talinum portulacifolium (Forssk.) Aschers ex Schweinf in Bull. Herb.Boiss.4, App.2:172.1896. Basionym*: Orygia portulacifolia* Forssk., Fl.Aegypt-Arab.103.1775. Synonym: *Talinum cuneifolium* Willd. Family : Portulacaceae.**(Pl. II,Fig. 7)** Local names: *Alak pui, Pnui, Bilati pui* (*pnui*), *American palang, Australian pnui*in Bengali. N*ative country:* Africa.	Succulent branched perennial, mostly decumbent, with somewhat fleshy tuberous roots. Stems and branches terete, fleshy and soft. Leaves alternate, simple, spathulate, oblanceolate to ovate, 5-10 cm long and 2-3 cm wide, obtuse, entire along margin, fleshy, somewhat mucilaginous with prominent midvein, glabrous, petiolate. Flowers few, pinkish to rose-coloured, borne in axillary pedunculate branched cymes, 5-merous; petals 5, spathulate, elliptic. Fruits globuse to ovoid capsule, many-seeded. Flowering and fruiting both occur throughout the year, more frequent from February to May. Grown as pot plant.

Table-13

12. Names of plants with family, local names and native country	Identifying characters of plants
Tagetes erecta L. , Sp. Pl.ed.1:887.1753**. (PI. II,Fig. 6)** Family : Compositae (Asteraceae). Local names: *Gandha, Ganda, Chutkey gandha, Ganda phul* in Bengali and French marigold, African Marigold, Aztec in English. N*ative country:* Africa.	Erect, branched, aromatic annual herb, often with plenty of adventitious roots towards the basal portion of the stems. Stems and branches slightly angular-ribbed. Leaves opposite, pinnatisect and much segmented, 10-13 cm long and 5-8 cm wide, acute, serrate dentate along margin, prominently veined, green to rare often purple-green in colour. Flowers in capitula (heads); both homomorphic and heteromorphic; variously coloured as yellow, saffron to orange, or red, etc. Fruit cypsela. Flowering December to March,now throughout the year for some cultivars(Heywood., 1978).

1. Aerva javanica (Ref. Table No. 1)

The plant is reported to use to stop bleeding of fresh cut as mentioned in the Medicinal Plant Resources of South Bengal (Santhosha and Kar, 2017). It is also mentioned by Mabberlay (2018) as used for local medicinal purpose.

In the present observation the juice of fresh leaves is used to stop bleeding of fresh or immediate cut and the leaf paste is also used as poultice to relievethe pain and for the remedy of any inflammation of this cut. The poultice is usually continued with fresh preparationtill the complete remedy of this cut or wound. The haemostatic activity of the leaves is now more popular with the local people.

Another species *Aerva lanata* (L.) Juss.exSchultes (local name-*Chaya*) is very well known for its uses in kidney disease (Mabberley, 2018) and also to treat cough, sore throat, diabetes, headache, lithiasis (Anon., 2004) and the roots as diuretic, anti-inflammatory, anthelmintic effects (Prasad *et al*., 1986). Itis well known as *Bhadra*, in Ayurvedic system, long been used as anti-inflammatory (Sivarajan and Balachandran, 1994; Prajapati *et al*., 2003) and consideredas astringent, bitter, cooling,emollient, vermifuge, suppurative, diuretic and lithontriptic (Prajapati *et al.*, 2003; Singh, 2006).

2. *Agave sisalang* (Ref. Table No. 2)

It is already mentioned in the report of Medicinal Plant Resources of South Bengal (Santhosha and Kar, 2017) that the three species as *Agave americana, A. cantala* and *A. sisalana*

have some medicinal uses.

A. americana L. (commonly known as Century plant, American aloe) where both roots and leaves are used to treat different diseases(Anon, 2004; Santhosha and Kar, 2017).The roots of *A. cantala*Roxb. ex Salm-Dyek (known as Cantala, Manila magney, Bombay aloe) is used as diuretic and diaphoretic and the leaf juice is applied to bruises (Santhosha and Kar, 2017).In case of *A. sisalana* Perrine (known as Sisal – named after Mexican sea port) it is mentioned that the leaf juice is used to treat low blood pressure and the juice of roasted leaves used in swollening of throat of cattle along with black pepper (Santhosha and Kar, 2017).

In *A. sisalana* it is presently noted that the roasted leaf juice is used as massage in slightly warm condition for the remedy of muscular pain of human being and to relief from inflammation.This treatment is done specially whenever there is severe mascular pain due to heavy work along with some swelling as well as inflammation of the muscles of legs or hands. This practice is donefor 2-3 times in a day and continued for usually 3-5 days till remedy or relief. This observation is somewhat alike with the earlier findings asdone in case of cattle. Sometimes, the leaves of *A. americana* are used for the same purposes without knowing its proper identity as the leaves look alike.

3. *Ageratum haustonianum* (Ref. Table No. 3)

Ageratum L. is an American genus is now pantropic in distribution particularly for its two species *A. conyzoides* L. (known as billy-goat weed, blue top) and *A. haustonianum* P.Mill. It is a widely cultivated ornamental edging plant with blue and the cultivars with white and pink florets(Chadha, 2009) in condensed capitula (heads). It contains precocenes 1& 2 (based on 2,2-dimethyl-cromene)which interfere with juvenile hormone activity and cause precocious metamorphosis of insects and an oil very toxic against *Fusarium* wilts of *Cajanus cajan* (Mabberley, 2018). *Ageratum conyzoides* L., known as *Uchunti, Dochunti, Kukursoka* etc. in Bengali, is used medicinally (Santhosha and Kar, 2017; Prajapati *et al*. 2003; Anon., 2004).Being looked alike with *Uchunti,A. haustonianum* P.Mill. is used by the local people and the leaf juice is applied for the washing or cleaning of wounds and also the skin-diseases.

4. *Ayapana triplinervis* (Ref. Table No. 4)

Plant is propagated through vegetative means.The whole plant is considered as medicinal one as used in different diseases (Santhosha and Kar, 2017). Due to the presence of various chemical constituents this plant is variously used in Ayurvedic treatments(Prajapati *et al*, 2003).It is also used as medicinal tea and cultivated in Brazil (Mabberley, 2018).

In the present finding as additional report, the local peoples use the leaf juice to stop bleeding of any fresh cut and also use the leaf paste as poultice for the remedy of the cut or wound including the remedy of any pain and any inflammation due to cut.Moreover, the fresh leaf juice is taken by the local peoplefor the remedy of blood dysentery with the doses usually one teaspoonful twice in a day and continued for 3-5 days. The leaf juice is also taken in case of anaemic condition of any person. The opinion

of the local people is in support of the use of this plant mainly to overcome the iron deficiency of man.

5. *Barleria lupulina* (Ref. Table No. 5)

It is already reported that the crushed leaves are applied to fresh cut, wounds and ulcers as a haemostatic to stop bleeding (Santhosha and Kar, 2017). The present study also supports this previous finding.

Moreover, it is seen that the poultice of fresh, crushed leaves or paste of fresh leaves is kept as bandage for the remedy of pain and swelling of the cut. Poultice is done forusually 3-5 days with the fresh leaf-paste and is often continued till remedy and relief of pain.The fresh leaf juice is also used for the washing and cleaning of any type of wounds and even the skin diseases of human being.Of course, Mabberley (2018) has reported that both the leaves and roots are chewed against toothache in Mauritius and the aquous extract of leaves with bright red mid-vein works wounder as local anticeptic on wounds and helps in healing to regain natural condition of skin with the remedy of wounds or cuts as stated by Sinha and Sinha (2001).

The locally applied Bengali name *Bishalyakarani,* quoted from the Ramayana, is also often used to some other plants which have the capability to stop bleeding of fresh cut as for the plants like *Justicia gendarussa*Burm. *f.* (syn.: *Gendarussa vulgaris* Nees) which is also known locally as*Jagatmadan* and *Vetaghni*. So to distinguish as well as to characterise this property of hoemostatic to stop bleedingthis local name *bishalyakarani* is often used prefixing another distinctive features of the plants as *Lal bishalyakarani* for *Aerva javanica* and *Kanta bishalyakarani* for *Barleria lupulina* etc. *Justicia gendarussa* has the same property to stop bleeding of fresh cut (Santhosha and Kar, 2017) which is also growing as hedge plant in gardens.

6. *Calendula Officinalis* (Ref. Table No. 6)

Plant is an already established drug due to the presence of active principal chemicals – calendulin, calenduloside A – H (saponins) used for the remedy of many diseases, as stated in the review papers by Mehta *et al*. (2012) and Verma *et al*. (2018). Varlijen (1989) had reported that due to presence of calenduloside A it acts as anti-infectious diseases. it is used in fever in Portugal (Mabberley, 2018). It is also reported to relieve muscle spasms, to prevent haemorrhage and to help the healing of wounds, also used as astringent, detoxifying agent and mild oestrogenic (Prajapati *et al*. (2003).The plant is used in the treatment of ulcers, both internal and external, and open sores and an ointment made of flowers is considered to be useful for skin complexions (Singh *et al*., 1983).

The local peoples use the juice of florets and the leaves for the remedy of headache and rub over the forehead and take the smell of fresh crushed florets and leaves. The paste and juice of fresh florets are also used for skin diseases, wounds and fresh cut to stop bleeding. In case of any burn the paste of florets and the paste of the rhizome of turmeric (*Curcuma longa* L.) are mixed together and use as poultice for the remedy of the burn. Thus the antiseptic and anti-infectious properties are well noticed by the local men through their experiences.

7. *Catharanthus roseus* (Ref. Table No. 7)

The whole plant is medicinally used with 80 named alkaloids and notably vincristrine and others have returding effect on progress of leukaemia discovered when tested for alleged effects in diabetes and in Hadgkin's Disease (Mabberley, 2018).The medicinal uses are now various as recorded by Santhosha and Kar(2017), Prajapati *et al*., (2003) and Anon., (2004).The juice of the leaves is taken to reduce highblood pressure, to treat diabetes, blood dysentery, in griping pain of babies, to treat piles and root paste to treat fever (Anon., 2004).Pal and Jain (1998) had stated that the people of Lodha community uses the root paste of this plant for the curing of septic wounds, fever and the leaves for dysentery and the Santals use seeds for the treatment of epilepsy and latex for scabies and wounds and the leaf paste for piles.

It is seen that local people of this district believe that the white flowers have the capability to reduce sugar lebel of the blood and can help to control diabetes. So, adult people takes one white flower every morning and chewed to reduce the diabetic disease. Although it was mentioned that the plant has the capability for the remedy of diabetes. However, the results are not verifiedor tested for confirmation. Ghani (2003) had mentioned that the plant is used in treating diabetes in Bangladesh. So also by Sinha and Sinha (2001) had stated that the plant is traditionally used for the treatment of diabetes.

8. *Celosia argentea* (Ref. Table No. 8)

Although it is a garden ornamental plant, it has some medicinal properties with the presence of several acids, alkaloids, sterols and nitrogen containing anthocyanins, lathosterol and its derivatives (Anon., 2004). The flowers are astringent, used in diarrhoea and in excessive menstrual discharges (Anon., 2004).The seed is demulcent and is useful in painful micturition, cough and dysentery (Anon., 2004). It is used as aphrodisiac, diuretic, useful in blood disease and mouth sores, for clearing the vision and eye disease

(Singh *et al.*, 1983; Prajapati *et al.*, 2003; Santhosha and Kar., 2017). The roots are chewed in empty stomach, twice daily, as a pain killer (Anon., 2004; Santhosha and Kar, 2017).

It is further reported that plant shows good antilithiatic activity preventing stone formation and to remove the stones as done in experimental rats (Dubey *et al*., 1982).

Presently it is found that the leaves are taken and consumed by local people as vegetable as like different 'amaranthus'. It is considered to provide nutrients, regain appetite and to prevent anaemic condition.

9. *Coleus hadiensis* (Ref. Table No. 9)

The whole plant is used as expectorant, emmenagogue and diuretic (Santhosha and Kar, 2017). The tuberous roots are used to treat hypertension, glaucoma, asthma, congestive heart failures, metastatic condition, thrombosis (Prajapati *et al*., 2003), alsoas spasmolytic and to treat constipation. The decoction of the plant is used as tonic and to treat worms. The leaf paste is applied on boils, eczema and skin diseases. The leaves are also used for the treatment of intestinal disorders. The plant is traditionally used by the ethnic people to cure

cardio-vascular diseases and to treat high pressure, abdominal colic, insomnia and memory loss, etc. (Sinha and Sinha, 2001).

The uses of the plants are known to activate hormone-sensitive adenylate cyclase and to act as potent hypotensive agent and for the treatment of glaucoma (Bhakuni, 1990; Sinha and Sinha, 2001). Mabberley (2018) had reported that the infusion of plant is used as lice-remover in Uganda and due to presence of diterpene (forskolin) it is a potential drug for hypertension, glaucoma, asthma, etc.

The present findings reveal that the local peoples use the aromatic leaves variously as the fresh leaves are rubbed over forehead to get relieve from headache, cold and cough; paste of fresh leaves used as poultice to relieve pain and inflammation of body muscles and the fresh leaf paste rubbed on chest to get relieve from cold, cough, bronchial trouble, asthma, etc. The fresh leaves are often used by mixing with the fresh leaves of tulsi (*Ocimum tenuiflorum* L.=*O. sanctum* L.) and rub then over forehead to get relieve from headache as more effective and more quicker.

10. *Eryngium foetidum* (Ref. Table No. 10)

It is a very popular one as often grown in kitchen gardens for the uses of aromatic leaves as a substitute of the leaves of Coriander (*Coriandrum sativum* L., family – Umbelliferae) during off season and thus the local name in Bengali is applied as of coriander smell and flavouring materials of culinary use.Eryngo is variously used as culinary herb(Devi *et al.,* 2016 ; Santhosha and Kar, 2017). There are also many reports of its traditional uses as medicine, anti-inflammatory, analgesic, anti-convulsion,anti-clastogenic, anti-parasitic, anti-oxidant, antimicrobial, antibacterial, etc. (Devi *et al*., 2016; Singh *et al*., 2014).With different vernacular names as cilantro, fitweed, ngo-gai,thai coriander - leaves are taken as raw or steamed, pickled in Sikkim and as culinary herb in West Indies (Mabberley,2018).The review work on this plant *Eryngium foetidum* had mentioned various economic uses (Devi *et al*., 2016) including the medicinal properties.

The present findings on this plant are also of various uses as the rosette leaves are often used in curries while cooking and are made into paste to prepare 'chutney'. The fresh leaves are used as anti-vomiting purpose for children. The fresh leaves are rubbed on forehead to get relieve from headache and rare often to get remedy from cold and cough.All the local uses are not mentioned in the earlier report including the review work (Devi *et al*., 2016; Santhosha and Kar, 2017).Of course, a drug for the treatment of arthritis and skin diseases has already developed in which the essential oil derived from this plant is one of the main components (Singh *et al*., 2014).

11. *Stachytarpheta jamaicensis* (Ref. Table No. 11)

According to Singh (2006) in modern research the plant is used for antibacterial property.The whole plant is reported for some medicinal purposes and the leaves are used for cardiac troubles and to treat ulceration of nose (Santhosha and Kar, 2017).Ghani (2003) had added some more information for the medicinal

uses of this plant in Bangladesh.

Local peoples of Purba Medinipur district cultivate this plant in gardens and use the fresh leaf juice for the remedy of the pain, irritation and inflammation due to stinging of insect bites. Paste of fresh leaves is also used for few days, if required, to get relieve from pain. The insects are usually considered as any ants, red ants, bumble bee, honey bee and small spiders, etc. The juice of fresh leaves is also used to wash any wound of the body as considered with the antiseptic property of this plant.

12. *Tagetes erecta* (Ref. Table No. 12)

The whole plant and the leaves of *Tagetes erecta* are considered as medicinal and traditionally used in bleeding and menorrhagia as haemostatic activity (Singh, 2006).The presence of aromatic oil and the alkaloids find applicable against rheumatism, cold, bronchitis, hypotensive, spasmolytic, anti-inflammatory as well as purgative (Singh *et al.*, 1983).In Bangladesh this plant is also much used medicinally as reported by Ghani (2003).

The infusion of plant is used in rheumatism, colds and bronchitis. The juice of fresh leaves and flowers are considered as emmenagogue and used to cure bleeding piles and purifies blood. Leaves are also used in the treatment of kidney troubles, muscular pains and also applied to boils and carbuncles. The root extract is also used as laxative.

The present finding is that the local peoples of Purba Medinipur district use the fresh leaf juice to stop bleeding of fresh cut. They also make poultice of the paste of fresh leaves for the relief of pain and inflammation due to this cut and continued till remedy. The juice of fresh leaves is often used as nasal drop to stop bleeding from the nose that happened during high warm season as well as during winter season with heavy cold for the children and others. Sometimes the juice of fresh leaves of *Cynodon dactylon* (L.) Pers. (Poaceae) is also mixed together to promote better result. The haemostatic activity of the leaves is more popular among men. The juice of fresh leaves is also taken as one teaspoonful twice in a day for the remedy of anaemic condition and is continued for 3 to 5 days or till remedy.

13. *Talinum portulacifolium* (Ref. Table No. 13)

It is a cultivated garden plant, grown along borders in almost all the tropical climatic condition and propagated mostly by vegetative means with the fragments of lower branches, less often by seeds.It is used as avegetable with fleshy stems and leaves. In addition to its culinary use it possesses some medicinal importance as the whole plant is used as an aphrodisiac (Santhosha and Kar, 2017).

Local peoples , although taken this plant *T. portulacifolium* as vegetable, cooked and eaten just like *Basella alba L*. (Basellaceae) but they often considered this plant as a laxative one. Peoples believe that this vegetable is helpful against constipation, can increase appetite and the substitute of many of the minerals available from different other vegetables.

Discussion and Conclusion: Most of the rural population are still dependant on the available medicinal plants growing around their locality for their everyday uses for the remedial measures of their ailments or diseases. The

people are, although well known about the herbal drugs of the indigenous plants but they are not much aware about the exotic plants and their respective medicinal properties. With the local communication and their own inventorization the further uses of other unknown plants as medicine were developed by themselves. Moreover, by trial and error they have selected these to use as they have got remedial results without any harmful effects.

The amount, time, doses and duration of uses were also standardized by themselves for proper remedial measures as well as benefits too.Thus the people are in success by the choosing of medicinal uses of cultivated garden plants which are not indigenous to our country. The alternate or substitute materials are always beneficial as that are easily available at hand and also of satisfactory amount of requirement.

Amongst the studied 13 plants only *Agave sisalana* is a woody plant. Similarly *Barlaria lupulina* and *Stachytarpheta jamaicensis* are of shrubby habitat. All the rest plants are herbaceous. Only a few plants as *Ageratum haustonianum* and *Calendula officinalis* are available in a particular season of the year. Otherwise all other plants are available throughout the year. Out of these 13 plants the leaves are used for all except *Catharanthus roseus* where the flowers are eaten. In case of three plants as *Celosia argentea*, *Eryngium foetidum* and *Talinum portulacifolium* the leaves are takenas vegetable and eaten after cooking. Thus the maximum uses are of external, not internal. Moreover, in maximum cases the juice and paste of fresh leaves are used to stop bleeding of fresh cut and taken for the remedy of headache, cold and cough. So, the mode of application is almost external. The records of the various uses of these plants are supportive based on the earlier evidences of these plants as recorded in respect of their properties and uses. So by trial and error the peoples are able to gain knowledge of using a new plant for new medicinal application.

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Plate I Figures: 1- *Aerva javanica*, 2- *Agave sisalana*, 3- *Ageratum haustonianum*, 4- *Ayapana triplinervis*, 5- *Barleria lupulina*, 6- *Calendula officinalis*

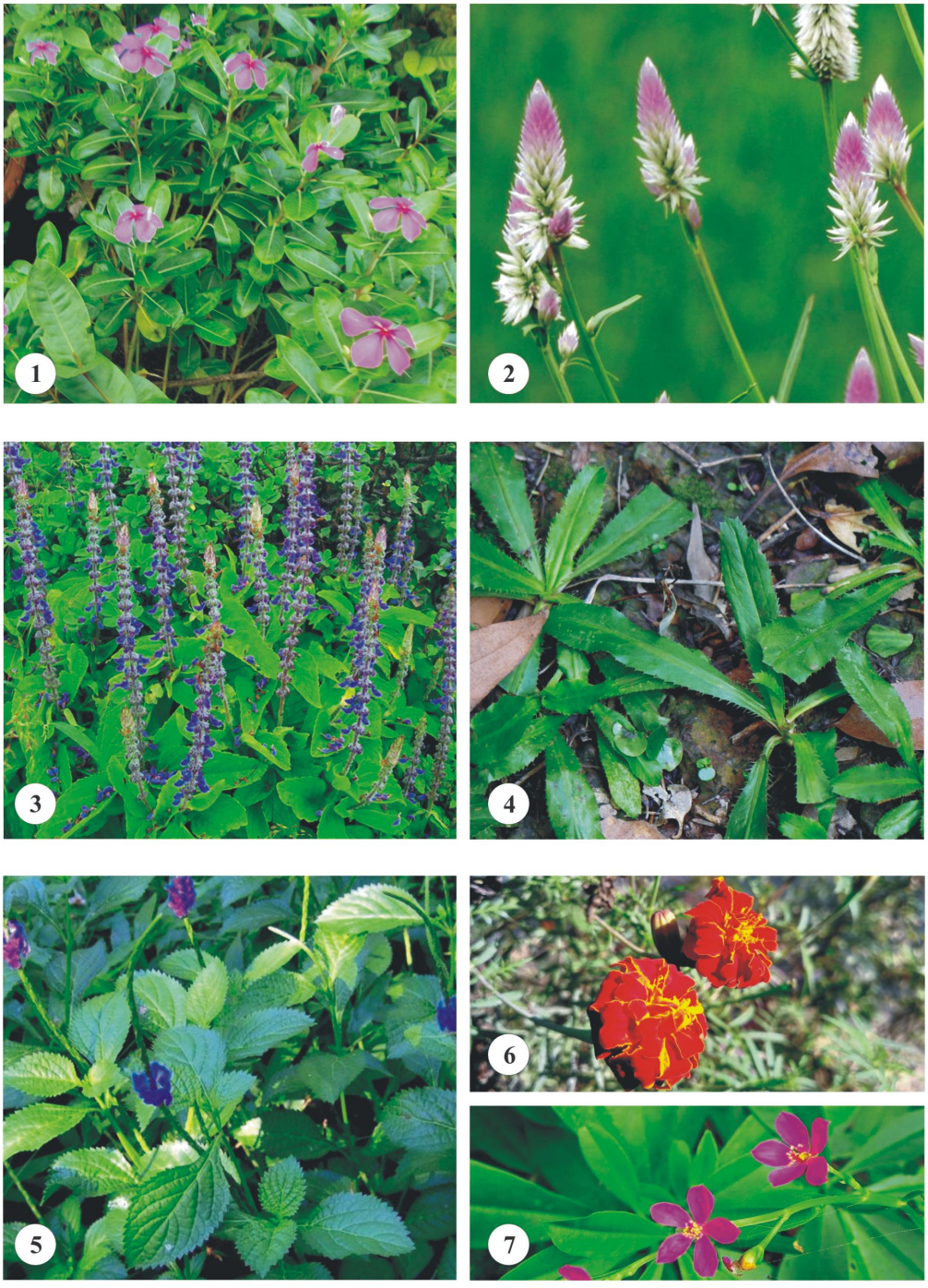

Plate II Figures: 1- *Catharanthus roseus*, 2- *Celosia argentea*, 3- *Coleus hadiensis*, 4- *Eryngium foetidum*, 5- *Stachytarpheta jamaicensis*, 6- *Talinum portulacifolium*

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Plate III. Images of author at various nurseries and garden: Fig. 1,2,3– At Malay Nursery, Panskura Fig. 4,5– At Arupam Nirupam Nursery, Panskura, Fig. 6- Author with Mr. Kalyan Maiti at Arupam Nirupam Nursery, Fig. 7- At one of the private gardens, Chandipur Fig. 8- At Sreejani Nursery, Panskura, Fig. 9- At Krishna Nursery, Panskura.

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Bivalve haemocyte based inexpensive biomarkers in aquatic environment monitoring: scopes and challenges

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ABSTRACT

The aquatic environment of this planet is constantly being challenged by pollutants from known and unknown sources which render threat to its biodiversity and bioresource. These natural waterbodies are densely populated with molluscs of diverse species, including bivalves. Bivalves are recognised as sentinel species in major global environment biomonitoring programme owing to their ability to accumulate aquatic pollutants from surrounding environment. Development and deployment of inexpensive biomarkers based on the circulating haemocytes of these molluscs is a popular and reliable biomonitoring approach, widely adopted by the western world.Data on haemocyte density profile,haemocyte functions, like, lysosomal stability, phagocytic efficiency, generation of reactive oxygen/nitrogen intermediates and haemocyte nuclear abnormalities are documentedto assess the species-specific reactions to pollutants.Such data is consulted to formulate effective environment biomonitoring and bioremediation management strategies against potential and actual xenobiotic insults. The present review work focuses on environmental monitoring against aquatic pollution with the help of haemocyte profile of bivalve as biomarker. It further reviews the scope of deployment of the concept for effective and sustainable bioremediation.

Introduction

Pollution is a major threat to the aquatic habitats causing the massive degradation of the biodiversity and bioresource(Naiman and Turner, 2000; Jackson et al., 2001; Malmqvist and Rundle, 2002; Rachel, 2002). The aquatic ecosystem throughout the world is experiencing dynamic stress mediated by diverse pollutants of natural and anthropogenic origin. It has attracted global attention which has escalated the efforts to monitor the environmental conditions through the development and deployment of selective biological and ecological measurements, or indicators. Biological indicators are thus considered as useful means to obtain effective information about the condition of an ecosystem. Bivalves are common aquatic invertebrate organisms widely considered as bioindicators in environment monitoring (Sanders, 1993; Chakraborty et al., 2012; Burgos-Aceves and Faggio, 2017). As filter feeders, these species are known to be ideal indicators of chemical contamination (Fournier et al., 2001; Guidia et al., 2010). Filter-feeding bivalves can accumulate chemical contaminants in tissues,

several times higher than those in the waterbody (Chakraborty et al., 2010; Bolognesi and Fenech, 2012). Chemical analysis of bivalve tissues can thus be used to describe contamination profiles for different sites (Al-Subiai et al., 2011; Politakis et al., 2018). This exposure monitoring approach was used in northern Europe and the United States under the International Mussel Watch Program (National Academy of Sciences, 1980), and continues as the National Mussel Watch Program conducted by the National Oceanographic and Atmospheric Administration. The measures of bivalve mollusc defence activities, such as haemocyte density, lysosomal stability, phagocytic activity and production of cytotoxic molecules have potential to be established as indicators and appear to be responsive to xenobiotic insults in the aquatic environment (Oliver and Fisher, 1999; Nicholson, 2001; Sauve et al., 2002;Guidia et al., 2010;Patetsini et al., 2013). It has been widely accepted that the measurement of physiological and biochemical responses of individual bivalves may be used as indicators of the health of the larger population and community (Bayne et al., 1980; Guidia et al., 2010). Such measurements are termed 'biomarkers' (McCarthy and Shugart, 1990; Huggett et al., 1992; Patetsini et al., 2013) and the overall impact of a specific pollutant on multiple biomarkers have been designated as the integrated biomarker responses (IBRs) (Beliaeff and Burgeot, 2002; Cao et al., 2018).

Bivalve Haemocyte

Haemocytes are the cells of the circulating haemolymph present in the haemocoel of the molluscs. In bivalves, the internal defence system is based on the structural and functional integrity of haemocytes which display phagocytic and microbicidal activities (Cooper and Knowler, 1992; Burgos-Aceves and Faggio, 2017). They act as the major immune effector cells (Cheng, 1977; Adema et al., 1991b) and mediate non-self phagocytosis that provides natural immunity in the bivalves (Lopez et al., 1997a,b). They also remain associated with a variety of physiological and pathological functions including nutrient transport, digestion, wound and shell repair, internal defence as well as excretion (Cheng, 1981; Bayne, 1983; Fisher, 1986;Glinski and Jarosz, 1997). In molluscs, bivalves in particular, haemocytes represent the major component of their immune system although their types (Figure 1)andspecific functions are not fully understood (Table 1). However, substantial information is with haemocytes of bivalves (Cheng, 1984; Adema et al., 1991 a,b; Jing and Wenbin, 2003). Cheng (1975) has demonstrated that apparently different types of haemocyte (hyalinocyte or granulocyte) may actually represent the same type of cell during different functional or maturational stages. However, it is not clear if the variety of haemocytes described in the literature represents distinct cell lineages, or due to the differences in the maturation and/or physiology of the haemocytes, or variations in the techniques being applied (Cheng, 1975). Bivalve haemocytes are believed to be responsible for the transport of contaminants from the organ of entry (e.g. gill, mantle, digestive gland) to the kidneys or other tissues where detoxification

or accumulation may occur (Pirie et al., 1984). Alterations of the immunosurveillance have been reported for bivalve molluscs exposed to metals (Cheng and Sullivan 1984; Pipe et al.,1999) and xenobiotics (Fries and Tripp, 1980; Beckmann et al., 1992; Cimaet al.,1998). It has been established that the efficiency of haemocytes may be affected by environmental contaminants (Anderson et al., 1988; Canesi et al., 2003). Xenobiotics may alter functional profiles in molluscan haemocytes, such as phagocytosis (Fries and Tripp, 1980; Anderson, 1988; Cimaet al., 1998), lysosomal enzyme activity and lysosomal membrane stability (Lowe et al.,1995; Grundy et al.,1996; Nicholson, 2001; Sauve et al., 2002; Guidia et al., 2010; Patetsini et al., 2013). Consequently, toxic effects on haemocytes potentially affect the survival of these animals. Haemocytes act as the major immune effector cells in invertebrates including molluscs (Cheng, 1977; Adema et al.,1991b). Characterisation of haemocyte function is an important step towards understanding their immune capacity and its potential failure during toxic exposure and disease development.

Haemocyte Diversity and Count

Morphological analyses and functional consequence of molluscan blood cells under toxic exposure is poorly understood. Leydig (1850) studied circulatory haemocytes of mollusc and provided the basic information in understanding the function of haemocyte in healthy state, disease condition and toxic exposure. An ideal analysis of haemocyte population dynamics involves the simultaneous approach to a series of parameters throughout the animal's life, namely, total haemocyte count (THC) and differential haemocyte count (DHC) (Shapiro, 1979; Arnold and Hinks, 1976; Liu and Zhao, 2018). Marked variations in the density of haemocytes may be related to irregular haemocyte release from haemocytopoietic organs into the open circulation (Crossley, 1975). Detoxification and phagocytosis have been attributed primarily to granular haemocytes and the proportion of this cell type is reported to be elevated in polluted environment (Pirie et al. 1984; Liu and Zhao,

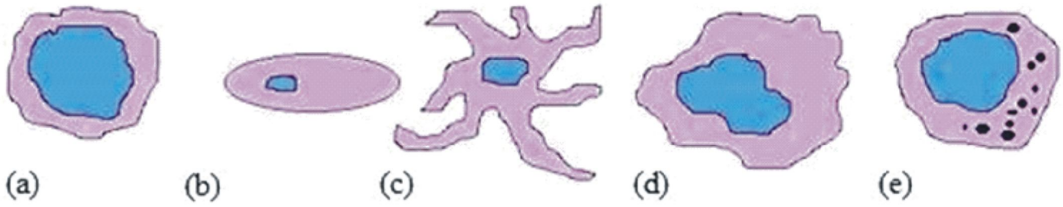

Figure 1. Illustration onthe variety of circulating haemocytes in the bivalve molluscs **(a)** blast like cell or pro-haemocyte **(b)** hyalinocyte **(c)** spreading haemocyte or asterocyte **(d)** agranulocyte **(e)** granulocyte [Source: Illustration is author's own creation]

Haemocyte types	Characteristics	Reference
Blast like cell	i. Small cells, non-spreading in nature ii. Often designated as the pro-haemocytes and molluscan undifferentiated/stem cells	Caraballal et al., 1997; Hine, 1999;Cima et al., 2000;Martin et al., 2007; Chang et al., 2005; Chakraborty et al., 2008
Agranulocytes	i. Cells are large and have ovoid to round nuclei ii. Cytoplasm with scarce secretory granules	Auffret 1988; Chang et al., 2005;Martin et al., 2007; Chakraborty et al., 2008;
Hyalinocytes	i. These cells are ovoid in shape ii. Pale hyaline cytoplasm with small and distinct nucleus iii. Cytoplasm with scattered secretory granules iv. High phagocytic ability and reactive oxygen intermediate (ROI) and nitric oxide (NO) generation	Hine, 1999; Caraballal et al., 1997; Chang et al., 2005; Martin et al., 2007; Lambert et al., 2007; Liu and Zhao, 2018
Granulocytes	i. Cells have variable in size and shape - spherical or oval ii. Small nucleus with granular cytoplasm iii. High phagocytic ability ROI generation	Cheng, 1981; Caraballal et al., 1997; Hine, 1999; Martin et al., 2007; Liu and Zhao, 2018
Asterocytes or spreading haemocytes	i. Cells are spreading and variable in their morphology ii. Projects pseudopodia/filopodia iii. Cytoplasm contains few granules	Hine, 1999; Chang et al., 2005; Mahilini and Rajendran, 2008**;** Chakraborty et al., 2009

Table 1. The structural and functional attributes of the molluscan haemocyte as bio-indicators under the influence of diverse environmental challenges.

2018). An understanding of the types of haemocytes in molluscs is essential in studying basic cell responses to environmental changes (Fisher et al., 1989; Pamparinin et al., 2002), handling of the animals (Ballarin et al., 2003; Malham et al., 2003); and infections (Canesi et al., 2002; Cochennec-Laureau et al., 2003). In freshwater ecosystem, bivalves are dominant filter-feeders that exert control over ecosystem structure and function (Strayeret al., 1999). Considerable data obtained from controlled exposures have demonstrated that oyster defence activities do respond to anthropogenic chemicals such as heavy metals. Cheng (1988 a, b) reported lower percentage of hyalinocytes in oysters exposed to 1 ppm copper sulphate and significantly higher percentage of hyalinocytes in oysters exposed to 1 ppm of cadmium chloride. Coles et al. (1995) reported a significant increase in circulating haemocyte numbers in mussels *Mytilusedulis*resulting from exposure to 400 ppb of cadmium for 7 days. Haemocytes of freshwater zebra mussels (*Dreissenapolymorpha*) exposed to lead and zinc contained enlarged and/or more numerous lysosomes compared with controls (Giamberini and Pihan, 2005). Exposure to 40 ppb of cadmium suppressed the release of degradative enzymes from the haemocytes during phagocytosis. Mussels (*Mytilusedulis*) exposed to copper also had increased granular blood cells by factors of three to four fold over unexposed controls (Pickwell and Steinert, 1984). Shift in haemocyte count of bivalves on interaction to pollutants and environmental cue are often found to be species-specific (Chakraborty et al.,

2008; Matozzo et al., 2010) while differential sensitivity of the haemocytes of clam *Chameleagallina*and the mussel *Mytilusgalloprovincialis*to acidification and temperature alterations are in report (Matozzo

of lysosomal membranes may cause undesired release of hydrolases into the cytosol, resulting consequent damage of self-cells (Lowe et al.,1995). Lysosomal hydrolytic phosphatase enzymes remain compartmentalised within

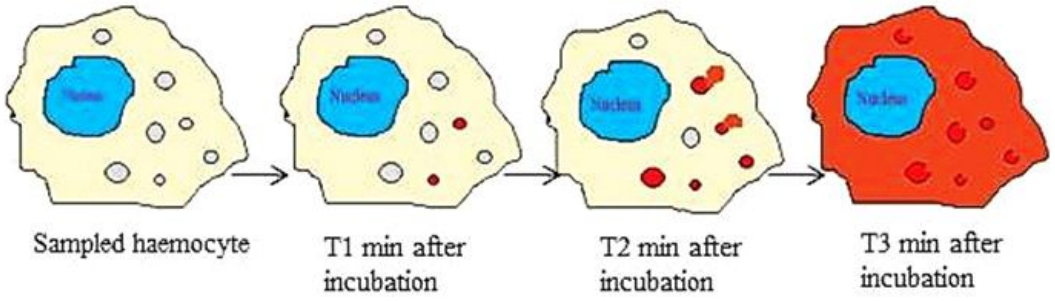

Figure 2. Schematic representation of the method of analysing the lysosomal stability of haemocyte following the principle of linkage of neutral red cationic probe from the lysosomes (T-time interval) [Source: Illustration is author's own creation]

et al., 2012). Total and differential haemocyte count of the freshwater mussel *Anodonta woodiana* has been examined to analyse the water quality of river and waterbodies in Taiwan (Wijayanti *et al.*, 2018).

Lysosomal Stability of Haemocyte

Lysosomes play an important role in the immune responses of bivalve molluscs. On phagocyte stimulation, lysosomal hydrolases are released out of cells to degrade foreign materials (Mohandas et al.,1985) or into phagosomes, thus participating in the degradation of internalized foreign particles (Cheng, 1981). It is known that the haemocytes of bivalve molluscs may accumulate high levels of metals, mainly in lysosomes (Moore, 1990; Bordin *et al.*,1996). Alteration of the integrity

electron-dense specific granules of haemocytes and granular cells (Pipe, 1990). Cytochemical studies have demonstrated the occurrence of several lysosomal enzymes associated with the cytoplasmic granules in haemocytes of several bivalve molluscan species (Moore, 1985; Gelder and Moore, 1986). Degranulation is associated with the release of lysosomal enzymes in the serum during phagocytosis which is vital for maintenance of tissue homeostasis (Cheng and Dougherty, 1989).

The neutral red retention assay (NRR) is a useful technique applied for monitoring the alterations in the permeability of the lysosomal membrane caused by environmental pollutants (Figure 2.). It has been recognised as a sensitive indicator to estimate and assess the period of

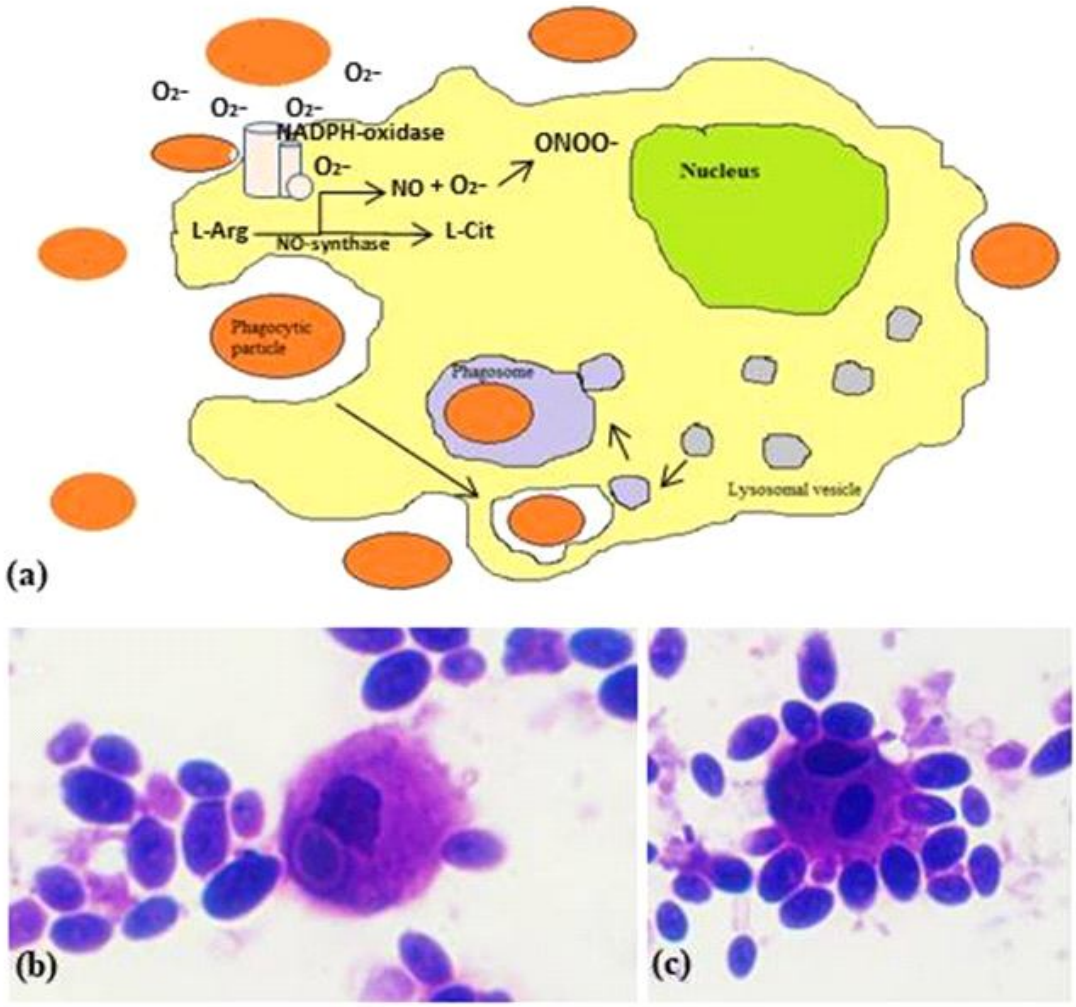

Figure 3. The event of phagocytosis of foreign particles by haemocytes is coupled with the generation of reactive oxygen intermediate (ROI) and reactive nitrogen intermediate (RNI) **(a)** illustration representing the process of phagocytosis by a haemocyte mediated by its lysosomes supplemented with the generation of ROI (O_2^-) and RNI (NO, $ONOO^-$) with the assistance of the membrane bound NADPH oxidase system and nitric oxide synthase enzyme **(b, c)**the phenomena of phagocytosis by the granulocytes of a freshwater bivalve *Lamellidensmarginalis*when experimentally challenged with yeast particles. [Source: Illustration (a) is author's own creation; photographic plates (b, c) are author's own unpublished work]

contaminant exposure (Fernley et al., 2000; Nicholson, 2001; Guidia et al., 2010; Matozzo et al., 2012). Reports suggest that the NRR assay is least affected by natural factors, like temperature and salinity, but is mainly influenced by pollutants (Ringwood et al.,

1998). This assay has been applied to several studies to examine the effects of diverse toxins (Fernley et al., 2000; Wedderburn *et al*., 2000) and heavy metals (Svendsen and Weeks, 1995). A reduction in NRR time under exposure to xenobiotics was observed in similar kind of studies conducted on molluscs reared in the laboratory (Lowe *et al*., 1995; Chakraborty and Ray, 2009) as well as specimens from natural habitat (Fernley *et al*., 2000). A reduction in lysosome membrane stability has been reported in mussels and oysters exposed to heavy metals (Regoli,1992; Ringwood et al.1998), pesticides (Patetsini *et al*., 2013) and expired drugs (Politakis *et al*., 2018).

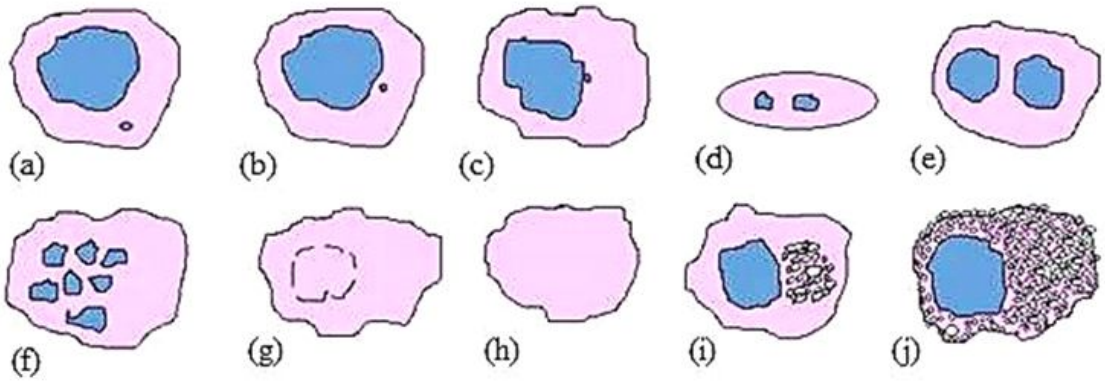

Figure 4. Illustration depicting the different types of nuclear anomalies and cellular damages as observed in the haemocytes of molluscs **(a, b)** micronuclei formation **(c)** nuclear bud formation **(d, e)**binucleation **(f)** nuclear fragmentation **(g, h)** karyolysis or nuclear dissolution **(i, j)** necrotic agranular haemocyte. [Source: Illustration is author's own creation]

Phagocytosis and Generation of ROI/RNI

In cell-mediated immune responses, non-self phagocytosis by circulating haemocytes (Figure 3) is one of the main defence reactions against pathogens and foreign materials (Cheng, 1981). Phagocytes are supposed to be ancient immune defence cells which are widely distributed throughout the body of multicellular animals (Bayne *et al*., 1979). The importance of phagocytosis in immune defence of molluscs and its sensitivity to environmental xenobiotics exposures have made it a major biomarker in ecotoxicological studies. Toxin and pollutant mediated modulation of haemocyte number and function in relation to non-self recognition, phagocytosis, respiratory burst activity etc. are in report by various workers (Adema *et al*.,1991a; Oliver and Fisher, 1999; Parisi *et al*., 2008; Chakraborty *et al*., 2009).

In invertebrates like bivalve molluscs, haemocytes mediated phagocytosis and respiratory burst activities form the major line of defence against invading pathogens andxenobiotics(Cooper and Knowler, 1992; Sami *et al*., 1992; Parisi *et al*., 2008). Nitric oxide (NO) has been described as a key component of the vertebrate immune system (Colasanti *et al*., 2002) and it has also been from

invertebrates and plants where it provides effective protection against bacterial infections (Tafalla *et al*., 2003; Zeidler *et al.*, 2004). In invertebrates including bivalve molluscs,it can kill pathogens byitself or by generating reactive nitrogen intermediate (RNI) combining with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$) (Figure 3a), a strong bactericidal agent (Arumugam *et al.*, 2000; Bogdan, 2001; Donaghy *et al*., 2015). Chakraborty *et al*. (2009) reported the toxicity of arsenic in the haemocytes of *Lamellidensmarginalis*in relation to phagocytosis and NOgeneration while Ciacci *et al*. (2012) have reported differential NO generation under experimental exposure of the marine bivalve *Mytilusgalloprovincialis* to nanoparticles of metallic oxides. In many bivalve species, phagocytic cells can be activated by foreign particles, or organisms and their antigens resulting in release of oxidative chemicals% this response is often referred to as an 'oxidative burst'. An oxidative burst leads to production of reactive oxygen intermediate (ROI), catalyzedby the membrane-associated enzyme NADPH oxidase. The initial metabolite O_2^- is dismutated to hydrogen peroxide (H_2O_2), which may then be converted to other toxic ROI, such as hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$) (Buggé *et al*., 2007). ROI act as killing agents, either alone or in combination with lysosomal enzymes and are important in the elimination of viruses, bacteria, yeast, fungi, and protozoa (Chu, 2000). Production of ROI in a number of molluscan species like *Crassostrea virginica, Crassostrea gigas, Ostreaedulis, Mytilusedulis, Mytilus galloprovincialis, Pecten maximus* and *Mercenaria mercenaria,* has been studied where luminol-dependent chemiluminescence was measured in a liquid scintillation counter or the optical density of the reduction of nitroblue tetrazolium (NBT) to measure production of ROI (Bachere *et al*., 1991; Pipe, 1992; Buggé *et al.*, 2007).

Nuclear Anomalies as Genotoxicity Marker

End point of cytogenetic damage is detected by a micronucleus test, an important and authentic assay (UNEP/RAMOGE, 1999). Appearance of micronucleated apoptotic cell in mussels (Figure 4) from polluted areas has been attributed to exposure of the animals to the hazardous environmental contaminants (Steinert *et al*., 1996; Baršiene et al., 2006). The micronuclei assay is simple and relatively rapid, and is suitable for routine screening and monitoring purposes (Heddle *et al*., 1983; Bolognesi and Fenech, 2012). Micronuclei are produced from the chromosome fragments whose occurrence may be due to the defect in cytokinesis or centromere damage (Heddle *et al*., 1991). Toxic chemicals can cause genotoxic impacts on organisms by modifying the structure of DNA, consequently resulting in irreversible damage to the integrity of chromosome (Hus, 1982). These responses can be considered as biomarkers of adverse effects on the scale of cellular changes and thus can be applied as biological endpoints in genotoxicity assays (Shugart *et al*., 1992). The assay have been utilised as a biomarker of genotoxicity in marine monitoring programme (Kalpaxis *et al*., 2004; Baršiene *et al*., 2006; Schiedek *et al*.,

2006; Rocha *et al*., 2014). However, enumeration of viable, apoptotic and necrotic cells must have to be done carefully following structural conformity of the haemocytes (Figure 4).

Comet assay is another popular non-specific biomarker of genetic damage which has been shown to be applicable in the detection of DNA single strand breaks/alkali labile sites (Lee and Steinert, 2003; Cheung *et al*., 2006).It was Rydberg and Johanson (1978) who were the first to directly quantify DNA damage in individual cells on a microgel. Comet assay is advantageous over other cytogenetic methods for DNA damage detection because it requires a small number of cells for the study and the studied cells need not be mitotically active (Pavlica *et al*., 2001). The assay has been effectively used in bivalve haemocyte based biomarker study against diverse pollutants and stressors of marine and freshwater environment (Pavlica *et al*., 2001; Lee and Steinert, 2003; Cheung *et al*., 2006;Parolini *et al*., 2009; Kumar *et al*., 2014; Martins and Costa, 2015).

Conclusion

An effective biomarker based biomonitoring has the potential to provide the vital information on the temporal and spatial effect of pollutants on an ecosystem. Such information can help the environmentalist and concerned agencies to device bioremediation strategy to prevent unwanted environmental perturbation by pollutants (Galloway, 2006).Bivalve molluscs play pivotal role in aquatic ecosystems and thus they are particularly susceptible to environmental stressors (Gagnè *et al*., 2006). In this context, the biomarking approach based on the bivalve mollusc'shaemocyte structure and function provides a great opportunity for tropical countries like India for sustainable biomonitoring of its rich aquatic bioresource and biodiversity. Assays carried out on bivalve haemocytes are easy for preparation and does not necessitate expensive equipment for observation and documentation (Parolini *et al*., 2009). Simple equipment like microscope, haemocytometer, stage micrometer, spectrophotometer and some dyes/probes are good enough for designing haemocyte based cellular assays in field stations although sophistication demands expenses. The haemocytes of the bivalves remain in direct contact with contaminants and their multifunctional roles make them more sensitive than other cell lines (e.g., gills and digestive glands) to internal and environmental factors (Venier *et al*., 1997; Parolini *et al*., 2009). It is thus considered as a useful cellular tool to assess the ecotoxicity of potential environmental stressors (Parolini *et al*., 2009). However, reports suggest that though popular in Europe and North America, effective use of molluscan biomarker based aquatic environment biomonitoring has not attracted due attention in the Indian subcontinent (Verlecar *et al*., 2006). Generation of database on available molluscan species in a geographic region and the nature of interaction with potential pollutants and stressorsare the prerequisites to implement such biomonitoring strategy. Bivalve mollusc based data on the shift in haemocyte density, lysosomal stability, phagocytosis,

generation of ROI/RNI, genotoxic assay are inexpensive to document and it provides direct information on the health of an aquatic environment. The emerging concept of evaluation of multiple biomarkers (star plots) is gaining rapid acceptance (Beliaeff and Burgeot, 2002; Cao *et al*., 2018) as it has the potential to generate robust and realistic data on the wholesome reaction of a species against a stressor.

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Cytotoxic Effects Induced By The Fungicide Dithane M-45 To Gram (*Cicer Arietinum* L.)

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ABSTRACT:

The cytotoxic effects of Dithane M-45, a fungicide were investigated in the mitotic cell division in gram (*Cicer arietinum* L.) root tip cells. The gram grains were treated with different concentrations of fungicide at room temperature. For this aim, the gram seeds were treated with four different concentrations (5%, 10%, 15% and 20%) of Dithane M-45 for 4, 8, 12 and 16 hours treatment periods. About 1- 1.5 cm length of root tip were cut,stained according to aceto-orcein squash procedure. About 400 cells were scored for each treatment and classified into normal and aberrant division stage. Calculate the mitotic index (MI) and the number of abnormal cells were counted in each phase of cell division. It produces several chromosomal abnormalities in mitotic divisions and the MI is reduced when the concentrations of Dithane M-45solution is increased.The obtained results indicate that Dithane M-45 had the ability to cause production of a large number of mitotic abnormalities. The chromosomal abnormalities were found to be increased as the concentration and treatment periods of the fungicide increased when compared to control. Various abnormalities on chromosomes like fragmentation, condensed chromatin, chromatin granulation, c-metaphase, chromosomal bridges, lagging chromosome, sister chromatin distaining etc. were seen among mitotic divisions treated with Dithane M-45. This result suggests that Dithane M-45 has some negative effects on mitotic divisions in *Cicer arietinum* L. root tip cells.

INTRODUCTION:

The chickpea (*Cicer arietinum* L.) is an annual legume of the family Fabaceae. Itis the important food grain legume and rich in protein. Chickpeas (*Cicer arietinum* L.) are one of the most widely consumed pulses in the world and protein content varies from 21.7 to 23.4% (El-Adawy, 2002). It is a pulse commonly used in Indian cuisine. Chickpea protein is rich in lysine and arginine but most deficient in the sulfur-containing amino acids, methionine and cystine (Manan *et al*., 1984). Chickpea contains twice the amount of protein than that of cereals, hence it can balance the amino acid and may improve the nutritive value of a cereal-based diet (Singh *et al*., 1988). In the last decades, the use of fungicides in agriculture for fungal diseases control has become crucial. Large amount of these chemicals is released into the environment and many of them affect non-target organisms, being a potential hazard to human health. When some chemicals accumulated within food chain to a toxic level, these

chemicals affect directly the public health (Fisun and Rasgele, 2009).

Fungicides produce a diverse range of products with novel modes of action. The extensive use of these compounds in the agriculture system raises public concern because of the harmful potential of such substances in the environment and human health (Mendes *et al.*, 2005). Pesticide exposure is ubiquitous, due not only to agricultural pesticide use and contamination of foods, but also to the extensive use of these products in and around residences (Pastor *et al.*, 2003).The extensive use of fungicides in plant protection against fungal disease generates long term residues in food and in the environment (Petit *et al.*, 2008). Fungicides may also influence to change plant genetic system due to their mutagenecity and carcinogenicity. Cytogenetic studies have been carried out to detect harmful effects of different pesticides on different plant species (Rank *et al.*,2002; Marcano *et al.*, 2004). There are several studies aiming to explain and to understand the effects of fungicides in plant systems. Rayburn *et al.*, (1993) stated out that amount of nuclear DNA is decreased by the fungicide, captan and this fungicide has been mutagenic, carcinogenic and teratogenic effects on many organisms. The present study has been carried out to investigate the influence of Dithane M-45 in *Cicer arietinum* root tip cells during mitotic cell division.

MATERIALS AND METHODS:

Healthy and dry seeds of *Cicer arietinum* L. were pre-soaked in tap water and then treated with Dithane M-45 at four different concentrations (5, 10, 15 and 20%) for 4, 8, 12 and 16 hours. After treatments, the seeds were thoroughly washed with running tap water to remove the excess amount of fungicide from the seeds. One set of seedswere kept untreated to act as control for comparison. Both the treated and controlled seeds were transferred to the Petridishes having the moist filter papers for germination. Forty seeds were used from each dose and control. The Petridishes were kept at room temperature (28-30^{0}C) for three days.

After three days the root tips of germinated seeds (both experimental and control) having a length in about 1.0-1.5 cm were excised and pretreated with aqueous para-dichlorobenzene for three hours, washed with distilled water, fixed with glacial acetic acid:ethanol (3:1) solution and kept for 24 hours. After 24 hours the root tips were transferred to 70% ethanol and stored in a refrigerator. For examination, the root tips were first treated with 2% aceto-orcein and 1(N) HCl (9:1) and just warmed over a flame of sprit lamp.

After proper fixation and staining, appropriate squash preparations were made for each of the treatment and control. Effects of fungicide treatment and control on different slides were observed under light microscope and 400 cells were counted from each treatment. The mitotic index (MI) was calculated and different types of chromosomal aberrations were also observed and scored. Mitotic index was expressed in terms of divided cells/total number of cells x100. These four different concentrations were chosen according to their dose of application in cultivated field to control different diseases.

All experiments were conducted with five replicates and average results were taken.

RESULTS:

The mitotic activity was normal in control roots of *Cicer arietinum* L. A wide spectrum of chromosomal abnormalities were noted in fungicide treated roots (Table 1). The increase of mitotic abnormalities and decrease of mitotic index (MI) was dependent on the increasing concentrations and treatment periods of Dithane M-45 fungicide. The mitotic index in control was observed to be maximum with no chromosomal abnormalities. All concentrations of fungicide cause a decrease in MI when the different division stages were examined. The percentage of abnormal mitotic stages was seen to increase respectively with increasing fungicide concentration. The treated root tips showed various types of metaphasic and anaphasic aberrations at each dose of treatment. Mitotic index of control set was 38.98 in 4h, 38.73 in 8 h, 39.55 in 12 h and 38.67 in 16 hrespectively. At lowest concentration of Dithane M-45 (5%), the mitotic index is reduced to 36.50 in 4htreatment period and further increase in concentration, resulted in decline in mitotic index. When the seeds were treated with 20% of Dithane M-45, the mitotic index was greatly reduced and found to be 16.43 in 16htreatment period. The fungicide Dithane M-45 was found to reduce the MI, irrespectively of the concentration or exposure time, compared to the control. We can infer that there is a direct correlation between the increase of exposure time, fungicide concentration and mitotic activity reduction (Figure 4).

Dithane M-45 significantly increased the percentage of aberrated cells at all concentrations and treatment periods in mitotic cell divisions when compared with control. In this study, the most common cytotoxic abnormalities like chromosomalfragmentation (Fig. 1), condensed chromatin, chromatin granulation, c-metaphase, chromosomal bridges (Fig. 2), lagging chromosome (Fig.3), sister chromatin distainingetc were observed. The treated root tips showed various types of aberrations at each dose of treatment. Increase in concentration of Dithane M-45 significantly increased the mitotic inhibition and ensured the harmful effect on mitotic cycle. The most prevalent aberration caused by Dithane M-45was 4fragments at metaphase in 16 h treatment period (20%), 5bridges at anaphase in 12 h treatmentperiod (20%) and 4 stickiness at anaphase 4in 16 h treatmentperiod (15%) respectively (Table 1).At all treatment periods, the highest concentration of Dithane M-45 (20%) decreased mitotic activity more than other used concentrations. The highest chromosomal anomalies were recorded at a higher concentration and longer exposure (48.49 at 20% concentration in 16 h treatment period).The fungicide Dithane M-45 was found to reduce the MI, irrespectively of the concentration or exposure time, compared to the control. We can conclude that there is a direct correlation between the increase of exposure time, fungicide concentration and percentage of total chromosomal aberration increase (Figure 5)

DISCUSSION:

The mitotic index is a reliable predictor of the cell proliferation in the tissue or organ. Decrease the mitotic index in root tip meristemsof *Cicer arietinum* L were observed in treated seeds when compared to the control and it decreased gradually in all the treatments with increasing concentrations and treatment periods of Dithane M-45. Similar type of results were also found on *Allium cepa* by using fungicide tilt (Pulate and Tarar, 2014) and the root tip cells of *Helianthus annuus* with copper chloride (Inceer *et al*., 2003). In the present study, the chromosomal aberrations induced by the fungicide Dithane M-45 included sticky metaphase, anaphase bridge and fragments may also be observed. Similar results have also been reported in *Trigonella* sp. by Abbasi and Anis (2002); Jabee *et al*., (2008). Various abnormalities on chromosomes like lagging early anaphase, chromosomal bridges, c-metaphase, sticky metaphase, multipolarity, fragment, vagrant etc. were seen among mitotic divisions treated with Calixin (Pulate and Tarar, 2014). Chromosomal stickiness is characterized by chromosomal clustering during any phase of the cell cycle. Stickiness and clumping may be caused by genetic and environmental factors. Several agents have been reported to cause chromosomal stickiness (Panneerselvam *et al*., 2012). The chromosome bridges were recorded at all the concentrations of the treated fungicide and it produced due to chromosomal breakage and joining of incorrect ends (Gill *et al*., 2000). However, Dithane M-45 fungicide has different effects on cell division mechanism. It may be concluded that as has been stated above, Dithane M-45 fungicide has harmful effects on the root tip meristem cells of *Cicer arietinum* L and it acts almost like a mutagen.

CONCLUSION:

Cytogenetic activities of fungicide Dithane M-45 were investigated in root meristems of *Cicer arietinum* L. Higher concentration and longer duration of treatment is toxic to cells. The present investigation clearly showed that there was a significant reduction in the mitotic index of the dividing cells and the chromosomal abnormalities were found to be increased as the concentration of the fungicide increased. These results indicated that Dithane M-45 should be regarded as a mutagenic agent for plants.

Table 1: Mitotic Index (MI), type and percentage of mitotic abnormalities in the root tip cells of *Cicer arietinum* L. exposed to Dithane M-45.

Treatment		No. of cells examined	No.of division cells examined	Mitotic Index (%)	Types and percentage of abnormalities.			Total aberration (%)
Time	Concentration				Fragment	Bridge	Sticky chromosome	
4h	Control	21.06	8.21	38.98	1	0	0	4.75
	5%	18.38	6.71	36.50	1	1	-	10.88
	10%	17.94	6.05	33.72	0	1	1	11.12
	15%	18.05	5.34	29.58	1	2	0	16.62
	20%	18.66	5.12	27.43	1	1	2	21.44
8h	Control	21.25	8.23	38.73	1	0	0	4.70
	5%	20.54	7.21	35.10	0	3	0	14.60
	10%	18.33	6.34	34.59	1	1	2	21.82
	15%	17.45	5.81	33.29	2	2	1	28.65
	20%	18.15	5.25	28.92	2	2	2	33.06
12h	Control	18.33	7.25	39.55	1	0	0	5.45
	5%	19.51	5.90	30.24	0	3	2	25.63
	10%	19.05	5.53	29.03	2	2	3	36.74
	15%	17.34	4.59	26.47	1	5	1	40.36
	20%	16.71	4.02	24.06	1	**5**	2	47.87
16h	Control	21.54	8.33	38.67	0	1	0	4.64
	5%	18.41	4.85	26.34	2	2	3	38.02
	10%	18.73	4.10	21.89	3	3	2	42.71
	15%	20.21	4.23	20.93	2	3	**4**	44.53
	20%	18.56	3.05	16.43	**4**	2	3	48.49

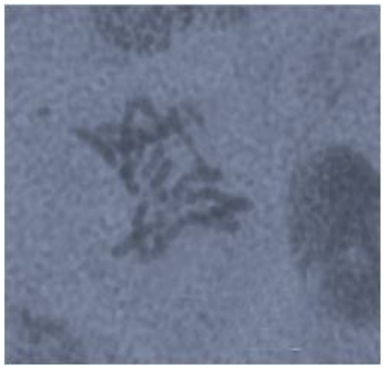

Fig. 1. Chromosome fragmentation

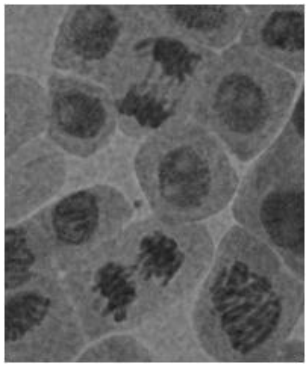

Fig. 2. Chromosomal bridges

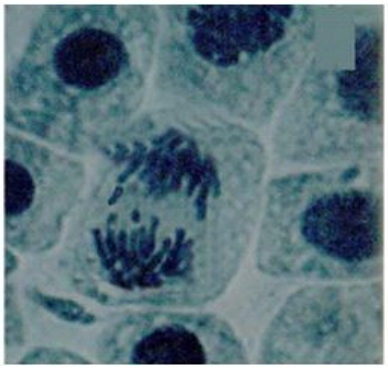

Fig.3. Lagging chromosome

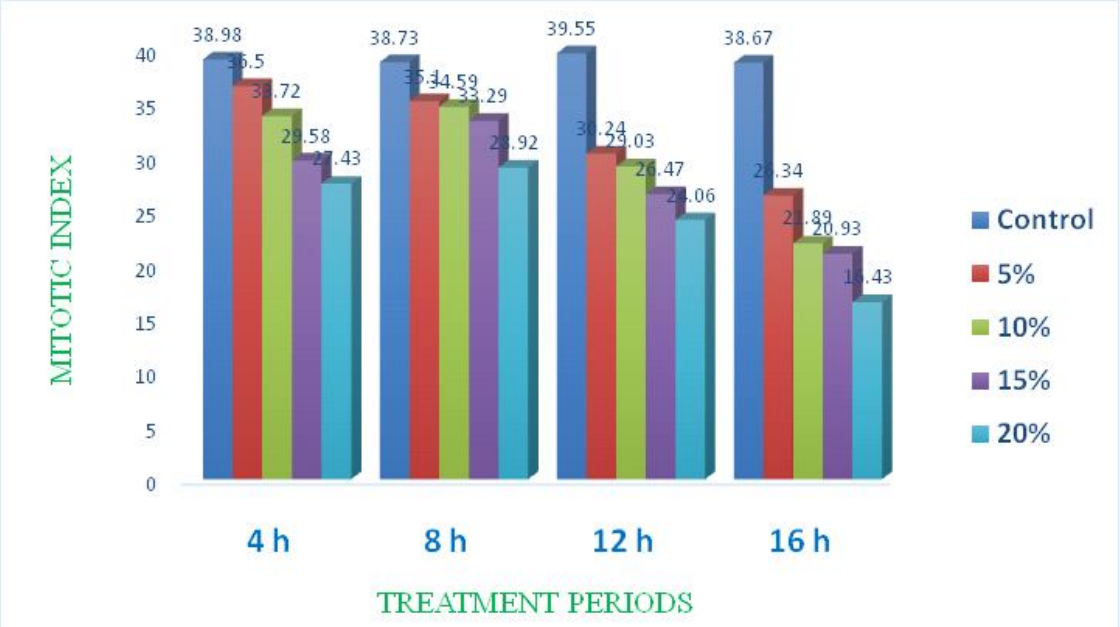

Figure 4: Mitotic index of C*icer arietinum* L. root meristem cells treated with Dithane M-45 at different times and concentrations.

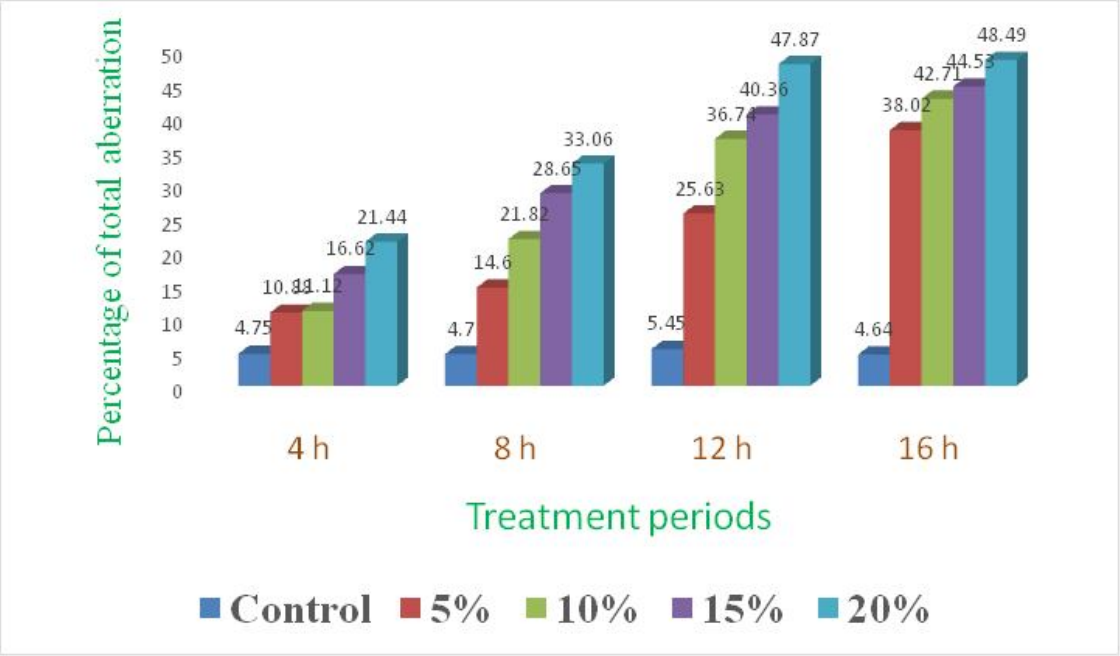

Figure 5: Percentage of total chromosomal aberrationsof C*icer arietinum* L. root meristem cells treated with Dithane M-45 at different times and concentrations.

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A Review on Reproductive Strategies in Ferns

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ABSTRACT

Reproductive strategies in ferns include biological discussion of all reproductive methods i.e. sexual, asexual, vegetative and other unusual special methods which leads to the development of new generations. In ferns, there are various works in different fields of reproductive biology, such as in mating system of ferns-populational, ecological, genetic adaptation in different types of apomixes i.e. apogamy, apospory, agamospory, vegetative reproduction and vivipary etc. A brief overview of the reproductive strategies taken by the ferns in different climatic and experimental conditions is represented here.

INTRODUCTION

From the point of view of reproductive strategies, the extant pteridophytes can be categorized in two major groups-homosporous forms and heterosporous forms. Heterosporous pteridophytes consist of eight living genera,such as *Selaginella, Isoetes, Stylites, Marsilea, Pilularia, Regnellidium, Azolla and Salvinia*. The life cycle of heterosporous pteridophytes is analogous genetically to the seed plants than to the rest of the pteridophytes. Megasporangia give rise to megaspores, which develop into endosporic female gametophytes bearing archegonia, while microsporangia give rise to microspores which develop endosporic male gametophytes and form antheridia and antherozoids respectively. Thus, maximum amount of inbreeding (self fertilization) that can occur in such a way that a plant is the fusion of egg and sperm coming from sib gametophytes. In contrast, ferns have sporangia, which form spores having the capacity to give rise to gametophytes that are exosporic and hermaphroditic. Union of two gametes produced by the same gametophyte, resulting in a zygote that is completely homozygous. Therefore, homosporous ferns have the capacity to form fully homozygous zygotes in one generation of selfing.

The pioneer works in the field of modern of reproductive strategies in ferns were done by Edward J Klekowski Jr. in the middle of 1960s (Klekowski & Baker, 1966; Klekowski & Lloyd, 1968). Since that time numerous studies had appeared on a variety of species and phenomena by a limited number of works (Cousens & Horner, 1970; Duckett, 1970, 1972; Ganders, 1972; Holbrook-Walker & Lloyd, 1973; Klekowski, 1969a, 1969b, 1970a, 1970b, 1970c).Due to the complexities, which are possible in fern reproductive biology, very specific terminology has been derived to describe various levels of crossing and selfing that are possible in ferns. Selfing can be defined

as the fusion of gametes from gametophytes derived from the same parental sporophytes, but due to the possibility of forming hermaphroditic gametophytes two levels of selfing are possible i.e. the fusion of gametes produced by the same gametophytes and the fusion of gametes produced by sib-gametophytes. The probability of a genotype being homozygous for a given gene where selfing is a result of the fusion of gametes formed by sib-gametophytes is half (1/2), where as the possibility that a gene locus is homozygous where the gametes originate from the same gametophyte is one (1).An additional point is that the zygote resulting the fusion of the gametes coming from the same gametophyte is homozygous at every gene locus, where as the probability of completely homozygous zygote occurring, where the gametes come from sib-gametophytes is $(1/2)^n$, where n=number of heterozygous loci in the parental sporophyte genotype. The following types of selfing, crossing and mating are found in pteridophytes.

1. **Intragametophytic selfing:** The fusion of sperm and egg from the same gametophyte. Normally this results in a completely homozygous zygote.
2. **Intergametophytic selfing:** The fusion of sperm and egg from gametophytes with both genotypes being sib i.e. originating from the same parental sporophyte. This is analogous to the self-fertilization of a seed plant.
3. **Intergametophytic crossing:** The fusion of sperm and egg from different gametophytes with each gametophyte originating from a different parental sporophyte. This is analogous to cross-fertilization of a seed plant.
4. **Intergametophytic mating:** The fusion of sperm and egg from different gametophytes with the origin of gametophytes not specified.

Although the genetic consequences of selfing of a gametophyte have been long recognized (Lang, 1923), previous discussion of evolution in ferns have emphasized hybridity (interspecific) and have assumed that intragametophytic selfing is of little consequence in nature (Manton, 1961). This opinion regarding intragametophytic selfing still held many pteridologists and is based primarily upon a kind of "Fern Chauvinism" against homozygosity.

MATING SYSTEM IN FERNS:

A. POPULATIONAL:

Homosporous ferns offer a particular interesting comparison with seed plants. They differ from higher vascular plants in producing highly dispersible haploid spores, which generate free-living potentially bisexual gametophytes. Thus mating systems in ferns are quite different from those of angiosperms and gymnosperms and similar in some ways to those of bryophytes. In fact, three types of mating are possible (Klekowski, 1969; Lloyd, 1974 a).

1. **Intergametophytic crossing:** the cross-fertilization of gametophytes produced by different sporophytes.
2. **Intergametophytic selfing:** the cross-fertilization of gametophytes produced by a

single sporophyte.

3. **Intragametophytic selfing:** the self-fertilization of a single gametophyte.

Intergametophytic crossing is genetically equivalent to outcrossing (cross fertilization) in seed plants and Intergametophytic selfing is genetically equivalent to selfing in seed plants.

As archegonia and antheridia are borne on the same thallus it has long been maintained that Intragametophytic selfing is predominant mode of reproduction in natural population of Homosporous pteridophytes (Klekowski and Baker, 1966; Klekowski, 1973). This would have major implications for the genetic structures of these populations, because Intragametophytic selfing results in the production of completely homozygous sporophytes by act of fertilization. Experimental evidence indicates that some ferns like *Botrychium virgianum, B. dissectum* and species of *Asplenium* reproduce predominantly by self-fertilization (Soltis and Soltis, 1986).

Majority of the adaptations, which influence the breeding systems of ferns are aspects of the gametophytic generation. There are sporophytic adaptations, which can play a prominent role. An example of this is the reproductive biology of *Mettuccia struthiopteris*. This fern begins to shed their propagules in January and continue to do so until March. If snow is collected from beneath these fronds in January, it is found to contain full, unopened sporangia rather than spores, whereas collection of snow in March will contain spores and sporangia. The sporangia contain viable spores, which will germinate within the sporangium and rupture it, resulting in a cluster of male and female gametophytes. Thus in this fern two kinds of propagules are formed, sporangia that are distributed when the snow melts and the wind disseminated spores. The reproductive biology of sporangia is genetically analogous to an inbreeding seed plant (the predominant level of mating is between sib-gametophytes, Intergametophytic selfing, Klekowski, 1979).

Gametophytic adaptations also influence the nature of fern mating system (Lloyd, 1974). The adaptations include the gender, ecology, distribution of gametangia, longevity of the gametophytes, capacities for vegetative reproduction and polyembryony (Klekowski, 1969).The basic morphological syndrome or characteristic which leads to a predominance of Intragametophytic selfing is a uniform gametophyte population with respect to gender, the differentiation of antheridia initially and the later attainment of the hermaphroditic condition by differentiation of the archegonia with the continued proliferation of antheridia and a relatively short-lived gametophyte generation with limited capacities for vegetative reproduction and simple polyembryony. Such a mating system often characterizes weedy fern species, which rapidly colonize open, recently disturbed habitats (Lloyd, 1974b). *Pteridium aquilinum* and *Ceratopteris thalictroides*), which is aggressive weed in the respective environments, have capacities for extensive multiplication and antheridogen system, which can promote Intergametophytic mating (Schedlbauer and Klekowski, 1972). It is

interesting to note that amount of genetic variability in the populations of these four weedy species varies greatly and only *Pteridium aquilinum* populations consistently have genetic load (Klekowski, 1972).

No prior evidence was found regarding genetic self-incompatibility system present in homosporous ferns with free-living hermaphrodite gametophytes. Wilkie (1956) hypothesized that a genetic self-incompatibility system was present. Tryon (1941) did not support 'Wilkies hypothesis'. All the hybridization data could be explained best by assuming that sporophytes of this species are heterozygous for various combinations of recessive sporophytic lethals (genetic load) and that occasionally a spore sample is obtained that forms a trans heterozygote for a pair of closely linked lethal alleles Ab/aB. The spores of such a sporophyte will be ½ Ab and ½ aB and isolated gametophytes will not form viable homozygous sporophytes upon Intragametophytic selfing. If the gametophytes are paired randomly the following expectations will be realized – ¼ (Ab x Ab), ½ (Ab x aB), ¼ (aB x aB). Thus only 50% of the random pairs will be heterozygous at both loci and form sporophytes (Ab x aB). Crossing over will generate two recombinant spore genotypes (AB and ab) of which one (AB) will form viable sporophytes upon intragametophytic selfing. It should be noted that the above genetic load model accounts for all the hybridization data for a sporophyte heterozygous for a pair of self-incompatibility alleles S^1S^2 (Klekowski, 1979).

A population of isolated gametophytes will not yield viable homozygous sporophytes upon selfing (as their genotypes would be S^1S^2 or S^2S^2) and population of random pairs of gametophytes (were capable of forming a viable homozygous sporophytes upon) will result in 50% of the combinations being heterozygous (S^1 x S^2) yielding sporophytes. Wilkie (1956) found that a low frequency of gametophytes were capable of forming a viable homozygous sporophytes upon Intragametophytic selfing, this phenomenon is not accounted for the genetic self-incompatibility hypothesis, but readily explained under the genetic load hypothesis.Genetic load and self-incompatibility systems are closely related and one readily can envisage the evolution of such genetic incompatibility system from trans-heterozygotes for recessive sporophytic lethal. The tightening of the linkage relationships between such loci could well be a consequence of such evolution. The distinction between two phenomena, genetic load and genetic self-incompatibility for a species is based upon the ubiquity of sporophyte genotypes in the populations, which yield spore samples that are self-incompatible i.e. from gametophytes which fail to form viable homozygous sporophytes upon Intragametophytic selfing.

Among homosporous ferns three types of reproductive strategies can be found-

1. Those in which syngamy is eliminated and sporophytes originate apogamously.
2. Those which promote Intragametophytic mating, and
3. Those which promote Intergametophytic

mating.

Genetically apogamy results in the least genetic variability in the species, and Intergametophytic mating results in the greatest degree of genetic variability.

B. ECOLOGICAL:

Correlations between ecology of the species and length of the gametophyte generation discussed by Klekowski (1972, 1979), suggests that ferns occupying habitats less favourable to the survival of their gametophytes for a longer duration are expected to undergo predominantly Intragametophytic selfing, and these species would be characterized by shorter gametophytic generation time (Lloyd, 1974 a, b), indicates that the colonizing species may possess a shorter gametophyte phase. Moreover, considering the significance of polyploidy in the homosporous ferns as intimately related to homothallism of their gametophytes (Klekowski, 1973, 1979). Intragametophytic selfing might be expected to be more common in present day polyploids as compressed to the diploids. Masuyama (1979, 1986) has documented such a correlation with respect to 2X and 4X forms of *Phegopteris decursivepinnata*, where the diploid is favoured toward Intergametophytic mating and tetraploid toward Intragametophytic selfing.

Guillon and Raquin (2002) studied the environmental sex determination in Horsetails (*Equisetum*). Horsetails are Homosporous and sexual differentiation of *Equisetum* gametophytes is under the influence of environmental conditions. Still the environmental cues responsible for sex determination of *Equisetum* gametes *invitro* and in wild conditions have remained elusive. Here we show that significantly different sex ratios are obtained when gametophytes are grown on media with or without sugar.

C. HORMONAL:

A careful observation by Dopp (1950) led to the discovery of a specific organ-inducing substance in ferns. Dopp (1950) observed that juvenile gametophytes of *Pteridium aquilinum* bore antheridia. He thought that the induction to be mediated by the substance elaborated by older gametophytes and it has been found that the extract from mature prothalli of bracken fern hastened the onset of antheridium on newly formed gametophytes. This substance was organ specific, but not species specific. Naf (1959) rediscovered the biologically active factor, a natural metabolite specifically concerned with induction of antheridia. The "Pteridium factor" was designated as "Antheridogen". It has been found to be active in the members of Pteridaceae, Dryopteridaceae (Dopp, 1950), Gymnogrammaceae (Dopp, 1959), Aspleniaceae (Naf, 1959).The antheridogen either induce the formation of antheridia under conditions in which control plants do not form them, as in *Osmundasensibilis* or hastens the onset of antheridium formation. Extremely small amount of substance required for antheridial development. Investigations on fern reproductive biology revealed that, unresponsive to antheridogen led to the formation of a substance that controls antheridium formation in *Anemia phyllitidis*. The substance however is inactive towards

plants or gametophytes which respond to "Pteridium factor" (Naf, 1959). In *Lygodium japonicum* antheridium formation seems to be controlled by a second substance A_2 (=Antheridogen in *Lygodium*). From these studies it is apparent that antheridium formation is controlled by different substances in different groups of ferns.

Schraudolf (1962) introduced a new control of antheridial differentiation by Gibbarelic Acid (GA). GA induces antheridium formation in *Anemia phyllitidis, Anemia rotundifolia* and *Lygodium japonicum* in exactly same manner as "Anemia factor". GA_3 has been found to be active in all members of Schizaeaceae (Voller and Weinberg, 1969). Among non-Schizaeaceous species, GA induces antheridia on *Dryopteris filix-mas*. The antheridia formed in response to GA_3 quite normal. Voller (1964) has shown that seven gibberellins GA_1, GA_3, GA_4, GA_5, GA_7, GA_8 and GA_9 are capable of inducing antheridia *Anemia phyllitidis* (Schraudolf, 1967).

Relative information about archegonial initiation in ferns is lacking except that the conditions favourable for growth, favour femaleness and fast growing prothalli are female and later become hermaphrodite. Schizaeaceaeous ferns are especially suitable for studies on sexuality as the native antheridogen is replaceable by giberellin. In polypodiaceous ferns indole auxins are known to inhibit the formation of antheridia (Dopp, 1962), but this was imperative in Schizaeaceae. However, in *Lygodium flexuosum* auxin, 2,4-D hastened three-dimentional growth, inhibited the formation of antheridia and instead archegonia appeared first (Rashid, 1970). These gametophytes rapidly initiated antheridia on transfer to auxin deficient medium. Antheridia formation was also suppressed when auxin and gibberellins were supplied together. Therefore, the prothalli, which are initially archegoniate, are to be looked for either high auxin content or inhibitor of antheridium inducing substances.

The genetic mechanism of regulation of antheridogen activity has been worked out in *Ceratopteris* by (Banks *et al*., 1993, Banks 1994, 1997). The epistatic pathway is interplay of two master regulatory genes that regulate the sexual phenotype of the gametophyte. The transformer gene (TRA), which when active simultaneously promotes femaleness and suppresses maleness. While, the feminization gene (FEM) when active promotes maleness and suppresses femaleness. The factor that determines which of these two master sex-regulatory gene is expressed is determined on the presence or absence of A_{ce} (Ranker & Geiger, 2009; Mukhopadhyay, 2009).

D. GENETIC:

Genotype of homosporous ferns contains the necessary genes for the development of both the haploid gametophyte and diploid sporophyte. It is expected that a considerable portion of the genotype is expressed only in the sporophyte generation. Physiological and genetic evidence supports the hypothesis that many of the genes in the Homosporous fern genotype are expressed primitively in either one or other generation (Mohr and Barh, 1962;

Mohr, 1971; Klekowski, 1972). This compartmentalization of the genotype allows the development of viable gametophytes inspite of recessives, which are lethal when homozygous in sporophyte generation. Various techniques developed by (Klekowski, 1973; Dobzhanski, 1970; Wallace, 1970) for studying phenomenon of genetic load in fern populations. The fundamental question asked in these studies is whether a given sporophyte genotype is heterozygous for deleterious genetic combinations, which when present in the haploid gametophyte or diploid homozygous sporophyte generations, decrease the viability of that generation.

Genetic load studies have two functions-

a. They allow an estimate to be made of the nature and extent of this component of genetic variability in fern populations.

b. They led to accumulations of gametophytes clones bearing interesting mutations.

The former function yields information of an evolutionary nature, specifically the extent to which the various gametophytic and ecological adaptations have resulted in a genetically heterozygous population structure and the later results in interesting mutation for ontogenetic and developmental studies.

Masuyama (1986) revealed that gametophytes of diploid plants of *Phegopteris decursive-pinnata* have a low capability for Intragametophytic selfing. Chiou et al (1998) worked out on the reproductive biology of the genus *Elaphoglossum*. Fixed heterozygocity in *Elaphoglossum palaeaceum* suggests that sporophytes of this species are polyploidy, but at least some outcrossing occurs. High genetic loads determined from a single gametophyte cultures of *Elaphoglossum califolium* and *Elaphoglossum crassifolium* indicate two probabilities of successful Intragametophytic selfing.

Sequence and duration of gametangia formation:

Sequence and duration of gametangia formation in gametophytes of ferns is a significant behavior for reproductive strategies. Klekowski (1969) conceived the classification of the ontogenetic sequence of gametangia on meristic-prothalli. In his classification most of the Homosporous ferns belongs to the category with the initial formation of antheridia followed by a prolonged hermaphroditic phase, which led to the notion that the Homosporous fern life cycle is largely tuned for intragametophytic selfing. Masuyama (1975 a, b) proposed a revised classification of the sequence of gametangia. Masuyama (1975 a, b) concluded that features of gametangial sequence (type, A, B, C of table-1) are species specific and do not vary under experimental conditions, whereas the three patterns of gametangial sequence on meristic prothalli recognized by Masuyama (1975 a, b) characterize most of the ferns and these may be regarded as the basic patterns. Accordingly, Verma (1985, 1989,2003), Verma et al. 1987, 2000; Ganguly and Mukhopadhyay (2005, 2009) added two more new types to Masuyama's classification and visualized finally eight different types. From this classification, intraspecific variation for

gametangial sequence may be explained particularly in all such species committed to versatility in mating and there would always be sufficient deviation within species in the pattern of gametangia production to provide flexibility in reproductive strategies.

Antheridial area and its correlation:

Distribution of antheridia on bisexual gametophytes plays a significant role in breeding system of Homosporous ferns (Nayar and Kaur, 1971; Atkinson and Stokey, 1964). Masuyama (1975 a, b) recognized four basic locations of antheridia on monoecious-prothalli-

1. Antheridia on the lower part of the body (L).
2. Antheridia on the lower half of the wing (LW).
3. Antheridia on the lower half of the margin (LM)
4. Antheridia on the upper half of the central cushion (UC).
5. Antheridia on all along the margin (M).

Masuyama (1975a) observed that a striking correlation between the four types of antheridial area (L, LW, LM, UC) and three types of (A,B,C) of gametangial sequence. The location of antheridial area restricted to the lower part of the gametophyte (L-position) was formed to be associated more often or generally with type-A gametangial sequence, which lack antheridium formation during archegonial phase, essentially ensures Intergametophytic mating. The type B sequence of gametangia, where indefinite and simultaneous formation of antheridia and archegonia, capable of intragametophytic selfing, was associated often with antheridial location as UC and LM. Moreover, the type A occurred more frequently among diploid species, whereas type B sequence commonly in the polyploids (msuyama, 1975 b, 1979). These correlations tend to suggest that the ancestral kind of mating in homosporous ferns as exemplified by the present day diploid is Intergametophytic mating, contrary to the view of habitual gametophytic self-fertilization (Klekowski and Baker, 1966; Klekowski, 1969, 1979).

As concluding remarks, it may stated that, the initial bearing of antheridia on eventual monoecious prothalli together with the location of antheridia in the lower part of the gametophyte (location L)cannot be taken as conditions predisposing such species to Intergametophytic selfing (Klekowski, 1969). Instead, the co-occurrence of the L-type location of antheridia commonly with the type A sequence of gametangia (lack of antheridium formation during the subsequent archegonial phase, i.e. protandry) suggests an adaptation for Intergametophytic mating.

ASEXUAL AND VEGETATIVE REPRODUCTION:

Departures from a normal sexual life cycle of a fern result in the customary cytological and or morphological alteration of generations being interrupted or modified. Such events include apospory, apomixes and vegetative reproduction.

1. APOGAMY:

Apogamy involves the production of sporophyte

from a prothallus without the intervention of oogenesis or fertilization. Like apospory this may occur or be induced sporadically, but is also found as a recurrent event in the obligatory apomictic alteration of generations, which characterizes many ferns.Apogamy was first reported by Farlow (1874) in *Pteris critica*. Apogamy occurs in nature and has also been induced under experimental conditions. It is common and widespread phenomenon in ferns. In some species of ferns apogamy appears to be a necessity and is a regular process. It is perhaps due to the inherited constitution of the plant. Natural apogamy is common in *Dryopteris, Pteris, Adiantum, Asplenium, Athyrium* etc.

Reasons for Apogamy:

Regarding the casuses of apogamy, several explanations have been put forwarded. Lang (1889) induced the formation of sporophytic buds, roots, sporangia and tracheids in the various fern prothalli, by avoiding watering of the prothalli. Brown (1923) summarized regarding the induction of apogamy by avoiding fertilization. The following conditions are thought to favour apogamy:-

1. Culture in bright light and at the higher temperature (Solni, 1900).
2. By lowering the vitality of the prothallus by fungal and algal infection and various unfavourable nutritional environments.
3. Willams (1938) suggested that in addition to the environmental factors there must also be some internal factors, such as the nature of inherent susceptibility due to abnormal nuclear composition and behavior, which bring about apogamy.
4. Ageing of the prothallus has also been regarded as one of the factors influencing apogamous development on the prothalli of some ferns.

Evans (1964) has reported an interesting case of apogamy in a species of *Polypodium*. He reported the formation of 32 mitospores in the sporangia. The sporangia have 16 spore mother cells, which do not undergo meiosis, but instead divide mitotically into 32 spores. This spore is reniform in shape and occurs in diads rather than tetrads. He reported this sequence to occur regularly in all sporangia. The spores germinate readily to form prothalli, which bear numerous apogamous sporophytic buds. The prothalli bear stomata, but produce no sex organs.

2. APOSPORY:

In apospory, gametophytic tissue is produced by the sporophyte without intervention of spores. The prothalli so produced are usually functional, although they may differ somewhat in morphology from those of the same species produced by spores. Apogamy only occurs sporadically in ferns, but it can be induced. Aposporous prothalli customarily bear normal sex organs and since they have the same chromosome number as the parental sporophytic tissue, they provide a means of inducing polyploid series. For example, production of triploid (3n) and tetraploid (4n) *Osmunda* without use of cochicine (Manton, 1950).Induced apospory in a normal sexual species cannot be repeated indefinitely from

generation to generation, because this would involve doubling of chromosome number in each fertilization. The phenomenon of apospory was recorded by Druery (1884) in fern called *Athyriumfilix-fomina* var *clarissima*. Bower (1885) reported apospory in two species of *Trichomanes*.

Reasons for Apospory:

Several factors seem to influence the aposporous development of gametophytes from vegetative tissues of sporophyte.

- Bristow (1962) demonstrated that minimal nutrition is responsible for the formation of prothalli from callus tissue obtained from the sporophytes of *Pteris critica*. When he supplied sucrose to such callus tissue it develops into sporophytes.
- Lawton (1932) and Beyerle (1932) demonstrated that there is a pronounced relation between the stages of development of sporophytic cells and kind of organs regenerated. They observed that in *Ceratopteris thallictroides* aposporous gametophyte development on decapitated young sporophytes with one or two leaves where as in older sporophytes only shoot bud developed.

3. AGAMOSPORY:

In some fern species apogamy may be repeated from generation to generation and from a regular method of reproduction. In such cases, it is accompanied by diplospory, involving certain well-defined cytological events during a modified sporogenesis such that the chromosomal number remains the same in both generations. It forms a major type of recurrent apomixes met within the ferns and described as apogamy by Love and Love (1977). During sporogenesis in sexuality reproducing ferns, the initial archesporial cell in a developing sporangium divides four times by meiosis resulting in 16 spore mother cells. Meiosis occurs, giving rise to 64 spores with half the original chromosome number. In agamosporous ferns modifications of either mitosis or meiosis occur, resulting in the production of viable spores, but which have the sporophytic instead of the reduced chromosome number. Dopp (1932, 1939) originally described the course of events in *Dryopteris remota* and the process was further amplified and fully illustrated by Manton (1950). This system is described as Dopp-Manton system. In the Dopp-Manton system, two main types of cytological behavior are shown-

- In one case the archesporial cell divides four times by mitosis to give rise to 16 spore mother cell and then meiosis occurs, just as in sexual species. However, meiosis is irregular and consequently only abortive spores are produced, whilst these events are of no reproductive significance, they are scientifically important in that the true chromosomal homologies are demonstrated and therefore can be used in experimental analyses of agamosporous species.
- In other type of behavior shown, a compensation mechanism occurs, resulting in regular meiosis and the production of viable spores. Here three mitotic divisions have given rise to cells. A further mitotic division starts

normally, the chromosomes move to the equation and divide, but there is no separation towards the poles and restitution nuclei are formed. As there is no cytoplasmic division involved during this mitosis the result is the formation of eight spore mother cells, each with double the original chromosome number, following meiosis. As a result 32 diplospores are produced which have the same chromosome number and genotype as the sporophyte.

Braithwaite (1964) described another type of agamospory in *Asplenium aethiopicum*, here only one type of sporangial development occurs. Here, the archesporial cell undergoes four normal mitotic divisions to produce 16 spore mother cells. At the first meiotic division the chromosomes move towards the equator without pairing. The cytoplasm fails to divide and restitution nuclei are formed with the result that there are still 16 without any alteration in chromosome content. The second meiotic system is normal, giving rise to up to 32 viable diplospores, arranged in diads and having the original sporophytic chromosome number. Evans (1963) independently described a method of agamospory in *Polypodium dispersum* and mentioned that it was an ameiotic process.

4. VIVIPARY:

The function of aphlebiae as vegetative propagules developing young sporophytes viviperously is reported in *Dennstaedtia scabra*. This type of alternative means of reproduction is an ecological adaptation, which is not unlikely in a situation where development of sexuality produced sporophytes, seems difficult in a snowy coverage. Detachment of aphlebiae as propagating organ seems possible, as the vascular tissue of aphlebiae is not continuous with the parent plant (D'Rozario *et al.,* 2005). Vivipary is a common phenomenon observed in mangrove plants such as *Rhizophora* and is considered as an ecological adaptation to halophytic habit. It is also noted in some ferns like *Asplenium*, *Woodwardia*, *Adiantum*, *Camptosorus, Cystopteris, Diplazium, Tectaria* and specially in plants growth on rocks in the sheltered areas in moist forests to overcome the unfavourable environmental condition. In these species, frond architecture is typically such that gemmae are brought easily into contact with the substrate, either during the life of the parent frond or by its eventual decay.

D'Rozario *et al* (2001) suggested that aphlebiae could function as vegetative propagules.Some *Dennstaedtiascabra* plants were found to bear aphlebiae on their leaves during winter. The most interesting part of this observation is that the development of some young sporophytes from the surrounding surface of these aphlebiae. Aphlebiae have been reported earlier from fossil coenopterids *Stauropteris* and in some Cyatheoid ferns.

5. VEGETATIVE:

In addition to the sexual gametophyte-sporophyte life cycle, some pteridophytes have developed various vegetative means of propagation to increase the extent and the number of their population. This is advantageous where seasons are favourable, or the environment is otherwise not conductive to gametophyte production and in some cases it

may just be advantageous to generate numerous genetically identical individuals.A number of quite unrelated species produce vegetative buds or bulbils that are capable of producing roots and new plants. The bulbils are leaves derived structures and are produced in leaf axis or at various places on the leaf surface. Plants may chain along as successive bulbils produce roots, establish themselves and produce fronds and bulbils of their own. Proliferating bulbils can be found in *Asplenium*, *Camptosorus*, *Diplazium*, *Polystichum*, *Ampelopteris*, *Huperzia* etc. proliferation is possible from root stolons, tubers and similar structures. This occurs in some species of *Nephrolepis* and *Blechnum*. More of the survival strategies than a means of propagation, some ferns of arid areas are able to dry out almost completely without actually dying. Crisp and brittle to touch, they resuscitate rapidly when rain comes and continue their growth. A number of species of *Cheilanthes* behave in this way.

Special reproductive strategies in some epiphytic Polypodiaceae:

Genetic load estimates from sporophyte production by isolated gametophyte cultures indicate mating systems of intragametophytic selfing in *Campyloneurus angustifolium*, *C. phyllitidis*. Polyploidy characterizes the intragametophytic selfing species; whereas the intragametophytic mating taxa are diploid. The duplicate loci of polyploidy taxa may mitigate the expression of recessive lethal alleles caused by intragametophytic selfing; whereas genetic load probably maintains the mating system of intergametophytically mating taxa. Enzyme electrophoresis patterns of fixed heterozygosity suggest allopolyploids origin of *C. phyllitidis* and *Polypodium leuceanum* and confirm their intragametophytic mating systems. Antheridogen present in both groups may promote Intergametophytic mating in diploids through promotion of the early development of the male plants in gametophyte populations and bisexuality in isolated gametophytes of polyploids of the gametophytes delay or do not attain insensitivity to their own antheridogen. In the poliploids, antheridogen may also alleviate low genetic variability through promotion of occasional outcrossing. The perennial clone forming habit of epiphytic Polypodiaceae increases the duration and the physical space occupied by derivatives of a single spore, thus expanding, the chances of interaction with a later migrant. Genetic load, duplicated genes and antheridogen together with a perennial and clone forming gametophytes growth habits interact to produce successful breeding strategies of the epiphytic species.

Several fern species are known to exist over parts of their ranges as gametophytes only, without development of the sporophyte stages of their life cycle. Gametophytes of *Hymenophyllum wrightii* have been collected from several places along the pacific coast. Farrar (1967) has demonstrated the occurrence of populations of *Trichomanes* gametophytes in England over 800 km from any known sporophytes of the genus.In this case the gametophytes have the characteristic capacity to produce gemmae by which they maintain

local populations vegetative and independently of the sporophyte generation. Species of *Vittaria* also have gemmae producing gametophytes. In peninsular Florida *Vittaria lineata* produce both sporophytic and gametophytic stages. Here gametophytes produce the gemmae characteristic of the genus but also produce sporophytes in normal sexual life cycle (Farrar, 1974). In Appalachian Mountain plataue region of the eastern US, a third species of *Vittaria* is represented by the gametophytes only. This species is known as *Vittaria appalachiana* (Farrar and Mickel, 1991).

CONCLUSION

The gametophyte generation of Homosporous ferns is the most delicate part of the life cycle and the sporophyte generation would be at the mercy of their gametophytes to the extent that latter expresses any portion of the genome they convey (Mukhopadhyay, 2013; Ganguly, 2013).

Homosporous ferns produce sporophytes intragametophytic selfing, Intergametophytic mating or apogamy, gametangium ontogeny, genetic load and polyploidy affects the mating system of each species. Gametangium ontogeny causes different sexual expressions and may permit or prohibit intragametophytic selfing. Only in the situation, which the male and female gametangia on the same gametophyte mature at the same time and release sperms, is intragametophytic selfing likely to occur. Genetic load is a measure of the effect of deleterious genes on fitness. The lower the genetic load, the higher the probability of intragametophytic selfing. As the genetic load increases, so does the frequency of Intergametophytic mating. Intragametophytic selfing is a characteristic feature of polyploidy species, whereas most diploid species reproduce by Intergametophytic mating. Antheridogen promotes antheridium and male gametophyte formation. Thus Intergametophytic mating is more frequent and more successful in those species with high antheridogen production. In addition to sexual reproduction some ferns produce sporophytes by apogamy. Each reproductive strategy is favourable for creating a new population (intragametophytic selfing, apogamy), increasing genetic diversity (Intergametophytic mating) or produce offspring's in drier habitats (apogamy).

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Phytochemistry and Antibacterial Activity of Crude Extracts and Extracted Phenols of *Selaginella bisulcata* Spring

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ABSTRACT

Phytochemical analysis and Antibacterial activities of crude extracts and extracted phenols from sporophytic parts of *Selaginella bisulcata* Spring belonging to the family Selaginellaceae were studied in summer and winter seasons against *Bacillus subtilis* AR-2 (Gr +ve) and *Escherichia coli* XL1-Blue (Gr -ve). Both the crude extracts and extracted phenols from sporophytic plant parts showed antibacterial activities. In summer, the phenol content is minimum and in winter the phenol content is maximum. Each and every sporophytic plant parts showed antibacterial activities by crude extract as well as by extracted phenols. Detailed observations revealed that crude extract shows better antibacterial activity than extracted phenol. HPLC analysis for extracted total phenol reveals that 6 types of phenolic compounds were present in *Selaginella bisulcata* Spring.

INTRODUCTION

Selaginella a member of the family Selaginellaceae, is a very common fern-ally and according to the modern concept belongs to the Lycophytes (Smith *et al*., 2006), lithophytic in nature, mainly grows on shaded humid rocks and is distributed in India throughout the Himalayan region from Eastern Himalayas to Western Himalayas and also found in the Western and Eastern Ghats. This genus is represented by about 700 species throughout the world mainly in the tropical and subtropical climates (Alston, 1945; Jermy, 1990; Mukhopadhyay, 2017). This species isfound in between sea level to 2700 m altitude. Current microbial resistance to antibiotics has been a global concern, as all known classes of natural compounds for antimicrobial therapy are becoming resistant (Boller, 1995). Many fern species are important medicinal plants (Puri and Arora, 1961; Sharma, 1981; Asolkar *et. al.*, 1992; Guha *et. al*., 2004;Ganguly et. al. 2011, 2013). However, some of these plants have been tested for antimicrobial properties (Khandelwal *et. al*., 1985; Hain *et. al.*, 1993). As per literature concerned, there is no report of antimicrobial property of this species of *Selaginella*. Only Homoeopathic medicines are available in the market but not as antimicrobial drug.So,there is urgent need of new compounds for antimicrobial therapy. This study was conducted to test potentiality of this plant as an antimicrobial agent. The present study was conducted to reveal thephytochemical properties, types of phenolic compounds and antibacterialeffects of extracted phenols and

crude extracts of different parts of sporophytic plant body of *Selaginella bisulcata.*

MATERIALS AND METHODS

The study material *Selaginella bisulcata* Spring was collected from different parts of Darjeeling Himalayas at the altitudes of 1900 -2100m and identified with the help of Alston's key (1945) and with the help of the book of Dixit (1992).

CRUDE EXTRACT: Different parts of the sporophytic plant body (Leaves, sporophyll, stem, rhizophore and root) were takenfrom *Selaginella bisulcata* Sring. All the experiments were done byextracts of fresh plant materials. In one set, 100 mg of each of leaf, sporophyll, stem and rhizophore were collected in summer. Each sample of this 100 mg plant parts was crushed with mortar and pestle and extracted in 80% boiled ethanol. This ethanolic mixture was centrifuged at 4000 RPM for 10 min. Then the supernatant was taken out and its total volume was made to 5 ml with 80% boiled ethanol. Then 4 ml distilled water was added to this alcoholic extracts and was kept on a hot plate at 40^{o}C to evaporate the alcohol. Thus the crude extract comes in water solution with a concentration of 2.5% v/v. For each extraction of plant parts, 10 replicates were made for summer and winter.

TOTAL PHENOL: In second set of experiment, total phenols from each 100 mg fresh wt of different plant parts collected and extracted according to the method of Bray & Thorp, 1954. The biochemical analysis of the crude extracts was done (Vyas, *et.al.*, 1989; Britto *et.al.*, 1994; Patric *et.al.*, 1995;). Plant materials were extracted in 80% boiled ethanol, the extract contains plants total protein, total phenols and total soluble and insoluble carbohydrates. As extracted phenols and crude extracts were made from 100 mg plant tissue, the phenol contents were same in both. Total protein content was determined by method of Moore and Stein,1948. Total carbohydrate content was determined following the methods of Mc Cready *et al*., 1950. For all the biochemical analysis, 10 replicates were made for each season. The treatment consisted of three factors- (1) Sporophytic plant body (2) Plant parts (Rhizophore, stem, sporophyll, leaves, root) (3) Bacteria (*Bacillus subtilis* AR-2 and *Escherichia coli* XL-1 Blue).

ANTIBACTERIAL ACTIVITY: Antibacterial activity was measured using 'Agar cup' method (Tortura, *et. al.*, 2001). In agar cup assay, nutrient agar plates of 2 cm thickness were prepared. One set was inoculated with *Bacillus subtilis* AR-2 and the other with *Escherichia coli* XL-1 Blue. Cups of 9 mm diameter were made in the plates in a systematic manner with cork borer. The 0.1 ml water extract of the different parts of sporophytic plant body and their extracted phenols were applied in separate cups and incubated at 37^{o}C. After 24 hrs, diameter of the hallow zones formed due to bacterial lyses were measured. Distilled water was used as control. In each of the experiments 5 replicates were made.

PHYTOCHEMISTRY: Total protein was estimated following Moore & Stein, (1948) and Berenbaum& Zangri's, (1996) method. Soluble and insoluble carbohydrates were estimated

bythe methods of Mc Cready *et al.* (1950).

HPLC: HPLC analysis was performed by using available standards of phenolic compounds following Boudet (2007) and Boligon & De Brum (2012). The extracted total phenol from *Selaginella bislcata*was dissolved in 10 ml HPLC grade methanol and was passed though Whatman membrane filter before injecting in the C18 column. Analysis of extracted total phenol was performed by using 515 HPLC pump and 2489 UV/VIS detector of Waters Company. HPLC analysis of crude extract was not performed as several unknown compounds may be present and requirement of standards to identify them. Further work is needed in this respect.

RESULTS

From the present study it has been revealed that the different parts of *Selaginella bisulcata* Spingaccumulate different amounts of secondary metabolites in different climatic conditions (summer and winter) (Table: 1). Stem and Rhizophore contain maximum amount of phenols in both the seasons. In two seasons*Selaginella bisulcata* stem contains 250.43 µg/mg fresh wt phenol in winter, where as in summer it contains 240.51 µg/mg fresh wt phenol, which is also highest amount found in different parts of the plant parts studied in *S. bisulcata*. In both the seasons the sporophylls contain significant amount of phenols, it is about 195.23 µg/mg fresh wt phenols in summer and 211.17 µg/mg fresh wt phenols in winter followed by leaf which contain 187.32 µg/mg fresh wt in summer and 199.41 µg/mg fresh wt in winter respectively and root 147.41 µg/mg fresh wt in summer and 141.44 µg/mg fresh wt in winter.

Spophyllsand sterile leaves of *Selaginella* are small in size, microphyllous, very thin in textureand probably for these reasons both sporophylls and sterile leaves accumulate very little amount of proteins, it is about 11.21 µg/mg fresh wt in summer and 10.98 µg/mg fresh wt in winter.In both seasons *Selaginella bisulcata* accumulates highest amount of phenols (250.43 µg/mg fresh wt in summer and 240.51 µg/mg fresh wtin winter) in stem followed by rhizophore (230.32µg/mg fresh wt in summer and 235.47 µg/mg fresh wt in winter) and root (10.91µg/mg fresh wt in summer and 10.0791µg/mg fresh wt in winter).Sporophylls stood third position in respect to accumulation of phenols (195.23 µg/mg fresh wt in summer and 211.17 µg/mg fresh wt in winter. In rhizome a little variation is found in accumulation of phenols among the two seasons, which is 230.32 µg/mg fresh wt of in summer and 235.47 µg/mg fresh wt in winter. The total concentration of phenols was highest in summer season i.e. in heat stress condition and protein in winter i.e. in cold stress condition. On the other hand, total concentrations of soluble and insoluble carbohydrates were maximum in winter i.e. in heat stress condition (Table: 1).

Accumulation of biochemical compounds in different parts of the sporophytic plant body of *Selaginella bisulcata*Spring shows a definite seasonal trend. Phenols and proteins accumulation was highest in heat stress condition i.e. in summer season, whereas the

condition was reverse i.e. in cold stress condition in cases of soluble and insoluble carbohydrates. Increase in protein content was very little in winter in respect to summer season. Here also the Sporophyll accumulates highest concentration of protein 15.32µg/mg fresh wt in summer and 15.78 µg/mg fresh wt in winter, followed by leaf (12.51-13.00 µg/mg fresh wt) > Rhizophore (13.12-12.40 µg/mg fresh wt) >Stem (47.27-53.87 µg/mg fresh wt). Soluble and insoluble carbohydrates follow the reverse trend i.e. increase in cold stress condition in respect to phenols and proteins. Rhizophore which is the main accumulator of soluble carbohydrate contains 23.32-26.30 µg/mg fresh wt, followed by stem(21.72-14.30 µg/mg fresh wt) >sporophyll (12.24-10.60 µg/mg fresh wt) > leaf (11.67-10.44µg/mg fresh wt). Accumulation of insoluble carbohydrates was greater than soluble carbohydrates in leaf and sporophyll but reverse in other parts. Sporophyll accumulates maximum amount of insoluble carbohydrates (23.31-1732 µg/mg fresh wt) during winter and summer respectively among the plant parts studied, followed by leaf (14.41-18.62 µg/mg fresh wt) >stem (19.73-18.21 µg/mg fresh wt) >rhizophore (20.32-18.79µg/mg fresh wt) (Table: 1).

The results found in antibacterial activity was that the, both crude extracts and extracted phenols showed bacterial lyses against gram positive (gr +ve) and gram negative (gr –ve) bacteria. Here, crude extracts showed higher inhibitory property than extracted phenols. It has also been found that gr+ve bacteria are more prone to extracted phenols than gr –ve bacteria (Figure: 1). Inhibition zone (hallow area) created by fertile leaf extract was larger than any other parts of the plant in respect to crude extracts as well as extracted phenols (Fig: 1).

DISCUSSIONS

From the above results it is found that, the plant extracts of *Selaginella bislcata* Springshowed significant inhibitory activity against bacterial grm +ve bacterial strain, but show less significant inhibitory activity againstand gr – ve bacterial strain. Crude extracts of the plant parts were more potential than extracted phenols regarding antimicrobial property. It is probably due to the presence of some unknown compounds and phenols which cumulatively inhibit bacterial growth.

HPLC analysis of extracted total phenol reveals that there are about six phenolic compounds present in the plant extract namely Ascorbic acid, Gallic acid, Resorcinol, Catechol, Vanillinand Benzoic acid.

Antibacterial property of this plant might be attributed due to the presence of number of phenolic compoundsatmoderate levels. This is supported by the presence of moderate phenol concentration (240.51-250.43µg/mg wt) in each parts of the plant.

Now a day's microbial resistance to antibiotics has been a global concern, as it spans nearly all known classes of natural compounds. So, in this alarming situation, need of new compounds for antibacterial therapy is very urgent. It has been found that phenolic compounds in plants have strong antibacterial properties against gr+ve and gr–ve bacteria. Pharmacological,

pharmaceutical, phytopathological and food processing industries are some of the fields where phenolic compounds can be applied as bio- preservatives. *Selaginella bisulcata* Springshowed potential evidence for presence nine different kinds of phenolic compounds which has ethno-pharmacological use and promising broad spectrum antibacterial drug. Further work is needed to isolate those active principles responsible for antibacterial properties present in crude extract.

CONCLUSION

From the above result and discussion we can conclude that *Selaginella bisulcata* Spring have the potentiality to establish itself as a broad spectrum ethno-pharmacological antibacterial drug. Current microbial resistance to antibiotics has been a global concern, as it spans nearly all known classes of natural compounds. So, this type of research work for searching new sources of antibacterial agents and new antibacterial compounds is required to enrich the medical science. Further work is in progress to isolate the causal compounds responsible for antimicrobial properties.

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Table: 1- Total phenols, total protein, soluble and insoluble carbohydrate contents in different plant partsof*Selaginella bisulcata* Spring.

Plant Parts	*Selaginella bisulcata* Spring	
	SUMMER	**WINTER**
	Concentration of Phenols (µg/mg fresh wt)	
1. Leaf	187.32	199.41
2. Sporophyll	195.23	211.17
3. Stem	250.43	240.51
4. Rhizophore	230.32	235.47
5. Root	147.61	141.92
LSD at 5%	1.5	1.2
	Concentration of Protein (µg/mg fresh wt)	
1. Leaf	11.21	10.98
2. Sporophyll	15.32	15.78
3. Stem	12.51	13.00
4. Rhizophore	12.40	13.12
5. Root	10.91	12.07
LSD at 5%	0.78	0.69
	Concentration of Soluble carbohydrate (µg/mg fresh wt)	
1. Leaf	10.44	11.67
2. Sporophyll	12.24	10.60
3. Stem	21.72	14.30
4. Rhizophore	23.32	26.30
5. Root	19.25	21.71
LSD at 5%	0.95	0.78
	Concentration of insoluble carbohydrate (µg/mg fresh wt)	
1. Leaf	14.41	18.62
2. Sporophyll	17.32	23.31
3. Stem	19.73	18.21
4. Rhizophore	18.79	20.32
5. Root	09.32	10.47
LSD at 5%	0.87	0.91

Table: 2. Retention times of phenolic compounds present in ethyl acetate extract of *Selaginellabisulcata* Spring.

Phenolic compounds	Retention time	Area	Height	Concentration
Ascorbic acid	2.875	52,900	16,881	84.446
Gallic acid	6.097	3353	227	0.534
Resorcinol	12.850	1638	154	0.625
Catechol	16.200	559,222	26,580	24.276
Vanillin	28.254	294,220	14,324	22.544
Benzoic acid	39.809	517,865	19,835	348.303

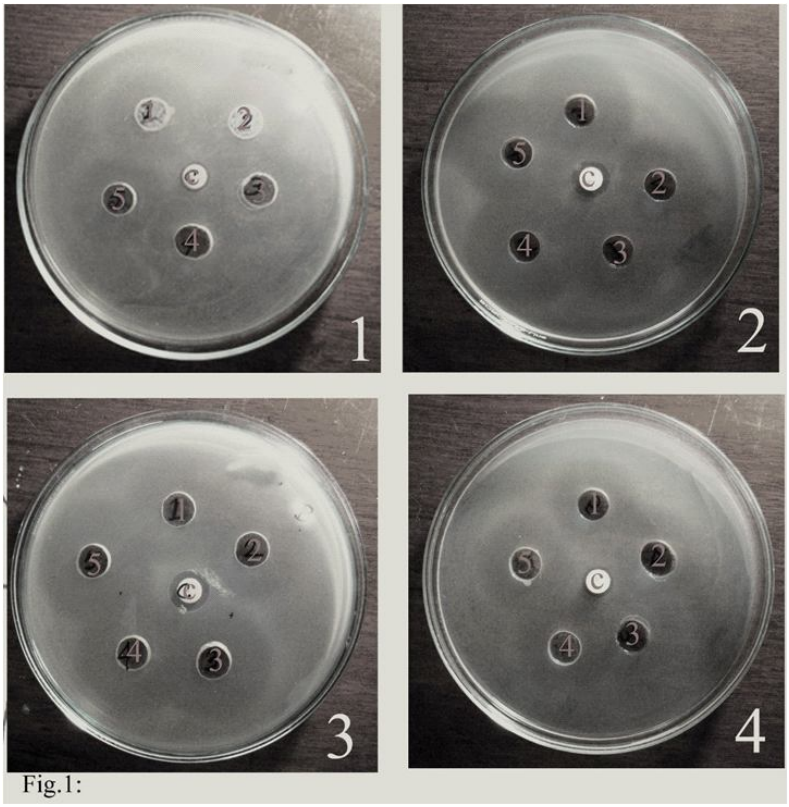

Fig.1:

Legend to the Figure: 1

1: Effect of Crude extract from different parts of *Selaginella bisulcata* on *Bacillus subtilis* AR-2

2: Effect of Crude extract from different parts of *Selaginella bisulcata* on *Escherichia coli* XL-1 Blue

3: Effect of Extracted Phenol from different parts of *Selaginella bisulcata* on *Bacillus subtilis* AR-2

4: Effect of Extracted Phenol from different parts of *Selaginella bisulcata* on *Escherichia coli* XL-1 Blue

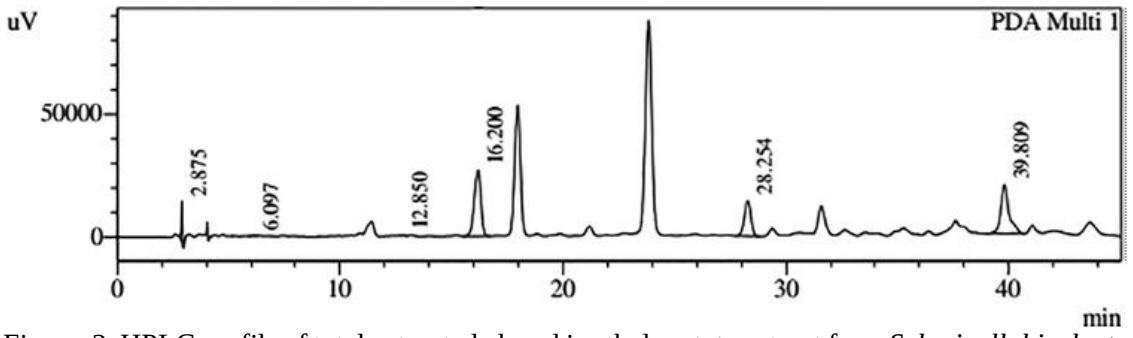

Figure: 2. HPLC profile of total extracted phenol in ethyl acetate extract from *Selaginellabisulcata* Spring

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Biodiversity with Special reference to Genetic Biodiversity and Sustainable Development- A brief review

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ABSTRACT

Biodiversity is the variety and variability of life on Earth. Biodiversity is the variability among living organisms from all origins, including terrestrial, deserts, marine, and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species, and of ecosystems. The diversity in the genetic makeup of a species is called genetic diversity. There are often a number of varieties or strains within a species that differs from each other. The differences are due to very minute variations in their genetic setup. The greater diversity in genetic constitution within a species enables it to adopt and survive the changing environment more effectively. Effective conservation of plant genetic resources there must be a clear understanding of extent genetic diversity of concerned species and it distribution, structure and material that it to be conserved either ex situ or in situ conservation. Sustainable development is the need of the present time not only for the survival of mankind but also for its future protection. By conserving, restoring and sustainability using biodiversity, we ensure that we have viable solutions to present and future challenges, including climate change, food scarcity, water scarcity, sustainable development, peace and security.

Key words:
Biodiversity,
conservation,
genetic biodiversity,
sustainable development

Introduction

In nature there we find a composite structure of flora and fauna along with abiotic components which ultimately constitutes a natural ecosystem. At present this ecosystem is clearly facing a wide and disconcerting array of environmental problems and ultimately culmination of biodiversity loss in their natural habitats.Until recently, biodiversity was a word not widely heard outside circles of ecologists and conservationists but now it is almost a buzzword used by a wide spectrum of environmentalists and policy maker.

Actually Biodiversity is the abbreviated word of Biological Diversity. Norse and McManus (1980) first defined biodiversity. Its abbreviation into 'biodiversity' was apparently made by Walter G. Rosen in 1985 during the first planning meeting of the 'National Forum on Biodiversity' held at Washington DC in September 1986 (UNEP 1995). Later Wilson and Peters 1988 introduced the notion of biodiversity in their book of Biodiversity and popularized the word among the scientific

community as well as the common people.In a word Biodiversity is the totality of genes, species, and ecosystems in a region.In simple terms, biodiversity is a measure that attempts to describe a holistic way the total variety of life on the planet.

Biodiversity describes the richness and variety of life on earth. Biodiversity includes the number of different organisms and their relative frequencies in an ecosystem. It is the variation among living organisms from different sources including desert, terrestrial and marine ecosystems, and the ecological complexes of which they are a part.There are three levels of biodiversity. These are-

Species richness and species diversity in a particular area or ecosystem; species richness is the simpler one, as it only counts the number of species; but species diversity is more complex in that it also takes the number of organisms for each species into account. Species diversity refers to the variety of different types of species found in a particular area. It is the biodiversity at the most basic level. It is the biodiversity observed within a community. It stands for the number and distribution of species. The number of species in a region varies widely depending upon the varied environmental conditions. It includes all the species ranging from plants to different microorganism (Hamilton, 2005).

Ecosystem diversity represents the entire biological and physical content of a locality (Sloep and Dam, 1995). Ecological biodiversity refers to the variations in the plant and animal species living together and connected by food chains and food webs. It describes the assemblage and interaction of species living together and the physical environment a given area. It relates varieties of habitat, biotic communities and ecological processes in biosphere. It also includes the diversity within the ecosystem.

Genetic diversity represents the biological variation or capacity for variation, within each species, genetic diversity is vital to the maintenance of ecological stability enabling different species to respond to environmental change and to fulfill different functions within the biosphere. It also helps speciation or evolution of new species and important for agricultural productivity, development, etc. Genetic diversity describes how closely related the members of one species are in a given ecosystem. In simple terms, if all members have many similar genes, the species has low genetic diversity. Because of their small populations, endangered species may have low genetic diversity due to inbreeding. This can pose a threat to a population if it leads to inheritance of undesirable traits or makes the species more susceptible to disease. Having high genetic diversity helps species adapt to changing environments.

Besides the above mentioned levels, the biodiversity concept incorporating the multiple levels of organisation and many different spatial as well as temporal scales (Noss, 1990).

I. Structural: It encompasses landscape

patterns, physiognomy habitat structure, population structure and genetic structure;

II. Compositional: It includeslandscape types, communities ecosystems,species populations and genes;

III. Functional: It comprises—landscape processes and disturbances land use trends,inter-specific interactions, ecosystem processes,demographic processes, life histories andgenetic processes.

The terms alpha, beta, and gamma diversity are used to describe the spatial component of biodiversity.*Alpha diversity* refers to the average species diversity in a habitat or specific area. Alpha diversity is a local measure.Beta diversity represents the differences in species composition among sites.It is the variation of the species composition between two habitats or regions. It takes into account the alpha diversity of the habitats and the number of unique species on each habitat. Gamma diversity is the diversity of the entire landscape (regional species pool).It is a measure of the overall number of species (the diversity) within a region. It is basically the sum of all the species of all habitats within the region of interest. Landscape ecology is the science of studying and improving relationships between ecological processes in the environment and particular ecosystems. This is done within a variety of landscape scales, development spatial patterns, and organizational levels of research and policy.

Genetic diversity

Gene diversity is the total number of genetic characteristics in the genetic makeup of a species, it ranges widely from the number of species to differences within species and can be attributed to the span of survival for a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary.

Genetic diversity refers to the variation in the nucleotides, genes, chromosomes, or whole genomes of organisms. Genetic diversity serves as a way for population to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of males that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals. The huge varieties of different gene sets also define an individual or a whole population's ability to tolerate stress from any given environmental factor.

While some individuals might be able to tolerate an increased load of pollutants in their environment, others carrying different genes, might suffer from infertility or even die under the exact same environmental conditions. Whilst the former will continue to live in the environment the latter will either have to live it or die. This process is called Natural selection and it leads to the loss of genetic diversity in certain habitats. However, the individuals that are no longer present might have carried genes for faster growth or for the ability to cope better with other stress factors.

Any change in the environment natural or

human induced causes a selection of events that only the fittest survive. Anthropogenic impacts are particularly apparent in the coastal zone and increase the number of changes occurring to individuals and populations. Such pressure is exerted by artificial selection (harvesting, aquaculture), degradation of habitats (leading to a reduction of total stocks and thus increasing the likeliness of inbreeding) and the release of framed fish into the wild. These activities reduce the sum of genes available, thus leaving behind the population that is less capable of tolerating any further natural and anthropogenically caused changes in the environment. These activities reduce the sum of genes available, thus leaving behind the population that is less capable of tolerating any further natural or human disturbances in the environment (Zavaleta, 2000; Vellend, 2005).

The loss of genetic diversity is difficult to see or measure. In contrast, the reduction and extinction of population is far easier to see. Extinction is not only the loss of whole species, but is also preceded by a loss of genetic diversity within the species. This loss reduces the species ability to perform its inherent role in the whole ecosystem. Furthermore the loss of genetic diversity within a species can result in loss of useful and desirable traits (resistance to parasites). Reduced diversity may eliminate options to use untapped resources for food production, industry and medicine.

Darwin's The Origin of Species begins with a discussion of variation. Without variation, populations cannot evolve. Soon after Mendel's principles were rediscovered, biologists began to document genetic variation in natural populations. Initially, these efforts focused on conspicuous features of the phenotype-pigmentation, size, and so forth. Later, they emphasized characteristics that are more directly related to chromosomes and genes.

A population is group of individuals belonging to the same species that live in a defined geographic area and actually or potentially interbreed. The genetic information carried by members of a population constitutes that population's gene pool. At first glance, it might seem that a population that is well adapted to its environment must be highly homozygous because you assume that the most favourable allele at each locus is present at a high frequency. In addition, a look at most populations of plants and animals reveals many phenotypic similarities among individuals. However, a large body of evidence indicates that, in reality, most populations contain a high degree of heterozygosity. This built in genetic diversity is often concealed because it is not necessarily apparent phenotypically; hence, detecting it is not a simple task. Nevertheless, the diversity within a population can be revealed by several methods.

The larger the gene pool, the larger the genetic diversity. The higher the chances are that some members of the population will survive or even flourish in time of environmental change and challenges. Basically, the bigger the population, the more likely there will be individuals with some unique combination of genes that will

allow their survival. But what happen when the gene pool is much smaller, in other words, when the gene pool is shallow. In the wild, in a small isolated population of organisms, the choice of mates with whom to breed tends to be restricted to closely related members of the same population. So the genetic makeup of the individuals becomes more and more uniform. And what's a worse, flaw or disabilities that those individuals might be carrying in their genetic information become expressed or appear in the population more frequently and this is known as inbreeding. Evidence shows that maintaining not only large numbers of species in ecosystems, but large numbers of individuals within populations of those species is important to preserving biodiversity overall. Lots of different studies have shown that extinction, permanent loss of a species is preceded by a drop in genetic diversity a decrease in the gene pool, within the threatened species. Degradation of habitats can cause a decrease in population size and promote inbreeding. When the population size of a species is reduced to the point that is almost goes extinct, the species is said to have gene through a genetic bottle neck. American bison are a classic example of such a bottle neck. The drop in the bison population reduced their genetic diversity which makes them more vulnerable to environmental changes and diseases. Just as we cannot get back species that's been lost to extinction , it's very difficult , and in most cases impossible , to get back the genes that are lost when that species goes extinct or when individuals carrying unique genetic combinations die. As human encroach on wild habitats, populations consequently become smaller and so does the gene pool. This represents a loss of options for a population to respond to stresses, whether those are natural stresses or stresses caused by humans. Ecosystem services arguments go straight to this idea of conserving genetic diversity. Gene controls the production of substances that human use in medicine and food and even as energy sources. Preserving genetic diversity increases the likelihood that new substances can be found among wild populations and that the supplies of useful substances human already have can be maintained. The erosion of genetic diversity in agriculturally important species can come in two forms. One is due to artificial selection for species traits that humans find desirable to the exclusion of other traits. The other source of erosion is that we've focused our dependence on only a few organisms. For example, only about 100 or so species of plants account for 90% of our food crops and only three different species corn, rice and wheat account for something close to 70% of the calories consumed by humankind and these include 50% of the plant proteins human eat (Rao and Hodgkin, 2002; Pal, 2012).

One of the most significant questions addressed in population genetics is how much genetic variation exists within natural populations. Genetic variation within populations is important for several reasons. First, it determines the potential for evolutionary change and adaptation. The amount of variation also gives us important clues about the relative

importance of various evolutionary processes because some processes increase variation while others decrease it. The manner in which new species arise and contemporary populations become extinct may depend on the amount of genetic variation harboured within populations. In addition, the ability of a population to persist over time can be influenced by how much genetic varitionm it has to draw on should environments change. For all these reasons, population genetics are interested in measuring genetic variation, attempting to understand the evolutionary processes that affect it, and understanding the effects of human environmental disturbance that may alter it.

Genetic diversity serves as a way for populations to adapt to changing environments. With more variation, it is more likely that some individuals in a population will possess variations of alleles that are suited for the environment. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals.

The academic field of population genetics includes several hypotheses and theories regarding genetic diversity. The neutral theory of evolution proposes that diversity is the result of the accumulation of neutral substitutions. Diversifying selection is the hypothesis that two subpopulations of a species live in different environments that select for different alleles at a particular locus. This may occur, for instance, if a species has a large range relative to the mobility of individuals within it. Frequency-dependent selection is the hypothesis that as alleles become more common, they become more vulnerable. This occurs in host–pathogen interactions, where a high frequency of a defensive allele among the host means that it is more likely that a pathogen will spread if it is able to overcome that allele.

Gene diversity is the proportion of polymorphic loci across the genome. Heterozygosity is the fraction of individuals in a population that are heterozygous for a particular locus. Allele per locus is also used to demonstrate variability. Nucleotide diversity is the extent of nucleotide polymorphisms within a population, and is commonly measured through molecular markers such as micro- and minisatellite sequences, mitochondrial DNA, and single-nucleotide polymorphisms (SNPs). Furthermore, stochastic simulation software is commonly used to predict the future of a population given measures such as allele frequency and population size (Govindaraj *et al.*, 2015).

Genetic diversity can also be measured. The various recorded ways of measuring genetic diversity include i. Species richness is a measure of the number of species, ii. Species abundance is a relative measure of the abundance of species, iii. Species density is an evaluation of the total number of species per unit area.

Actually where biologists found variation there is the every possibility the evolution of new species. Variations are the raw materials for the evolution of new species according to Darwin's

view. Evolutionary importance of genetic diversity: i. Adaptation: Variation in the population's gene pool allows natural selection to act upon traits that allow the population to adapt to changing environments. Selection for or against a trait can occur with changing environment – resulting in an increase in genetic diversity (if a new mutation is selected for and maintained) or a decrease in genetic diversity (if a disadvantageous allele is selected against). Hence, genetic diversity plays an important role in the survival and adaptability of a species. The capability of the population to adapt to the changing environment will depend on the presence of the necessary genetic diversity. The more genetic diversity a population has the more likelihood the population will be able to adapt and survive. ii. Small population: Large populations are more likely to maintain genetic material and thus generally have higher genetic diversity. Small populations are more likely to experience the loss of diversity over time by random chance, which is called genetic drift. When an allele (variant of a gene) drifts to fixation, the other allele at the same locus is lost, resulting in a loss in genetic diversity. In small population sizes, inbreeding, or mating between individuals with similar genetic makeup, is more likely to occur, thus perpetuating more common alleles to the point of fixation, thus decreasing genetic diversity. Concerns about genetic diversity are therefore especially important with large mammals due to their small population size and high levels of human-caused population effects. iii.Mutation**:** Random mutations consistently generate genetic variation. A mutation will increase genetic diversity in the short term, as a new gene is introduced to the gene pool. However, the persistence of this gene is dependent of drift and selection. Most new mutations either have a neutral or negative effect on fitness, while some have a positive effect.A beneficial mutation is more likely to persist and thus have a long-term positive effect on genetic diversity. iv.Gene flow: Gene flow, often by migration, is the movement of genetic material. Gene flow can introduce novel alleles to a population. These alleles can be integrated into the population, thus increasing genetic diversity (Govindaraj *et al*., 2015).

The maintenance of genetic diversity is essential to facilitate adaptations of species to this environmental change in order to increase the probability of longterm sustainability of ecosystem structure. Preserving the genetic diversity of species can enhance species' ability to adapt to new environmental conditions and thus influence their survival over these time frames.Genetic diversity originates as a result of recombination or mutation during the process of cell multiplication. Genetic diversity is the only way to comprehensively describe bacterial diversity in any ecosystem. Molecular data have revealed the lot of scope of microbial diversity. Often sequences of 16S ribosomal RNA (rRNA)—or of the corresponding DNA—are determined to assess genetic diversity in bacteria. Unique rRNA sequences are termed ribotypes. Genetic diversity is the basis for all evolution. It provides the means for populations to adapt to their ever-changing environment.

The more genetic diversity, the better the chance that at least some of the individuals within the population will have an allelic variant that is suited for the new environment, and will produce offspring and continue the population into subsequent generations. Populations with low genetic diversity can become so well adapted to local conditions that any environmental change may suffice to destroy them. Thus, for preserving biodiversity at all levels, genetic diversity is of great importance.

Biodiversity assessments have included rudimentary studies of genetic diversity, mainly limited to species counts because morphologically differentiated forms are easily recognized and the evolutionary significance of species is understood. Genetic diversity studies based on molecular population genetic and phylogenetic methods, the database itself is sparser and more diverse because molecular population genetics and evolutionary genetics are new fields. Numerous allozyme studies have been conducted in all types of aquatic environments; the database of genetic diversity using mtDNA RFLP data is large and still growing; microsatellite DNA studies are becoming the standard for investigating population genetics using nuclear DNA; and the database for DNA sequence data is expanding exponentially (Bert *et al*., 2002).

Because genetic variation is the raw material of natural selection, biodiversity ultimately is genetic diversity. Populations are natural entities that should be preserved for sustenance and they are essential components of ecosystems that provide basic life-support systems and ultimately depend on preserving genetic variation both among and within species (Bert *et al*., 2002).

The causes of biodiversity depletion-by anthropogenic activity-over exploitation of natural resources, indiscriminate felling of trees causing wanton clearance of forest cover, rapid urbanization in consonance with industrialization, gradual increasing of Global Trade through over exploitation of natural bio-resources, increasing population in an alarming rate, environmental influences i.e. acidification & Climate Change and natural calamities like tsunami, cyclone, super cyclone, forest fire, earth quake,land-slides, ozone depletion, etc are also the warning signals for the gradual habitat changes which in the long run invite the extermination of valuable species from our earth.

Biodiversity Conservation is worthwhile, this can be done by sustainable management of ecosystems, by taking afforestation programme terrestrial as well as coastal areas so as to stop tsunami, cyclone etc, by judiciously using natural resources and by introducing MAB, sanctuary, National Parks etc (Krishnamurthy, 2004).

Genetic diversity Conservation

Genetic diversity has a great importance from the individualistic and population point of view. All the phenotypic plasticity is dependent on the genetic variability of any organisms which also helps it to adapt to and evolve in different environmental pressures. Mainly three lines of

evidences are there which support the ecological consequences on genetic diversity. Information about genetic diversity is necessary for the development of appropriate strategies in conservation biology as well as in many other applied fields. From a basic evolutionary standpoint, genetic diversity is assumed to be crucial for the evolutionary potential of a species (Mukhopadhyay & Bhattacharjee, 2016). An understanding of genetic diversity and its distribution pattern is essential for its conservation and use. It will help scientists and policy makers in determining what to conserve as well as where to conserve, and will improve our understanding of the taxonomy and origin and evolution of valuable plant species. In order to mange conserved germplasm better, there is also a need to understand the genetic diversity that is present in collections. This will help us to rationalize collections and develop and adopt better protocols for regeneration of germplasm seed. Through improved characterization and development of core collections based on genetic diversity information, it will be possible to exploit the available resources in more valuable ways (Rao and Hodgkin, 2002). The most powerful catalyst in the field of conservation has been the advances in genetic and molecular technologies, leading to a wide variety of molecular methodologies for application in conservation and population genetic studies. Recently, molecular methods have been applied intensively in conservation biology and genetic diversity studies, primarily as selective molecular tools, in resolving the empirical questions of conservation and evolutionary concern. Several analytical statistical tools and genetic diversity indices are now available to estimate or quantify genetic diversity of an organism with more sophisticated way (Mukhopadhyay &Bhattacharjee, 2016).

The genetic wealth and traditional knowledge of India need to be protected through patenting. Our traditional knowledge of plants should be protected for which efforts need to be taken on priority basis. In situ conservation of agricultural bioheritage can be accomplished by the maintenance of the various cultivars, races and the genetic diversity present in and among populations of the many species used directly in agriculture or used as sources of genes, in habitats were such diversity arose and continues to grow (Zavaleta, 2000; Balakrishnan, 2012). Scientists and other citizens should collaborate with governmental organizations, from local to national levels, in developing and implementing policies and regulations that reduce environmental deterioration and changes in biodiversity. For example, more stringent restrictions on the import of biotic materials could curb the rate of biotic invasions, and improved land and watershed management could reduce their rates of spread (Chapin *et al.*, 2000).

Conclusion

Biodiversity conservation and sustainable development are two inter-related branches focusing on social progress, economic growth and environmental protection on one side, and ecosystem conservation on the other.

Conservation includes the efforts carried out in protected areas such as national parks and community reserves, and in other areas with reach and important biodiversity where conservation is not the main focus. It is in these latter productive landscapes where sustainability is needed most. Sustainable agriculture, sustainable fisheries and sustainable management of natural resources are main approaches for preserving these landscapes for long-term social, economic and ecological benefits.

So considering the overall threats on our earth it is the need of the hour that if we don't take necessary precautionary measures for the conservation of our natural bio-resources (i.e. biodiversity) the time is not far away when our future generations have to face a lot of consequences and subsequently there will be no next button to them to restore conducible environ on the earth. So the first and foremost point is the massive involvement of the local people as an aid to not only conserve our biodiversity but also for the interest of environmental stability and subsequently to ensure the sustainable development by extensive local area exploration, documentation of floral and faunal components and their utilisation for the future generation. The most important measure would be an impartial and true assessment of the impact of human activity on the environment and formulation of optimization and management schedules, since a cleaner environment is sure to be conducive to maintain biodiversity at the optimum level, conserving our bio-heritage intact (Matta *et al*., 2011; Mukherjee andChakraborty, 2017).

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Comparative Study of Foliar Venation Pattern and foliar Anatomy of Two Species of Anaphalis Dc. (Asteraceae)

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ABSTRACT

Leaves are the indissoluble and constant part of a plant body. The gross form of angiosperm leaves include features like size, shape, nature of margin, form of the apex, base and petiole, positioning of glands and nature of venation pattern. They have the potential of diagnostic characters within their venation. The objective of this paper was to describe and compare the foliar morphology, venation pattern and foliar anatomy of *Anaphalis margaritacea* (L.) Benth. & Hook. f. and *Anaphalis triplinervis* Sims. ex C. B. Clarke. to distinctly identify the plants. The major venation pattern from fresh as well as dried leaves along with areoles, minor veins, marginal venation, veinlet endings, the 'very tips' and other associated characters were analyzed for the two species. Three primary veins, sparsely placed areoles arranged in the longitudinal direction of the lamina, free vein endings consisting of one spirally thickened tracheid and some free vein endings associated with sclerotracheoids atre the distinctive characters of *A. margaritacea***.** Whereas, in *A. triplinervis***,** the number of primary veins are five with dense areolation, arranged randomly. The free vein endings consist of four to five spirally thickened tracheids and covered with distinct sclerenchmatous sheath cells throughout. Other characters of the leaf of two species in addition to the above stated features can be elaborated asan identification key.

Introduction

Generally the floral characters of the plants are used for the identification of angiosperms. In 1974 Radford et. al. have pointed out that due to lack of detailed classification features of leaf, they are neglected organs in taxonomic and comparative morphological studies. The arrangement of veins in the lamina is an important component of the study of leaf architecture. Though the leaves are present in plants for much greater part of its life span than the flowers and fruits, still its use in identification of plants is very limited.After the publication of venation pattern terminologies by Ettingshaesun (1861) and systemization of these terminologies by Hickey (1973) attempts have been made to study the leaf architecture of some dicotyledonous families (Sehgal & Paliwal,1974; Jain, 1978). In 1976, Melville gave the venation pattern terminologies for both monocots and dicots. In the recent past attempts have been made to study the leaf architecture of different species belonging to different genera

under family Asteraceae by Banerjee & Deshpande (1973), Banerjee (1978) Ravindranath &Inamdar (1982), Akinubi et al. (2014) and others. A similar kind of work was done by Maitra and Mukherjeein 2017 on 4 species of *Spilanthes.*

So farno work has been done on leaf architectural pattern of the members of*Anaphalis* sp. In this paper the foliar anatomical characters of. *Anaphalis margaritacea*(L.) Benth. & Hook. *f*. and *Anaphalis triplinervis* Sims ex C. B. Clarke. are studied and considered as an additional tool for identification ofthis genus

Materials and Methods

Materials were collected from Darjeeling districts of West Bengal and East Sikkim in sets of 4-5 in numbers and identified with the local floras. These specimens were matched with the collections of the Department of Botany, University of Kalyani, Nadia. Author citations were matched following literature of Brummitt and Powell (1992) and website of the International Plant Names Index (www.ipni.org) and The Plant List (www.theplantlist.org).The voucher specimens were kept at the departmental herbarium of Government General Degree College, Keshiary. The specimens are listed in Table 1.

For future use matured leaves are preserved in FAA solution where the ratio of commercial formaldehyde: acetic acid: 70% alcohol is 5:5:90. Ten leaves of each species collected from five different plants were cleared following the techniques of Dilcher, 1974.

For studying theleaf vascularization, the entire lamina along with petiole was soaked overnight in 4% NaOHand transferred to chloral hydrate solution (Arnott,1959) and finally stained with 1% aqueous safranin. After gradual dehydration, permanent slides were prepared and mounted in Canada balsam. For representation of the vein orders upto 3rd order, magnifications with 5x eye piece and 4x objective of the microscope has been used and for higher order venations a suitable median portion of the lamina, 1/3 from apex and 2/3 from base of the laminawere selected and the vascular pattern drawn from the 3rd order onwards along with epidermis and trichomes, under mirror type camera Lucida (Fig.1). For microscopic photographs Olympus CX21i microscope and camera UCMOS 10000 KPA of Topcam were used (Plate 1).

In the same way, marginal venation pattern and free vein endings have been sketched under higher magnification (10x eyepiece and 40x objective) of the microscope. For anatomical descriptions, work ofMetcafe and Chalk (1950) has been followed and for foliar venation patternpublications ofthe followingauthors were consulted[Hickey (1973, 1979) Melville (1976), Prabhakar and Ramayya (1982), Annamani and Prabhakar (1991a;b, 1993, 1994a;b), Ferzana*et al* . (1991)and Dilcher (1974)].

Table 1. List of Specimens Collected

S. No.	Name of Species	Localities	Field No.
1.	*Anaphalis margaritacea* (L.) Benth.& Hook. *f.*	Happy Valley Tea Estate, Darjeeling Dist. Rishav, Darjeeling District Juluk, East Sikkim	76 112 145
2.	*Anaphalis triplinervis* Sims ex C. B. Clarke.	Tipindara, Rishav, Darjeeling District Juluk, East Sikkim	116 143

OBSERVATIONS

The identity of the two species of *Anaphalis* viz. *A. margaritacea* and *A. triplinervis* has been established on the basis of leaf morphology (Table 2) and anatomy (Table 3).An artificial key to the species was alsodeveloped:

Table 2. Leaf Morphology of *Anaphalis* spp.

Name of the species / Characters	*A. margaritacea*	*A. triplinervis*
Leaves	Leaves alternate, simple, narrowly linear or oblong,1 - 3 cm long x 0.1-0.5 cm wide, acute at apex, margin entire,auriculate at base, herbaceous, densely woolly on lower surface, less on upper side, sessile.	Leaves alternate, simple, lanceolate to elliptic-lanceolate,3-5 cm long x 1-1.5 cm wide; acute to acuminate at apex, margin entire, cuneate at base, leathery, usually 3-veined, woolly-white above and rusty-grey beneath, sessile.

DISCUSSION

Leaf anatomy according to Carlquist (1961) provides a variety of features that could be used for taxonomic purposes. This is evident from the leaf characters present in the two species of *Anaphalis*. Leaf morphology to leaf anatomy of both the species have lots of contrasting feature expressed in the observation table 2 and 3. The most eminent among them are the leaf shape, primary vein number, quaternary veins, areolar type, areolar density, veinlets and their association, epidermis and trichomes as mentioned inTable 2 and 3.Figure 1 and Pate 1 are also there to substantiate the observation. The most noticeable character among all, is the veinlets association with sclerotracheoids in *A. margaritacea*whereas in *A. triplinervis*,sclerenchymatous sheath cells covering each and every order of veins (Fig.-1C, C' and Plate 1 B, B').

CONCLUSION

It is proved that leaf venation pattern and leaf anatomical characters are stable in the studied species of *Anaphalis* Dc. From the present findings it may be concluded that leaf architecture characters are good taxonomic markers in plant identification and classification. However, it is also clear from the studies that these characters of leaves are potentially significant to delimit the taxa and

Table 3. Leaf anatomy of *Anaphalis* spp.

Name of the species / Characters	*A. margaritacea*	*A. triplinervis*
Venation type	Perfect-acrodromous	Palmate-acrodromous
Primary vein (1°)	3, basal, moderate, middle onestraight, lateral 2 slightly curved converging at the apex, unbranched.	5, basal, perfect, moderate, middle onestraight, lateral four curved, unbranched.
Secondary veins (2°)	10-14 pairs as connecting to primary veins, curved, branched or unbranched .	14-17 pairs as connecting to primary veins, curved, branched or unbranched
Intramarginal veins	Present as extreme lateral, continued from base to apex, merging with the primary laterals near the apex	Present as extreme lateral, primary, continued from base to apex.
Tertiary veins (3°)	Connecting the secondaries, mostly composite	Connecting the secondaries, mostly unbranched.
Quaternary veins (4°)	Rarely present	Thick, straight and relatively orthogonally oriented
Highest vein order (as free vein endings)	3°.	5°
Areoles	Areoles imperfect.	Areoles perfect
Areole shape	Mostly tetrangular rarely pentangular	Mostly tetrangular, rarely pentangular
Areoles/ sq. cm	1100	2678
Nature of free vein endings within the areole	Short in size, consisting of one spirally thickened tracheid of normal shape; the very tip normal and pointed or blunt due to coupling of very tip with sclerotracheoid	Short to medium in size, consisting of four to five spirally thickened tracheids of normal shape; the very tip normal and pointed, completely covered with sclerenchymatous sheath cells.
Associated features	The areoles in the margin linear-oblong, tetragonal, arranged according to long axis of leaves without free vein endings.	The areoles in the margin alike to the normal venation of laminar part
Upper epidermis	Consisting of compactly arranged thin walled corrugated to sinuate, elongated rhomboidal cells. Cell count ˜ 710 per sq. mm	Consisting of compactly arranged thin walled ovoid cells. Cell count ˜ 2253per sq. mm
Lower epidermis	Consisting of thin walled corrugated to undulated rectangular cells, associated with stomata. Cell count ˜ 800 per sq. mm	Consisting of compactly arranged thin walled ovoid cells, associated with stomata. Cell count ˜ 1972 per sq. mm
Stomata	Hypostomatic leaves with anomocytic stomata, stomatal frequency 160 per sq. mm	Hypostomatic leaves with anomocytic stomata, stomatal frequency 207 per sq. mm
Trichomes	Nonglandular flagellate trichome with one celled oval foot, 2-3 celled stalk whose basal cell is cylindrical and broader than median cell, head unicellular flagellate.	Nonglandular, flagellate trichome with one celled oval foot, 2-3 celled oblong cylindrical stack and unicellular long flagellate head.

on the basis of which identification keys are provided.

Key to the studied species of *Anaphalis* based on leaf morphology

1a. Leaves narrowly linear or oblong, 1–3 cm long x 0.1–0.5 cm wide, auriculate at base, herbaceous. ……***A. margaritacea***

1b.Leaves lanceolate to elliptic lanceolate, 3–5 cm long x 1–1.5 cm wide, cuneate at base, leathery, rusty-grey beneath. …***A. triplinervis.***

Key to the studied species of *Anaphalis* based on leaf anatomy

1a. Primary veins 3, areoles quadrangular and pentagonal, arranged usually in the longitudinal direction of the lamina, free vein endings consists of one spirally thickened tracheids, devoid of sheath cells, may associated with sclerotracheoids…..…***A. margaritacea***

1b. Primary veins 5, areoles quadrangular, arranged randomly, free vein endings consists of four to five spirally thickened tracheids, covered with sclerenchymatous sheath cells throughout…… ***A. triplinervis.***

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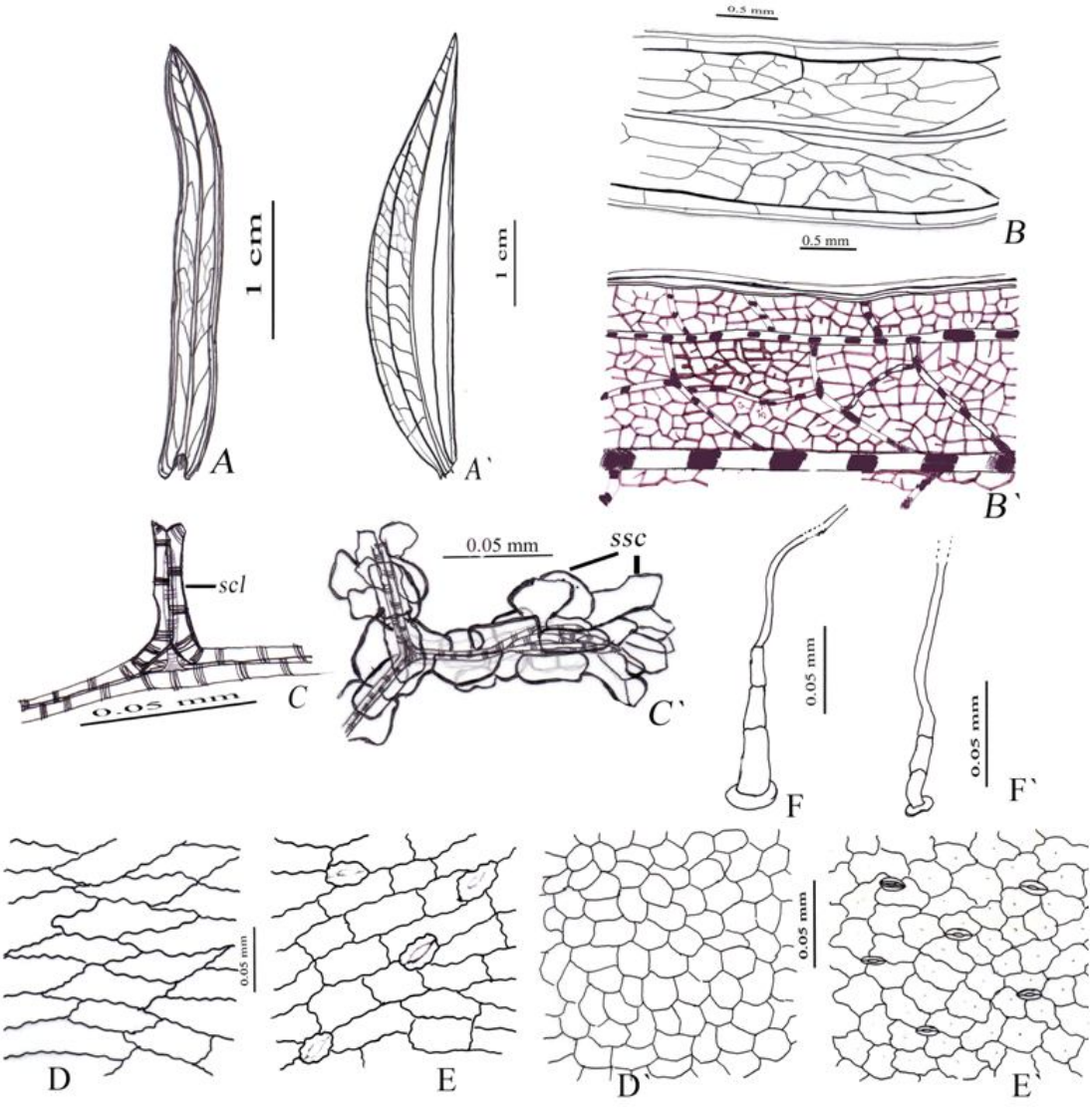

Fig 1: Foliar venation and foliar anatomy of *Anaphalis margaritacea* and *Anaphalis triplinervis*: A-F: *A. margaritacea*: A`-F`: *A. triplinervis*; A& A`. Leaf with major venations*; B-Fand B`-F` are the camera lucida diagrams.* *B & B`. Minor* venations; C & C` Free vein endings; D & D`. Upper epidermis; E & E` . Lower epidermis; F & F`. Foliar trichome;

(scl - Sclerotracheoids, ssc - Sclerenchymatous sheath cells.)

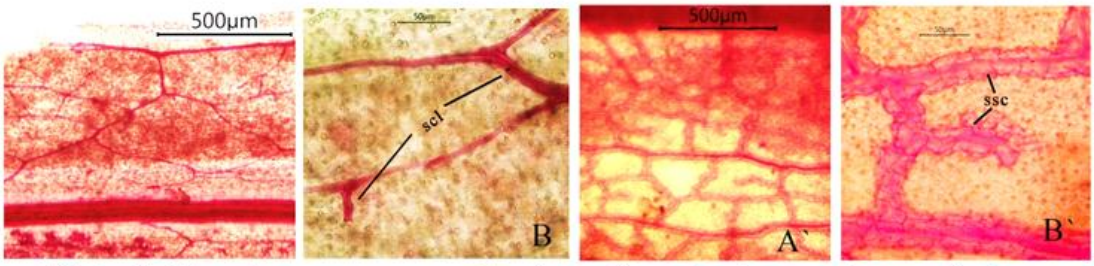

Plate 1: Venation, patterns and vein endings in the species studied: A-B. *Anaphalis margaritacea*: A. Venation pattern; B. Vein endings A`-B`. *Anaphalis triplinervis :* A`. Venation pattern; B`. Vein endings (scl - Sclerotracheoids, ssc - Sclerenchymatous sheath cells.)

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Pacu farming- a threat to local population in Purba Medinipur

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ABSTRACT

Recently introduced Pacu fish farming in Purba Medinipur district is picking up fast without envisaging scientific track to promote or regulate it. The unofficially introduced Pacu in India is projected to be *Piaractus brachypomus* relying on the identifying characteristics. However, the species available in aquaculture and that in aquarium trade is yet to be scientifically validated. The identification of juvenile fish available at hatcheries, farms and aquarium shops based on morphological features may not be correct particularly when possibility of existing different species of Pacu and their interspecific hybrids may exist. Monitoring, risk assessment and identification of the individual species should bescientifically conducted. In Purba Medinipur, the farm-raised Pacu grows well but its production differs from farm to farm. The fish can utilize diets high in carbohydrates and plant proteins, tolerates poor water quality conditions, varied environments and culture conditions. Breeding of the fish is now well established for its farm raising and propagation yet ornamental value of the fish has also further fascinated aquarium shopkeepers and hobbyists to keep and propagate it in Purba Medinipur. Farm raising, hatchery production and aquarium trading of Pacu facilitated inadvertent releases of introduced Pacu which has gravitated into several natural water bodies. It is proposed that the available Pacu farms and hatcheries need to be urgently registered in view of proper management and scientific regulations on Pacu farming. Based on the field oriented information presented in this study, there are several adverse ecological concerns and consequences are observed. The issues and concerns of rapid expansion of Pacu in Purba Medinipur draws attention of the policy makers and the scientific community to address them keeping in view of the larger interest of the farmer's community, society and the environment.

Keyword:
Pacu,
Culture,
Impact,
Local,
Diversity,
Purba, Medinipur.

Introduction

Introduction of exotic fishes in the Indian waters can be traced back more than a century old history. While the country was under the British rule, such fisheries were possibly introducedfor recreational fisheries. Sir Francis Day, the author of the classical work on the Fish fauna of Indian region (Day, 1889), was probably the first person who tried to introduce the brown trout, *Salmotrutta fario* in the Nilgiri waters in the year 1863, but his attempt was unsuccessful (Jhingran, 1975). This was followed by introduction of several exotic fish species from various parts of the world to different regions

of India for augmenting fish production through aquaculture, for sport fishery, for mosquito control, weed control, for ornamental purpose etc with successes and failures. The larvaecidal fishes, such as, *Poecilia retiuculta* and *Gambusia affinis* were introduced in the year 1908 and 1928 respectively, to control mosquito larvae in confined waters. But the larvaecidal value of these species is not well established. There are hundreds of ornamental fish species being imported to our country since the aquarium trade is in progressive growth stage but insecticidal value of these species is not well established. The ornamental fishes, although remain confined to aquarium tanks, their release into natural habitats is not uncommon and the impacts in case of escapee were not yet assessed.

The so-called fish Pacu, *Piaractus brachypomus*(Cuvier 1818) was unofficially introduced possibly during 2012 *via* Bangladesh (Chatterjee and Mazumdar, 2009;Singh and Lakra2011). As per information available in Fishbase, there are 12 species of Pacus (Froese, Rainer and Pauly. 2017). The popular species of Pacu are *Colossomama cropomum* (tambaqui), *Piaractus brachypomus*and Piaractus mesopotamicus. Red-bellied Pacuand black Pacuhave been reported from India (Singh, Dinesh, and Abubakar, 2012).Nevertheless, *Piaractus brachypomus* (Cuvier 1818) commonly known asPacu, pirapitinga, roopchand is understood to be available atmany of the farms, markets and aquarium shops of Indian states and the fish has got both food and ornamental value. In addition, another Pacu like speciescalled piranha is also available which belongs to the family Characidae and subfamily Serrasalmidae belonging to the samegroup (Jegu M. 2003). The morphological information, characteristics and biology of Pacus found in India are yet to be scientifically validated. At the juvenile stage, Pacu resembles piranha (*Pygocentrus natteri*), but differs greatly in behaviour and feeding habits even though they belong to the same family (Singh and Lakra 2011).Since the boundaries of the country are porous, Pacu piracy and unauthorized introductions have been carried out (Singh and Lakra 2011) and actually needed scientific information are lacking. The unofficial culture and breeding of Pacu in India has beenexpanding during recent past causing concern of local fish biodiversity management and encouraging aquaculture for food security. As per reports available, a significant subset of alienspecies can become invasive and have serious adverse impacton biodiversity and related ecosystem services, as well as haveother social and economic impact (Singh and Lakra 2011). Internationally, culture and breeding technology of Pacus, *Colossomama cropomum* (tambaqui), *Piaractus brachypomus* and *Piaractus mesopotamicus* is now well established andavailable (Hashimoto, Senhorini and Foresti, 2012). However, it is also important to mention that inrecent years, hybrids of Pacus have also been produced andreported to represent recent advances in aquaculture of Pacu (Hashimoto, Senhorini and Foresti, 2012). Since three species of Pacus *Colossoma macropomum* (tambaqui), *Piaractus brachypomus* and

Piaractus mesopotamicus are very common at farms and hatcheries inseveral countries, morphological distinction of such unintentional or deliberate hybrids production from the parents becomes highly unidentifiable, particularly between interspecific hybrids and pure species individuals. It is thus difficult to generate species specific information of the individuals available at the farms and markets. Hybrids Pacucan be erroneously identified as pure species in breeding facilities, which might reduce production on farms and negatively affect native populations due to escapes or unscientific stock ingpractices [Hashimoto, Senhorini and Foresti, 2014).These deliberate or unauthorized activities of hybrid production are considered to be more resistant tovaried environmental factors [Moraes, Avilez and Hori, 2006). Further, mislabelling of theexisting species may become a cheat to the market and farmersas the hybrids may not be as productive and remunerative for aquaculture as that of pure species (Hashimoto, Senhorini and Foresti, 2012). Nevertheless, genetic and environmental problems are alsoforeseen and since different species of Pacus are reproductively compatible (Hashimoto, Senhorini and Foresti, 2012). The propagule pressure on the Pacu fish farming in India isequal for aquaculture as food and also for ornamental keeping (Datta and Nandeesha, 2006; Ghosh, and Datta, 2014). It is quite likely that attempts are in operation or maybe made towards creating fancy appearance of the fish through crossbreeding of different species of Pacu for value addition especially in ornamental trade. It is therefore, imperative toensure what species farmers are cultivating and demonstrate authenticity. Absence of monitoring by competent agencies/authorities/scientific organizations, the warnings reported by Hashimoto *et al*. (2012) based on experience elsewhere particularly in the USA and Asia will be needed towards theimplementation of regulatory measures and managementon Pacu culture. Keeping in view of the above facts, presentscenario on the culture and breeding of Pacu in Purba Medinipur has been synthesized and presented in the present paper. Further, variousenvironmental conditions required for the fish to spread hasalso been generated so as to make out possible invasions. The instances of inadvertent releases of the fish in different naturalaquatic bodies are highlighted besides lessons to be learnt from the other countries and even other states of our country to contemplate scientific measures toregulate culture and propagation of Pacu in Purba Medinipur.

Materials and Methods

Present work is mainly based on the field and market survey during March, 2018 to February, 2019.Pacu fish were collected and surveyed in different blocks like Egra-I&II, Contai-I&II, Mahisadal, Moyna, NandigramI, Panskura, Potaspur-I&II,Bhawanpur-I&II,Tamluk of Purba Medinipur district. Different farm sides were also visited and their culture process was noted. Seeds were collected from fish hatchery of Onda, Bishnupur of Bankura district, reported by fish-farmers on Purba Medinipur.

Aquaculture of Pacu in PurbaMedinipur

Both red-bellied Pacu, *Piaractus brachypomus*

and the black Pacu, *Colossomamacropomum* have been found to grow wellin pond of Purba Medinipur. Pacu exhibit potential characteristics feature for use in aquaculture.

Pacu can:

- reproduce under captive conditions
- thrive low on the food chain
- accept prepared feed
- tolerate hares and hardyenvironmental conditions
- can easily be handled,
- grow rapidly
- be cultivated in high density
- be marketed and have consumer's acceptability
- fetch good price and
- be preferred as food as well as ornamental fish.

They exhibit fast growth, and are able to utilize diets havinghigh in carbohydrates and plant proteins. They are resistance topoor water quality conditions and diseases having good fleshquality. Theyhave high ornamental value and attractive aquaculture characteristics. Red-bellied Pacu, *Piaractus brachypomus*, is though native of Brazil is now introduced tostudy area via Bangladesh. It iscultivated in Purba Medinipur, under extensive or semi-intensive type ofculture both as monoculture andpolyculture system.Pacu in the study area is mostly cultivated in Inland water of Moyna, Tamluk, Panskura, Khajury, Bhawanpur, Egra and Contai block of the district.In most of the areas, Pacu is cultured in combination with mainly rohu (Labeo rohita) or even with catla (Catla catla) and mrigal (Cirrhinus mrigala) at a stocking density of 7000 and 5000 per hectare respectively with total production levels of 12-15 MT/ha. Although *Piaractus branchypomus* is one of the introduced species being cultured in the district alone in an area of over 1200 hectares, its compatibility with Indian major carps in mixed/poly culture has not been fully understood but still it is cultured. Nevertheless, there is no standard practice of its culture;the farmers are doing it at their own discretions andconvenience. The production of the fish varies from farm to farm and overall production in the district is assessed to cross 0.1 million tonnes/ha/year.

Invasion risks of Pacu in natural water bodies

Introduction of fish species is a globally widespread practicethat is now serious consideration as such practices cause lossesto native species and homogenization of diversity within andacross continents (Singh and Lakra 2011). In Florida, Pacu was first observed in the wild during the 1960's and initially the Pacu population was thought to be non-breeding and existed only as the escapees of residential aquariums and hence non-invasive. However, they later on colonised and today they are everywhere, from South Florida canals, to Lake Okeechobee and through-outmost of the continental United States (Howard and Brian, 2012). Invasions of red-bellied Pacu populations have further been reported from many other countries such as Philippines, Iran, and Hungary etc. Pacu being South American native fish

hasbeen reported to occur near Yuma, Arizona in June 2006 and inNew Jersy in June 2015 (Howard and Brian, 2012). The occurrence of Pacu has alsobeen reported in Denmark, Michigan and other places. However, invasiveness of the species has yet to be determined. So far as the ecological issues are concerned, there is a report from Papua New Guinea in 2011, where incidence of twohuman deaths was reported due to Pacu attack by biting off thetesticles of fishermen (Rick, 2013). The species possesses a powerfuldentition that can also cause serious bites to humans and otheraquatic organisms and damage to fishing nets such as gill andcast nets as reported in the reservoir of Pune, India (Singh and Lakra 2011). Someof the reports available from different countrieswhere Pacuwere introduced and escaped into natural aquatic bodies arepresented here so as to understand possible risks of its cultureand propagation keeping in view of the international scenario.

Therefore, Pacu culture in Purba Medinipur is at high risk of escaping from farm to the natural water bodies especially in flood prone blocks like Moyna, Panskura and Tamluk sadar. As like other invasive species Pacu also causes ecological adverse effect on local population.

Adverse effects on local species

In spite of their significant contributions in aquaculture and ornamental trading, there is debate over the introduction of non-native species. Any new species introduced to an ecosystem has an impact, although in most cases, the effects may remain unnoticed. Non-native fish species are often considered to pose a threat to biodiversity, and these effects on aquatic biodiversity can result from competition for food and space, habitat destruction, alteration of ecosystems and genetic interaction through hybridization. It is believed that the introduction of farmed non-native species such as Pacu has an adverse impact on small indigenous aquatic species of Purba Medinipur.

Although non-native species were introduced only for aquaculture in India, they are often found in inland rivers, reservoirs, floodplains, canals and wetlands. Purba Medinipur is a flood-prone district of West Bengal, especially Moyna block of the district where non-native species can easily spread from closed culture systems to open water resources during the monsoon season. Scientists have noted that this spread of the fast-growing, non-native fish like Pacu impacts native ecosystems and threatens native aquatic species. Populations of these introduced fish have seen exponential growth and are rapidly extending their range outside the points of introduction.

There is a worrying increase in the number of non-native fish species being detected in rivers, lakes, reservoirs, irrigational tanks and canals in recent years. The non-native fishes moved into open waters inadvertently or due to unawareness or lack of knowledge of the aquaculturists and farmers. The impact of such escapee fishes have been assessed and found to cause ecological problems in several natural aquatic systems. Ecological risks have been mainly caused by non-native species that have

become fully established and acclimatized in natural ecosystems and show naturally reproducing populations. In suitable conditions, such species produce abundant populations and causes a permanent alteration in the local ecosystem.

Discussion

Occurrences of red-bellied Pacu in Purba Medinipur have been recorded from several natural aquatic bodies raising alarms and concernsof scientists. It is recorded to occur in the Moyna,Tamluk and Panskura Block of Purba Medinipur District is the initial alarm to native aqua-fauna of the region. In India, Pacu has already been reported from Pamba River in Kerala, which is known for its inland fishery resources andgreat biodiversity (Rani, Dharan and Sherly, 2017). Zeena and Beevi (2014) reportedcaptures of Pacu from Muvattupuzha River, Kerala. The occurrence of *Piaractus brachypomus*has also been interestingly reported from the Vembanad Lake. The Vembanad Lake is the largest coastal lagoon on the southwest coast of India with a catchment area of 14500 km2 drained by seven rivers which are the Chalakudy, Periyar, Muvattupuzha, Meenachil, Manimala, Pamba and Achankovil, along with alarge number of canals originating and flowing through Western Ghats. The region is known as one of the 34 global biodiversity hotspots of the world (Srinivas, Revichandran and Maheswaran, 2003). Presence of red-bellied Pacu, *Piaractus brachypomus*has also been reported from Krishna and Godavari rivers of Telangana (Johnson, Paromita and Sivakumar, 2014; Laxmappa, 2016).

Therefore, like Kerala, Telangana and adjoining regions, West Bengal as well as Purba Medinipur are also infested by the illegally imported cultivable fish, *P. brachypomus.*

Conclusion

Some of the recent reports on the incidents of inadvertent releases of Pacu that gravitated into natural water bodie sespecially in Purba Medinipur are definitely considered as serious concern and are alarming in response to the heedless interventions of the aquaculturists and emanating environmental changes. The Pacu introduction in India has notbeen scientifically evaluated and hence its invasiveness stillremains a query. A plastic diet, large body size and longevity(up to 70 cm SL and 28 years) (Loubens, Panfili and Biologie,2001) and the capacity to achievelarge local abundances and wide distributions, are some of the characteristics that support the invasive characters of thespecies (Singh and Lakra 2011). Whatsoever may be the scenario, established selfsustaining populations *P. brachypomus* have not been recordedso far due to the low frequency of individuals occurring in natural aquatic habitats. Nevertheless, there is a great possibility that a gradual release of these long-living fishes provides favourable conditions for growth, spawning, and establishment. The possibilities of adversities on the biodiversity and ecosystem services attract serious attention of the policymakers and the scientific community to address them looking into larger interest of the

farmers community, society and the environment.

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Plants used against Intestinal Worms by Ethnic Communities in Paschim Medinipur District, West Bengal, India

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ABSTRACT

The district Paschim Medinipur is situated on the extreme south-western part of West Bengal, India and is located in between 22°57² 10³ N to 21°36² 35³ N latitudes and 88°12² 40³ E to 86°35² 50³ E longitudes. The total area of the district is 9786.28 square kilometres. A number of ethnic communities are inhabited here and the number of scheduled tribe communities in this district is 37. Major ethnic groups are Santals, Bhumijs, Mundas Savars, Koras and Lodhas. These indigenous people of the district have valuable traditional knowledge of their own. Their ethno-medicinal knowledge to cure numerous diseases is invaluable to strengthen present day system of medicine. Now this indigenous knowledge of thousand years old is also under threat of rapid invasion of modern civilized culture and therefore change of traditional way of life style.

Intestinal worms are very common among people of ethnic communities especially of children. They used some common plants found in their surroundings against intestinal worms. A number of ethno-medicinal formulations are found among various ethnic communities for treatment against such problems. *Ananas comosus, Andrographis paniculata* and *Luffa cylindrica* are most frequently used against intestinal worms. *Caesalpinia bonduc and Cuscuta reflexa* are considered most effective antihelmintic plants. The present work deals with 11 such plants used by different ethnic communities of Paschim Medinipur District.

INTRODUCTION

The district Paschim Medinipur is the second largest district of West Bengal, India and situated on its extreme south-western part. It is located in between 22°57² 10³ N to 21°36² 35³ N latitudes and 88°12² 40³ E to 86°35² 50³ E longitudes. It covers an area of 9786.28 square kilometres (Anonymous, 2003).

The district is bounded in east with Purba Medinipur and Howrah districts of West Bengal, on the west with Mayurbhanj district of Orissa and Purba Singbhum district of Jharkhand states, on the north with Hooghly, Bankura and Purulia districts of West Bengal and on the south with Purba Medinipur district of West Bengal and Baleshwar district of Orissa state.

The population of tribal people in Paschim Medinipur district is largest in West Bengal. About 17.55 % population of this district is of

ethnic communities (Hansda, 2004). 37 different scheduled tribes are found in this district is 37 (Mandal *et al*. 2002). Most of them are inhabited in the western forest region. Major Scheduled Tribe groups are Santals, Bhumijs, Mundas, Savars, Koras and Lodhas.

The district is rich in plant diversity also but many wild plant species are under threat due to the increasing human population.

The ethnic communities of the district have valuable traditional knowledge of their own. Their indigenous ethno-medicinal knowledge for curing numerous diseases (of both human and veterinary) is invaluable to strengthen modern science of medicine. But this traditional knowledge of thousand years old is also under threat of rapid invasion of urban culture and change of traditional way of life style.

Ethno-medicinal plants of the region was previously explored by several workers such as Chaudhuri and Pal (1975), Chaudhuri and Pal (1976), Pal (1988), Pal and Jain (1989, 1998), Ghosh (2003), Paria (2005), De and Bhattacharya (2005), De (2013, 2015, 2016); Adjacent areas of the district was also studied in this respect by different workers such as Bodding (1925, 1927, 1940), Maiti and Manna (2000), Chakraborty *et al* (2003), Chakraborty (2006), Chakraborty and Bhattacharjee (2006), Dey and De (2011).

Like the floristic components of the region, valuable traditional knowledge of ethnic communities about medicinal plants of the district is under threat of extinction. So the written documentation of this indigenous knowledge is very much necessary.

MATERIALS AND METHODS

Some plants are believed as useful against intestinal worms and are frequently observed to intake orally for this purpose. This paper deals with 11 such plants used by the different ethnic communities of the district. Field studies in this regard were done by the following way-

i) Interaction with people of different ethnic communities for preliminary information about plants used by them against intestinal worms.

ii) Consultation with traditional medical practitioners (like *ojha*, *gunin* etc.) among ethnic communities who regularly practice their traditional medicine as far as possible.

iii) Consultation with different ethnic groups like Santal, Lodha, Munda etc. in different locations of the experimental area to verify the medicinal utility of these plant.

iv) Medicinal importance of different ethno-medicinal plants was also critically cross checked from literature, if any.

v) Plant specimens were identified with the help of existing literatures (i.e. Haines, 1921-1925; Mooney, 1948; Prain, 1903) and botanical names have been checked and verified from these literatures as well as from available online (i. e. http://www.theplantlist.org/tpl1.1/record/kew).

vi) Ethno-botanical enumeration technique as proposed by Jain (1995) and Jain and Mudgal (1999) are followed in this study with some necessary modifications.

vii) Herbarium specimens are deposited in herbarium of Department of Botany, Government General Degree College, Mohanpur.

Intestinal worms are very common among people of ethnic communities especially of children due to unhygienic environment and life style. They used some common plants found in their surroundings against intestinal worms. 11 such plant species under 10 genera and 10 families are found to be used against intestinal worms by field study. These plants are arranged alphabetically by their botanical name, family, accession number, vernacular name, along with their traditional medicinal formulation/s.

List of abbreviations: B-Bangla; E-English; L-Lodha; M-Mundari; S-Santali; Syn.-Synonym.

RESULTS AND DISCUSSION

During the investigation a number of unique and interesting ethno-medicinal formulations were observed and recorded. The detailed description of the same is enumerated below:

1. *Ananas comosus* (L.) Merr. [Bromeliaceae] RPD-46

Vernacular name: Ânâras, Keyâ (B); Pine apple (E).

Leaf:

1. Warm leaf juice used as anthelmintic against small white worms, once in the morning before meal for continuous use up to cure. Raw leaf juice without warming is also used. Sometimes fresh leaf juice mixed with fresh rhizome paste of *Curcuma longa* is used.

2. Lodhas and other communities use paste of soft leaf base mixed with common salt as anthelmintic, 3-4 spoonfuls, once daily in empty stomach for 3-7 days. It applies mostly for children.

2. *Andrographis paniculata* (Burm.f.) Wallich *ex* Nees. Syn. *Justicia paniculata* Burm.f.; *Andrographis subspathulata* C.B. Clarke. [Acanthaceae] RPD-18

Vernacular name: Kâlmegh, Chiratâ (B); Creat, Kirayat (E).

Leaf:

1. Ethnic communities prepare small pills from leaf paste and prescribe these pills for the treatment of small intestinal worms (*gunrhi krimi*), 1-2 pills once in the morning in empty stomach for 2-4 days.

3. *Asparagus racemosus* Willd. [Asparagaceae] RPD-52

Vernacular name: Satamuli (B); Asparagus, Spiny asparagus (E); Gaisirâ (L);

Lubui kabar (S).

Root:

1. Lodhas prescribe root-juice mixed with molasses twice daily (morning and night) against

intestinal worms in children.

4. *Azadirachta indica* A. juss. Syn. *Melia azadirachta* L. [Meliaceae] RPD-26

Vernacular name: Nim (B); Indian lilac, Margosa tree, Neem (E).

Leaf:

1. Lodhas prepare green leaf juice and prescribe it against intestinal worms as required.

5. *Caesalpinia bonduc* (L.) Roxb. Syn. *Guilandina bonduc* L.; *Caesalpinia crista* L.; *C. bonducella* (L.) Fleming. [Caesalpiniaceae] RPD-47

Vernacular name: Gil, Kântâ karanjâ, Nâtâ (B); Bonduc nut, Physic nut (E).

Seed:

1. Ethnic and other communities fry seeds in hot sands or burn it in fire and then made into powder, which is prescribed as antihelminthic, a single dose of one seed is recommended, chewing or swallowing a single intact seed is allowed but sugar never added.

6. *Carica papaya* Linn. [Caricaceae] RPD-51

Vernacular name: Penpe (B); Papaw, Papaya (E); Piphou, Amrit (S).

Latex:

1. Kurmis prescribe latex as an anthelmintic. Some communities mix latex with milk and apply orally against intestinal worm of children for 3 days.

7. *Curcuma longa* Linn. Syn. *Curcuma domestica* Val. [Zingiberaceae] RPD-48

Vernacular name: Halud (B); Turmeric (E); Sasang (S).

Stem (Rhizome):

1. Bhumijs prescribe 10g fresh rhizome paste mixed with tender leaf juice (10 ml) of *Ananas comosus* and sufficient water as antihelmintic, once in the morning in empty stomach up to cure.

8. *Cuscuta reflexa* Roxb. Syn. *Cuscuta santapaui* Banerji & Das [Cucutaceae] RPD-45

Vernacular name: Alok latâ, Bândâ, Swarnalatâ, (B); Common dodder, Dodder (E); Bândâ, Bândhâ (L); Alâk jârhi (S).

Whole plant:

1. Ethnic and other communities prescribe whole plant paste as antihelmintic, once or twice daily as needed. It is bitter in taste.

9. *Luffa acutangula* (L.) Roxb. Syn. *Cucumis acutangula* L. [Cucurbitaceae] RPD-50

Vernacular name: Jhingâ (B); Ribbed gourd, Ridged gourd (E); Jhinâ (M); Jhingou (S).

Seed:

1. Mundas prescribe seed paste (of 10 seeds) as antihelmintic, once daily for continuous use, up to cure.

10. *Luffa cylindrica* (L.) M. Roem. Syn. *Momordica cylindrical* L.; *Luffa aegyptiaca* Mill. [Cucurbitaceae] RPD-49

Vernacular name: Dhundhul, Palta, Porol (B); Sponge gourd, Towel gourd (E); Pallâ, Dada (S).

Fruit:

1. Ethnic communities soak 25g bitter fruit in a little amount of water (25 ml) for overnight, then the soaked fruit after sieving mixed with 5 ml honey and juice is extracted. The juice is prescribed against all types of intestinal worms, 20ml juice used thrice daily.

11. *Mentha arvensis* L. Syn. *M. canadensis* L., *M. palustris* auct. non Mill., *M. agrestis*

Sole [Lamiaceae] RPD-53

Vernacular name: Pudinâ (B); Corn mint, Field mint, Wild mint (E).

Leaf:

1. Bhumijs prescribe leaf paste with honey and small amount of common salt as anthelmintic.

CONCLUSION

11 plant species under 10 genera and 10 families are used against against intestinal worms. These plants are very common and frequently in and around every village of the area under study. The medicinal formulations are also very simple, people prepare these medicines by themselves and they never depend on their traditional medical practitioners like *ojha*, *gunin*, *jan* etc. Mostly these are single plant medicines, other ingredients if any used are common salt, honey or milk. Amount and doses are not strictly followed. *Ananas comosus* and *Andrographis paniculata* are most frequently used antihelmintic. *Caesalpinia bonduc*, *Cuscuta reflexa* and *Luffa* spp. are used by some communities and these are believed as most effective.

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Total Synthesis of Elvirol: An Unique Bisabolene Sesquiterpene

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ABSTRACT

Elvirol is a potent antimicrobial compound which show antibacterial activity against *Staphylococcus aureus* and *Vibrio anguillarum*. It isa structurally exceptional natural product isolated from *Deliliabiflora*(L.) Kuntze (syn. *Elvirabiflora* (L.) DC). The compound is a bisabolene sesquiterpene. Synthesis of elvirol does not obey specialisoprene rule. This review describes the total synthesis of the elvirol employing different methodologies**.**

Introduction

Curcuphenol**2** and Elvirol**1** (Figure 1) have molecular structures which possess powerful antibacterial, antifungal, antitumor and antimalarial activities[1]. This prompted chemists to undertake the synthetic analogical approach to synthesise these natural products. Elvirol **1** is a bisabolene sesquiterpene metabolite, isolated from *Elvira biflora* [2]**.** Although elvirol has been assigned a terpenoid basis, it is somewhat unique in that it does not conform to the isoprene rule that goes to make up all the terpene constituents. Different synthetic strategies have been employed to mediate total synthesis of Elvirol **1**

Results and Discussions

It has been assumed that the biogenetic origin of elvirol**1** could be traced to α-curcumene **3** which undergoes an aromatic-epoxidation and a subsequent 1,2- alkyl shift (**Scheme1**).

Enantiomeric nature of elvirol has not been referred to in its isolation and as such no information is available on its possible biological activity. Interestingly, its more well-known structural sibling curcuphenol **2** displays interesting biological activities of both its enantiomers [3,4]. Several reports of synthesis of elvirol have appeared in literature[3,4,5,6,7]and recently a synthesis of both its enantiomers has also been disclosed.

Bohlmann et.al, who reported the isolation of elvirol, also disclosed its first synthesis [5]. The synthesis started with the crotenylcresyl ether **4**, which on Claisen rearrangement furnished the propenyl phenol **5**. Protection of the phenolic group **6** followed by hydroboration resulted in the alcohol **7** which was converted to the bromide **8**. Oxidation to the aldehyde **9**

and a subsequent Wittig reaction **10** followed by acid treatment afforded the homologated aldehyde**11**. Wittig olefination with isopropylidene phosphorane then furnished elvirol **1** (**Scheme 2**).

Reagents and reaction conditions: i. Heat, 250°C,

ii. 2-methyldihydropyran, H^+,iii. B_2H_6, H_2O_2, iv. PBr_3, v. Me_3NO, vi. Ph_3P=CHOMe, viii. H_3O^+, viii. Ph_3P-$CHMe_2I$, LDA, THF, 0°C.

Ho and Ho also reported a synthesis of elvirol which started with the cyclopentenyl cresyl ether **12**. Ozonolysis of this in methanol produced the aldehyde-ester**13** and the regio-isomer **14** in 2:1 proportion which were easily separated[6]. The aldehyde function in **13** was converted to a methyl group through first transformation to a dithiane **15** followed by desulfurisation with Raney nickel to furnish the ester **16**. Partial reduction of the ester to an aldehyde **17** with diisobutyl aluminiumhydride and a Wittig condensation resulted in elvirol methyelther **18** (**Scheme 3**).

***Reagents and reaction conditions*:** i. O_3, DCM, MeOH, ii. BF_3, Et_2O, propane-1, 3-dithiol, iii. Raney Nickel, EtOH, iv. DIBAL-H, Toluene,v. Ph_3P=CMe_2, THF.

The application of microwave and clay catalysts to effect organic transformations under milder and simpler conditions have been exploited by Singh et. al. to achieve a very short synthesis of elvirol [7]. Microwave assisted reduction of 6-methylhept-5-en-2-one **19** using alumina doped with sodium borohydride furnished the alcohol **20** which was brominated to **21**. Treatment of this bromoheptene **21** with p-cresol in presence of montmorillonite k-10 clay resulted in a facile Fridel-Crafts alkylation furnishingelvirol **1** in good yield alongwith its regio-isomer **22** as a minor component (**Scheme 4**).

Reagents and reaction conditions: i. $NaBH_4$, Al_2O_3, microwave irradiation (MVVI); ii. Py, anhy. Et_2O, PBr_3, 0°C; iii) *p*-cresol,K- clay,10 day, 200°C, 5h.

The first synthesis of both the enantiomers of elvirol has been reported by Ono et.al [8]. This relied on an enzymatic resolution to develop the absolute stereochemistry at the solestereogenic centre. Prolonged hydrolysis of the racemic acetate **23** furnished a separable mixture of the (*S*) alcohol **24** and the (*R*) acetate **25**. The enantioselectivity was improved to 96% through repeated acetylation and hydrolysis of the (*S*) alcohol (**Scheme 5**).

A two-step sequence involving conversion to the tosylate **26** followed by reduction converted the primary hydroxymethyl moiety in **24** to a methyl group. Saturation of the double bond and reduction of the ester function in**24** led to the alcohol **27** which was deprotected to the phenol **28**. The corresponding 3,5-dinitrobenzoate **29**, as a crystalline solid was purified by crystallisation to obtain an enantiomerically pure product. Protection of the phenol in **29** as the methoxymethyl ether **30** followed by basic hydrolysis delivered the alcohol **31** which on oxidation to an aldehyde and a subsequent Wittig condensation with isopropylidene phosphorane followed by deprotection

furnished (*S*) elvirol (**Scheme 6**). Similar transformations on the (*R*) isomer **25** led to the synthesis of (*R)* elvirol.

Reagents and reaction conditions: i. Ts_2O/Py, iia. H_2/ $Pd(OH)_2$, b.$NaBH_4$,DMSO, c. $LiAlH_4$, iii. EtSH, $AlCl_3$, iv. 3,5-dinitrobenzoyl chloride, v. MOM-Cl, ($^{i-}Pr_2NEt$), vi. K_2CO_3/ Acetone, viia. PCC/ DCM, b. $Me_2C{=}PPh_3$, c. 2M HCl, ^{i}PrOH.

Hagiwara *et al*. have reported a synthesis of both enantiomers of elvirol **1**, demonstrating the application of the catalytic enamine reaction they had developed [9]. The synthesis began with the preparation of the unsaturated ketone **35**, from addition of vinyl magnesiumbromide to (*R)* citronellal **33** followed by oxidation of the resulting alcohol **34**. Conjugate addition of propionalldehyde to this unsaturated ketone mediated by diethylamine under the conditions developed by them provided the keto-aldehyde **36** which was cyclised to the cyclohexenone **37** as a mixture of diasteromers. Finally, aromatisation of the six-membered ring resulted in a synthesis of elvirol **1** (**Scheme7**). The *ent*-isomer**1** was synthesised following the same sequence of reaction starting from (*S)* citronellal.

Reagents and reaction conditions: i. $H_2C{=}CHMgBr$, THF,0^{o}C, ii. TPAP, NMO, DCM, 0^{o}C, iii. DEA, Propionaldehyde, MeCN, sealed tube, 87^{o}C, iv. $^{n}Bu_4N^{+}OH^{-}$, THF, Et_2O, v. LDA, HMPA, THF, PhSeCl,

Dennison *et.al* synthesized elvirol **1**in only three steps from 3-bromo-4-methoxytoluene **38** and 6-methylhept-5-en-2-one [10]. They also proved that the proposed structure of elvirol, previously isolated from *Elvira biflora*, was correct. 3-bromo-4-methoxytoluene **38** was treated with phenyllithium to lithiate the *ortho* position to the methoxy group by halogen-metal exchange and condensed with 6-methylhept-5-en-2-one to form the alcohol **39** as a major product alongwith the olefine **40**. The mixture of these two compounds was reduced with Na in liquid ammonia to afford **41** solely followed by demethylation of **41** with a solution of sodium thiolate and dimethylformamide to lead to the formation of the elvirol**1** whose NMR spectral data was fully consistent with that of the natural product(**Scheme 8**).

Reagents and reaction conditions: ia.PhLi/ Et_2Ob. $MeCOCH_2CH_2CH{=}CMe_2$, ii. Na, liq NH_3/ EtOH, iii. NaSEt, $HCONMe_2$.

Ghosh et.al [11] also published a synthesis of elvirol employing deoxygenation of a hydroxy group attached to the eight-membered oxocane system as a key step. The synthesis began with 4,6 dimethyl coumarin **43**which on drastic alkaline hydrolysis of the coumarin **43** with potassium hydroxide in ethylene glycol at reflux afforded the styrenol **44**. This phenol was subjected to a Barghellini reaction [12, 13,14, 15] involving interaction with chloroform in presence of powdered sodium hydroxide in refluxing acetone and furnished the *gem*-dimethyl carboxylic acid **45**. This acid **45**was reduced in very good yield to corresponding alcohol **46** with lithium aluminium hydride. When this alcohol **46** was subjected to oxidation with PCC, it afforded the benzoxopinenone **47** which on treatment with diazomethane in the

presence of catalytic amount of palladium acetate furnished the cyclopropyl ketone **48**. Catalytic hydrogenation of this cyclopropyl ketone **48** resulted regioselective cleavage of the central bond revealing the benzoxocanone **49.** Reduction of this ketone **49** with sodium borohydride produced the alcohol **50.** This alcohol was transformed to the thionocarbonate **51** through interaction with carbon disulfide and methyl iodide in presence of sodium hydride. Treatment of this thionocarbonate **51** with tri-butyltinhydride in toluene under reflux furnished elvirol **1**.

Reagents and reaction conditions: i. KOH, $HOCH_2CH_2OH$, 120°C, ii. $CHCl_3$, MeCOMe, NaOH, iii. $LiAlH_4$, THF, iv. PCC, DCM, v. CH_2N_2, Et_2O, $Pd(OAc)_2$, vi. H_2/Pd-C, EtOH, vii. $NaBH_4$, MeOH, viii. NaH, CS_2, MeI, ix. TBTH, AIBN, toluene, heat.

Conclusion:

In conclusion, this review article described different approaches towards the racemic as well as stereoselective total synthesis of elvirol 1 adopting different methodologies in a very concise way.

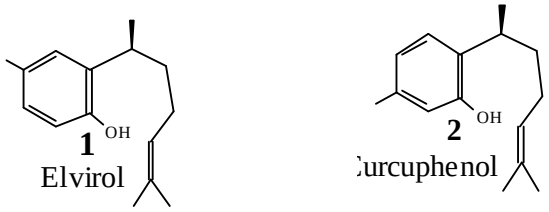
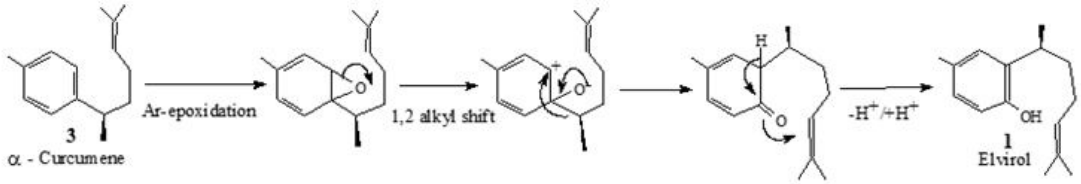
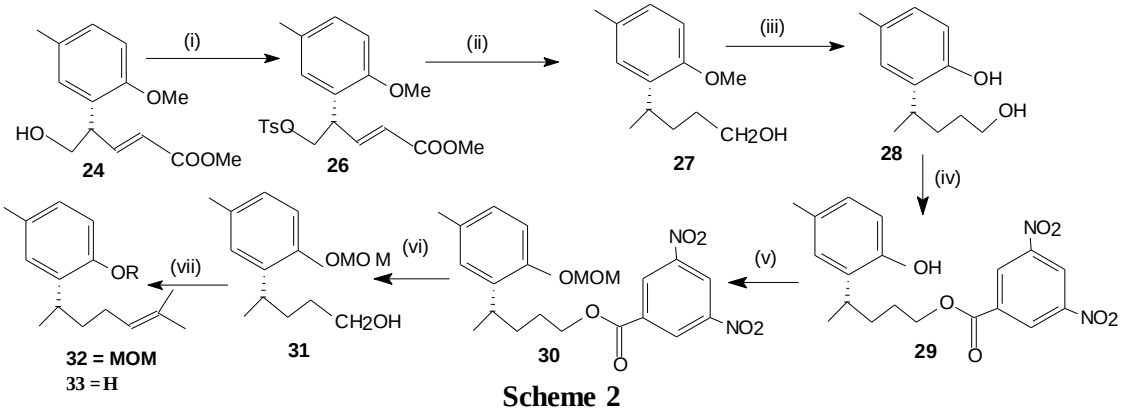


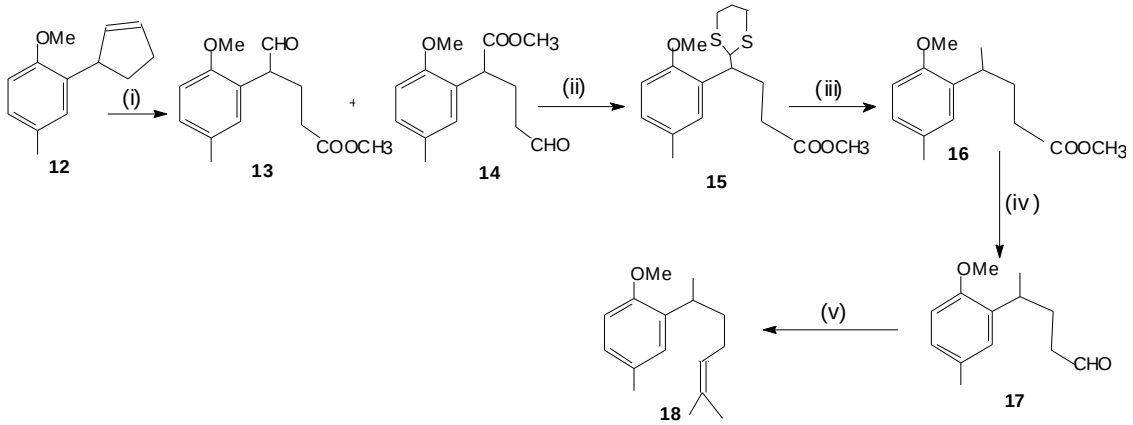
Figure 1



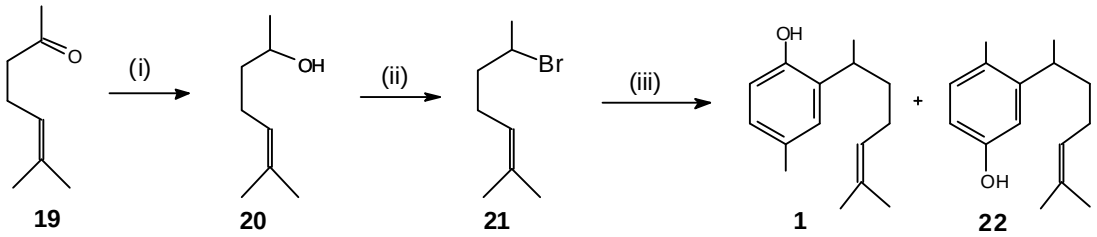
Scheme1



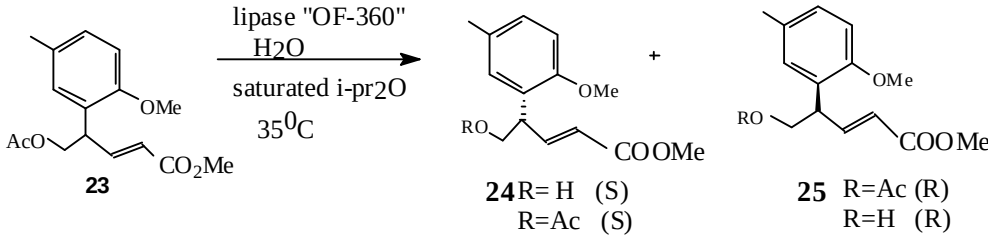
Scheme 2



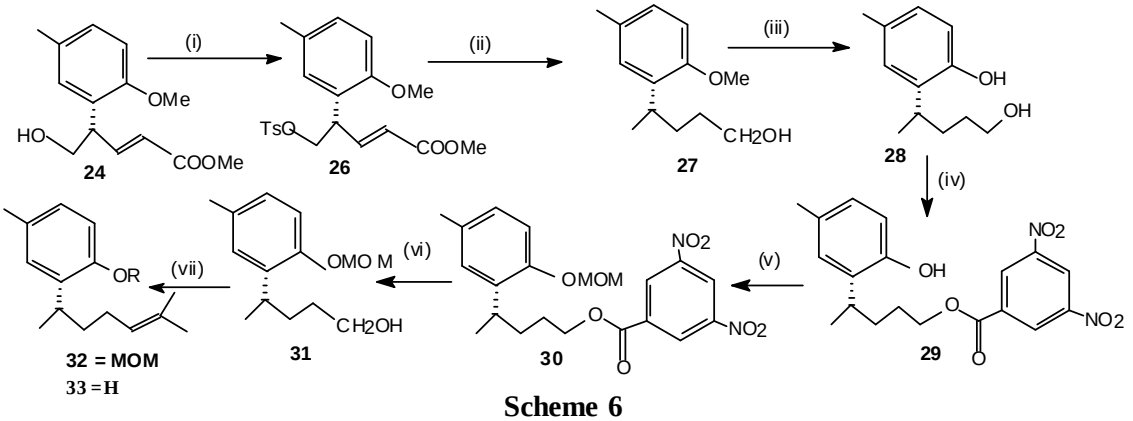
Scheme 3



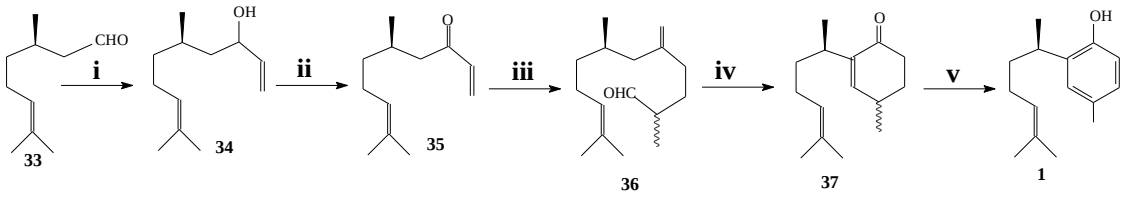
Scheme 4



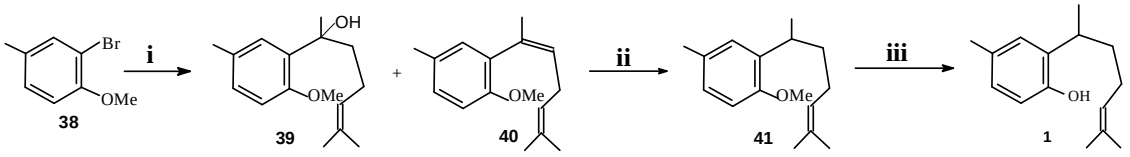
Scheme 5



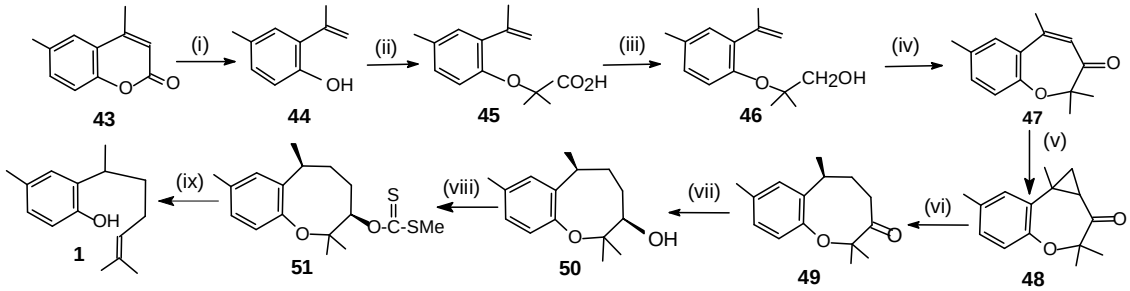
Scheme 6



Scheme 7



Scheme 8



Scheme 9

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A Preliminary Study on Wild Forms of Cultivated Plants in West Bengal, India

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wild relatives.

ABSTRACT

Cultivated plants are originated from wild plants. Usually a region having one or more wild species of a cultivated plant is considered its centre of origin. In this point of view investigation of wild types or wild relatives of cultivated plants is very much significant. Many plants are cultivated in West Bengal for different purposes like cereals, pulses, vegetables, spices, fruits, fibres, timbers, ornamentals etc. Some of which are found wild in different regions of West Bengal. In this present work 23 such wild plants are described which are mostly known as cultivated plants, with their common names, distribution and phenology. Several cultivated plants have other species found to grow wild; these are known as wild relatives. Such 9 wild relatives are also discussed. Some cultivated plants have potentiality to grow wild outside of cultivation. Some of these feral plants are also mentioned.

INTRODUCTION

A number of plants are cultivated in different parts of West Bengal for food (cereals, pulse, tuber, vegetable, fruit, seeds etc.), fodder, shelter, fibre, wood, medicine, beautification and so many purposes. These plants have must originated from some wild plants; these may be either indigenous or exotic. De Candolle (1881) and Vavilov (1949-50) worked extensively about the origin of cultivated plants around the world. Usually it is considered that a region with one or more wild varieties or species of a cultivated plant is its centre of origin, from where it spreads different parts of the world through cultivation. Through cultivation a number of cultivars are developed, which have remarkable morphological differences with its wild types. So the study of wild forms or wild relatives of cultivated plants is very significant. These are also important to improve cultivated plants gene tically.

AIMS AND OBJECTIVES

The aims and objectives of this work are as follows:

1. To properly documentation of wild forms of cultivated plant species before its disappearance, because most of these are

locally threatened in wild condition.

2. To determine the actual habitat and localities of these plants, it is very much helpful to understand the origin of cultivated plants.
3. To determine the distinguishing features between wild and cultivated forms, so wild types among cultivated varieties are easily understood.
4. To indicate locally threatened wild kinsman of cultivated plants that need conservation and protection before extinction.

MATERIALS AND METHODS

The investigation on wild form of cultivated plants of west Bengal is conducted from 2014, on the seasonal basis following floristic, phenological and community studies. Darjeeling, Jalpaiguri, Alipurduar and Coochbehar in the north and Bankura, Jhargram, Paschim Medinipur, Purba Medinipur and Purlia in the south are selected as survey area. During this work Herbarium, Museum materials and literatures studies were also done in the same time period. Plant species were identified following existing literatures (Cowan, & Cowan, 1929; Haines, 1921-1925; Hooker, 1872-1897; Mooney, 1948; Prain, 1903). Names of plant taxa have been verified from literature and available online (i. e. http://www.theplantlist.org/tpl1.1/record/kew). Common names of trees are collected from the survey and also from various literatures and online (i. e. Haines, 1921-1925; Mooney, 1948; Prain, 1903; http://www.flowersofindia.net).

List of abbreviations: B-Bangla; E-English; Fl. Flowering season; Fr. Fruiting season; N-Nepali; R-Rajbansi; S-Santali; Syn.-Synonym.

RESULTS AND DISCUSSION

During the survey a number of unique and interesting wild plant species were observed which are very common under cultivation. These are broadly divided into 3 categories, wild, wild relatives and feral plants. In this present work 23 such wild plants are described which are mostly known as cultivated plants. Some cultivated species have other species found to grow wild; these are considered as wild relatives. Such 9 wild relatives are also discussed. several cultivated plants have potentiality to grow wild outside the cultivation. Some of these feral plants are also mentioned. Plant species are describes under these 3 categories with their common names, distribution and phenology. The detailed account of the same is alphabetically enumerated below:

A. Wild

1. *Bauhinia variegata* L. Caesalpiniaceae.

Varigated Bauhinia (E); Ban korhol (S).

A medium sized deciduous tree. Leaves alternate, emarginated like most of Bauhinias. Flowers in leafless condition. Corolla pale pink with dark standard. Very beautiful in full bloomed leafless plants. A number of cultivated varieties are found in gardens. Wild plants are found in hilly areas of Jhargram.

Fl. March. Fr. October-January.

2. *Caryota urens* L. Arecaceae.

Râmguâ (B); Fishtail palm (E); Râng Bhâng (N); Châ guâ (R).

A tall solitary palm with long bipinnate leaves. Inflorescens very large and pendulous. Planted as ornamental, found wild in North Bengal.

Fl. and Fr. Almost throughout the year.

3. *Coccinia grandis* (L.) Voigt. Syn. *Cephalandra indica* Naud., *Coccinia indica* wight & Arn. Cucurbitaceae.

Telâkuchâ (B-wild plant); Kundri (B-cultivated plant); Scarlet gourd (E).

A gregarious tendril climber. Flowers white. Fruits small, oblong, bright red when ripe. It has several cultivated forms with smaller and greenish-white flowers. Fruits of cultivated forms are used as vegetable mostly in South West Bengal. Common throughout West Bengal.

Fl. and Fr. Almost throughout the year, gregarious in rain.

4. *Colocasia antiquorum* Schott. Araceae.

Ban kachu, Sarkachu (B-wild); Kachu (B-cultivated); Taro (E).

A common plant of marsh and wet lands. Spadix yellow. Cultivated varieties are grown on

terrestrial condition.

Fl. and Fr. Mostly June-December.

5. *Cucumis melo* var. *agrestis* (Naudin) Pangalo. Cucurbitaceae.

Ghumâri, Banphuti (B); Wild melon (E).

A week climber spread on the ground, very similar to cultivars of melon. Fruits are small, lemon size but also edible.

Fl. and Fr. March –December.

6. *Ficus elastica* Roxb. Moraceae.

Rabâr (B); Indian rubber tree (E).

A small tree with milky latex. Leaves thick alternate with a long bud scale on the apex of branches. Common ornamental tree with several cultivars, found wild in Darjeeling district.

Fl. and Fr. Not found.

7. *Hiptage bengalensis* Kuntze Syn. *H. madablota* Gaertn. Malpighiaceae.

Mâdhabilatâ (B); Hiptage (E).

A strong lianas with opposite leaves. Very similar to *Combretum decundrum* in flowerless condition. Flowers white with yellow tinge. Fruits are 3 winged samara, look like that of *Shorea robusta*. Rare in garden. Wild in the forest of Jhargram, not frequent. Locally threatened.

Fl. February-March. Fr. March-April.

8. *Ipomoea aquatica* Forsk. Convolvulaceae.

Kalmi (B-wild and cultivated); Dângâ kalmi (B-cultivated); Water spinach (E).

A common wild plant of marshy places. Flowers violate or sometimes white. Cultivated varieties are dwarf and terrestrial.

Fl. and Fr. October-December.

9. *Ixora coccinea* L. Rubiaceae.

Rangkâthi, Rangni, Rangli (B-wild); Rangan (B-cultivated).

Marshy shrub grows on the edges of water bodies. Internodes much longer than cultivated plants. Leaves are also larger. Flowers are red. Common ornamental plant with numerous

cultivars. Wild in southern part of Paschim Medinipur. Very rare and population decreasing rapidly.

Fl. and Fr. August-October.

10. *Jasminum angustifolium* (L.) Willd. Oleaceae.

Kathmali, Banmâli (B); Wild Jasmine (E).

A suffruticose shrub very similar to cultivated *Jasminum sambac*. Flowers white, corolla single. Usually grown in Sal forest but may found in urban region also.

Fl. May-June. Fr. June-August.

11. *Mangifera indica* L. Anacardiaceae.

Tusi âm, Tupri âm, Tusku âm, Deshi âm (B-wild); Âm (B-cultivated and wild); Mango (E).

One of the most common cultivated fruit tree with a number of cultivars. Wild types found throughout the South West Bengal with small to medium sized very sour fruits. Stone large and mesocarp fibrous. It is self grown, occasionally cultivated.

Fl. February. Fr. May.

12. *Momordica cochinchinensis* (Lour.) Spreng Cucurbitaceae.

Kânkrol (B); Spiny bitter-cucumber (E).

A slender annual dioecious tendril climber. Leaves cordate. Flowers large creamy-white. Cultivated throughout the West Bengal. Wild in Coochbehar and adjoining areas, very frequent. Also grown as wild in southern part of Paschim Medinipur, but not common, may be feral.

Fl. May-October. Fr. Mostly May-December.

13. *Momordica dioica* Roxb. ex Willd. Cucurbitaceae.

Ghi kâllâ (B); Spine gourd (E).

Similar to but very slender than the above. Flowers small yellow. Found wild throughout Paschim Medinipur and Jhargram. Mostly wild, occasionally cultivate for its highly esteemed fruits.

Fl. June-August. Fr. September-October.

14. *Murraya paniculata* (L.) Jack. Syn. *M. exotica* L. Rutaceae.

Ban kamini (B-wild); Kâmini (B-wild and cultivated); Chinese box (E).

A small aromatic shrub but cultivated plants may grow as large as a small tree. Flowers scented, white. Wild in the forest of Jhargram and south parts of Paschim Medinipur. Locally frequent.

Fl. and Fr. Repeatedly May-October.

15. *Musa paradisiaca* L. Musaceae.

Ânthia kalâ, Dayrâ kalâ, Majiâ kalâ (B); Banana (E).

Wild plants are very tall. Fruits are very thick with numerous seeds. Found throughout the West Bengal but more frequent in North Bengal.

Fl. and Fr. Throughout the year.

16. *Nyctanthes arbor-tristis* L. Oleaceae.

Banshiuli (B-wild); Shiuli (B-cultivated); Night jasmine (E).

Small shrub with opposite and scabrous leaves.

Corolla rotate, corolla tube orange and lobes white, fragrant, flowers at night. Cultivated plants are taller than wild plants. Found wild in hills of Bankura and Purulia.

Fl. August-November. Fr. September-December.

17. *Pachyrhizus erosus* (L.) Urb. Syn. *P. Angulatus* DC. Fabaceae.

Kânthâlu (B-wild plant); Keshar âlu, Ras âlu, Shânk âlu (B-cultivated plant); Yam bean (E).

A robust climber. Leaves trifoilolate. Flowers in elongated raceme, blue. Found wild throughout West Bengal, more frequent towards southern part of Paschim Medinipur. Similar to cultivated plants but tuberous roots of wild plant are smaller, more woody and hard due to low water content.

Fl. October-November. Fr. November-January.

18. *Pentapetes phoenicea* L. Sterculiaceae.

Dupuremani (B); Twelve o'clock (E); Borh bâhâ (S).

A slender annual plant of marshland or low land. Flowers are red. Internodes are much longer and flowers are paler than cultivated forms. A common ornamental plant mostly in the Santal villages. Wild plants are found in southern part of Paschim Medinipur. Rare and population decreasing rapidly.

Fl. and Fr. August-October, mostly in September.

19. *Putranjiva roxburghii* Wall. Syn. *Drypetes roxburghii* (Wall.) Hurus. Putranjivaceae.

Putranjib (B); Lucky bean tree (E).

A common road side planted tree, look very similar to *Polyalthia longifolia*. Dioecious medium sized tree. Found wild in the forest of Jhargram. Not frequent.

Fl. April. Fr. October-November.

20. *Sesamum indicum* L. Syn. *S. orientale* L. Pedaliaceae.

Kâthtil (B-wild); Til (B-cultivated); Sesamum (E).

An small annual erect herb. Flowers pink. Cultivated plants are taller and mostly white flowered. Common on the paddy field in Purulia.

Fl. and Fr. July-September.

21. *Tabernaemontana coronaria* Br. Syn. *Ervatamia divaricata* (L.) Burkill Apocynaceae.

Ban tagar (B-wild); Sâdâphul, Tagar (B-cultivated); Wax flower (E).

A low bush with milky latex. Similar to cultivated plants but much smaller. Flowers white. Fruits a pair of follicles, seeds red. Very frequent in Duars and its adjoining regions.

Fl. Summer. Fr. March (green)-December (ripe).

22. *Thunbergia grandiflora* (Roxb. ex Rottl.) Roxb. Acanthaceae.

Nil latâ (B); Bengal trumpet vine (E).

Robust climber, look like a cucurbit without tendril. Flowers white whereas cultivated ornamental plants have blue flowers. Found wild in Alipurduar, not frequent.

Fl. and Fr. June-September.

23. *Trichosanthes cucumerina* L. Syn. *T. anguina* L., *T. cucumerina* ssp. *cucumerina* Cucurbitaceae.

Ban chichingâ, Ban patol (B-wild); Chichingâ (B-cultivated); Snake gourd (E-cultivated).

An annual climber and wild form of cultivated plant (*T. cucumerina* ssp. *anguina*). Fruits are small about 5cm but sometimes elongated more than 20cm.

Fl. and Fr. July-October.

B. Wild relatives

Several cultivated plants have one or more wild species such as *Abelmoschus* spp., *Amaranthus* spp., *Amorphophallus sylvaticus* (Roxb.) kunth , *Artocarpus* spp., *Carissa spinarum* L., *Cestrum diurnum* L., *Cinnamomum* spp., *Citrus* spp., *Corchorus aestuans* L., *Crotalaria* spp., *Curcuma* spp., *Gardenia* spp., *Ipomoea* spp., *Lagerstroemia parviflora* Roxb., *Luffa* spp., *Magnolia* spp., *Nicotiana plumbaginifolia* Viv., *Passiflora foetida* L., *Saccharum* spp., *Solanum* spp., *and Zingiber* spp. Some other plants are mention bellow:

1. *Catharanthus pusillus* (Murray) G.Don *Vinca pusilla* Murray Apocynaceae.

Ban marich (B); Tiny periwinkle (E).

A very pretty small annual erect herb. It is a miniature of common *Catharanthus roseus*. Found wild in Jhargram and Purulia. Rare.

Fl. and Fr. July-September.

2. *Coffea benghalensis* B.Heyne ex Schult. Syn. *Psilanthus benghalensis* (B.Heyne ex Schult.) J.-F.Leroy Rubiaceae.

Bankowâ (B); Bengal coffe (E).

A common wild shrub and it is a wild relative of coffee plant. Grows in Duars forest in association with wild *Tabernaemontana coronaria*, and looks similar to it.

Fl. March-April. Fr. June-August.

3. *Crinum amoenum* Ker Gawl. ex Roxb. Amaryllidaceae.

Himalayan crinum lily (E).

An annual bulbous plant. Flowers white, blooms at evening. Found in North Bengal.

Fl. April-May. Fr. June-July.

4. *Crinum viviparum* (Lam.) R.Ansari & V.J.Nair Syn. *C. ensifolium* Roxb., *C. defixum* Ker Gawl. Amaryllidaceae.

River Crinum Lily (E).

An aquatic annual bulbous plant. Grow in pond and other water bodies. Flowers white. Found in southern part of Purba and Paschim Medinipur.

Fl. And Fr. Rainy season.

5. *Duchesnea indica* (Andrews) Focke Syn. *Fragaria indica* Andr. Rosaceae.

Indian strawberry (E); Pântâ (R).

A prostrate stoloniferous herb with trifoliolate leaves. A miniature of cultivated strawberry with yellow flowers instead of white. Fruits are very smaller, edible. Found wild in North Bengal.

Fl. February-April. Fr. April-May.

6. *Impatiens racemulosa* Wall. ex Hook. f. & Thomson Balsaminaceae.

Ban dopâti (B); Pink Raceme Balsam (E).

A common annual herb. Grown wild in North Bengal. It is similar to *Imapatiens balsamina.*

Fl. and Fr. March-December.

7. *Mangifera sylvatica* Roxb. Anacardiaceae.

Chuche ânp (N); Himalayan mango (E).

A wild species of mango. Fruits are elongated. Found in Darjeeling.

Fl. March-April. Fr. June-August.

8. *Mussaenda roxburghii* Hook. f. Rubiaceae.

Dhobi jhâr (N); East Himalayan Mussaenda (E).

Different species with many cultivars are found in gardens. This wild species is found in Northern part of North Bengal.

Fl. and Fr. Rainy season.

9. *Oryza rufipogon* Griff. Syn. *O. sativa* ssp. *fatua* (Prain.) De Wet Poaceae.

Urhâ dhân, Urhi dhân, Urhki dhân (B); Wild rice (E).

Common wild rice of West Bengal plains. It has a long awn at the tip of the spikelet otherwise similar to cultivated rice and its grains are also edible. Found in Paddy field and wet lands.

Fl. Rainy season. Fr. Autumn, before cultivated rice.

C. Feral plants

Several cultivated plants have trend to grown outside of cultivation. Some of these are *Annona squamosa* L., *Antigonon leptopus* Hook. & Arn., *Brassica* spp., *Bryophyllum pinnatum* (Lam.) Oken, *Caesalpinia pulcherrima* (L.) Sw., *Canna indica* L., *Catharanthus roseus* (L.) G. Don, *Ecbolium viridae* (Forssk.) Alston, *Lycopersicum esculentum* Mill., *Kalanchoe blossfeldiana* Poelln., *Quamoclit vulgaris* Choisy.

CONCLUSION

In this present work 23 wild plants are discussed which are mostly recognized as cultivated plants, such as *Hiptage bengalensis* Kuntze, *Ixora coccinea* L., *Pachyrhizus erosus* (L.) Urb., *Pentapetes phoenicea* L. and *Tabernaemontana coronaria* Br. Some cultivated species have other species found to grow wild; these are considered as wild relatives. Such 9 wild relatives are also discussed. Of which *Catharanthus pusillus* (Murray) G.Don, *Coffea benghalensis* B.Heyne ex Schult. *and Mangifera sylvatica* Roxb. are remarkable. Several cultivated plants have potentiality to grow wild outside the cultivation. Some of these feral plants are also mentioned.

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Ethno-medicinal plants used for the treatment of skin diseases from the southern parts of West Bengal, India

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ABSTRACT

Present investigation recorded 62 species (dicots 56 and monocots 6) of ethno-medicinal plants under 56 genera (dicots 50 and monocots 6) and 32 families (dicots 27 and monocots 5) for the treatment of skin diseases and its related diseases such as crack, eczema, pimples, ringworm, scabies and urticaria. Maximum number of species (8) was recorded in the family Fabaceae. As many as 20 plant parts were recognised as sources of ethno-medicines. Leaf (21) is the maximum source of such medicines for treating skin diseases. Present initiatives have been taken to record the plants to treat skin diseases and its related problems by using traditional medicines (phyto-medicines) which are generally used by the people of the southern parts of West Bengal.

Introduction

In nature there are bounties of plants with different habit groups. These plants bear different kinds of potential medicinal properties. Before the all-round human civilization, the aboriginal people are started their habitation near the forest and its vicinity. So they first started the uses of these plants and their different parts like root, stem, bark, flower, fruit, latex, etc for remedies of their daily life problems / or ailments. Initially they got satisfactory results by using these plants as medicines for the treatments of different diseases and problems. Subsequently as they were not very much aware of the uses of particular dose, efficacy, potentialities of traditional medicines, in most of the cases they failed to cure their ailments permanently. But with the accumulation of more and more knowledge on these plants they gradually are habituated to the use of these medicines and they realised that their diseases /problems can be tackled easily in their daily life. Later, with the development of modern medicines based on synthetic chemical molecules, they realised that these modern medicines are not only costly but may also have so many side effects. So, they depended more and more on the uses of traditional medicines and the remote village people gradually become accustomed with these medicines giving priority for use in their daily lives. In course of time these traditional medicines paved its entry to the civil society.

We the human beings face common diseases like diabetes, ulcers, diarrhoea, asthma, ophthalmic, kidney problems, piles, dysentery etc in our everyday life. The skin disease is one of them. Skin diseases are of different kinds

such as eczema, ringworms, scabies, etc. Traditional medicinal properties, especially of plants have been found to play an important role by their administration in dermatological conditions (Ram *et al.,* 2004*).* So to combat various skin diseases, proper uses of phyto-medicines are to be inspired to the local village people.

Literature survey:

A large number of papers, literatures of phyto-medicines / ethno-medicines have been published in India including West Bengal by eminent plant explorers, researchers (Pant *et al.,* 1993; Namhata & Ghosh, 1993; Pal & Jain, 1998; Paul, 2003; Pakrashi & Mukhopadhyay, 2001; Ghosh, 2003; Paria, 2005; Chakraborty & Bhattacharjee, 2006; Samanta & Biswas, 2009; Dey & De, 2010; Chanda & Mukherjee, 2011a; Das, 2012; Mallick *et al.,* 2012. Das & Ghosh, 2017; Biswas *et al.,* 2016; Mukherjee *et al.,* 2016; Bisaws & Mukherjee, 2017a; Biswas & Chatterjee, 2018; Chaudhury *et al.,* 2018). These literatures have been consulted and relevant references have been given.

Objectives of the present study:

Present initiatives have been taken to record the plants to cure skin diseases and its related problems by using traditional medicines (phyto-medicines) [Table-1] which are commonly used by the people of the southern parts of West Bengal.

Materials and Methods

The study areas:

The Southern parts of West Bengal consist of four districts (Purba Medinipur, Paschim Medinipur, Bankura and Purulia). Though the climatic variations, physiographic make up are not very much sound enough among them but there are growing different types of plant groups. An overall climate is of tropical type. The soils of the four districts are varied. The soils of Purulia, Bankura and Paschim Medinipur are mostly of lateritic type but in Purba Medinipur it is generally of alluvial type. Sal forest is very much common in Paschim Medinipur district. The temperature of four districts varies from 34 ^{0}C to 44^{0}C in summer and goes down to at around 9^{0}C in winter. The average annual rainfall is about 1400 mm.

Collection of specimens /data

Collections of specimens were made in different parts of study areas in different seasons of the year during January 2018 to December 2018. Field and herbarium techniques were followed as recommended by Jain & Rao (1977). The identification of collected specimens was made with the help of literatures (Parin, 1903; Haines, 1921-1925; Duthie, 1960). The collected specimens were deposited at Ramnagar College Herbarium. The website of The Plant List (http://www.plantlist.org) was consulted for updating the species names. The list of accepted names were arranged alphabetically along with their botanical name, local name, family, parts used and mode of administration for treating the types of skin diseases and were presented in tabulated form (Table-1).

Pertinent literatures, published papers of plant explorers, researchers (Chopra *et al.,* 1956;

Kirtikar & Basu, 1975; Jain, 1991; Martin, 1995; Anonymous, 2010; Lal & Singh, 2012; Panigrahi & Sahu, 2013; Tripathi *et al.*, 2013; Chanda & Mukherjee, 2014; Mandal *et al.*, 2014; Sannigrahi, 2014; Rahaman & Karmakar, 2015; Biswas & Mukherjee, 2017b) were consulted for more information regarding the medicinal values to cure skin diseases, in addition to our personal and local people's experiences.

Observations

Present investigation recorded 62 species (dicots 56 and monocots 6) of ethno-medicinal plants under 56 genera (dicots 50 and monocots 6) and 32 families (dicots 27 and monocots 5) regarding the treatment of skin diseases.

Table-1: List of plants used for the treatments of skin diseases in human beings

Sl. No.	Botanical name	Local name	Family	Parts used	Mode of applications
1.	*Abrus precatorius* L.	Kunch (Lal)	Fabaceae	Leaf	Fresh paste of leaves is used to cure scabies
2.	*Acalypha hispida* Burm.f.	Shibjata	Euphorbiaceae	Leaf	Fresh leaf extract is used to treat skin disease
3.	*Acalypha indica* L.	Muktajhuri	Euphorbiaceae	Leaf	Paste is used to cure scabies
4.	*Ageratum conyzoides* (L.) L.	Uchunti	Asteraceae	Leaf	Fresh leaves extract is used on infected skin
5.	*Allium sativum* L.	Rasun	Liliaceae	Bulb	*Allium* paste is used to treat urticaria
6.	*Aloe vera* (L.) Burm.f.	Ghritakumari	Liliaceae	Leaf	Fleshy/ succulent leaves paste is used over to eczema
7.	*Alstonia scholaris* (L.) R.Br.	Chhatim	Apocynaceae	Leaf Latex	Latex with kusum oil is used to treat scabies
8.	*Argemone mexicana* L.	Sial kanta	Papaveraceae	Root	Root is used to treat skin disease
9.	*Argyreia nervosa* (Burm.f.) Boj.	Bijtarak	Convolvulaceae	Leaf	Leaves paste externally used to treat skin disease
10.	*Azadirachta indica* A. Juss.	Nim	Meliaceae	Leaf	Neem leaves extract is used to cure skin disease
11.	*Bauhinia acuminata* L.	Sewtkanchan	Fabaceae	Bark & Leaf	Bark & leaves paste is used to cure skin disease
12.	*Bauhinia racemosa* Lam.	Ban Raj	Fabaceae	Bark & Leaf	Bark & leaves paste is used to cure skin disease
13.	*Bidens pilosa* L.	Phutium	Asteraceae	Whole plant	To treat skin related problems
14.	*Borassus flabellifer* L.	Tal	Arecaceae	Fruits	Pulp is used to treat skin disease
15.	*Caesalpinia bonduc* (L.) Roxb	Natakaranja	Fabaceae	Seed	Seed is used to treat skin disease
16.	*Calotropis gigantea* L.	Akanda	Asclepiadaceae	Leaf latex	Fresh leaf latex is used to treat skin disease
17.	*Cascabela thevetia* (L.) Lippold	Kalkephul	Apocynaceae	Leaf	To treat skin disease
18.	*Cassia fistula* L.	Bandar lathi	Fabaceae	Bud & Flower	Buds & flowers are used to treat skin disease
19.	*Cestrum nocturnum* L.	Rat Ki Rani	Solanaceae	Leaf	Leaf paste is used to treat skin disease
20.	*Cheilocostus speciosus* (J. Koenig) C.D. Specht	Kemuk	Costaceae	Root	Root paste is used to treat skin disease
21.	*Chrozophora plicata* A. Juss.	Kshudi okra	Euphorbiaceae	Leaf	Fresh leaf paste is used to treat skin disease
22.	*Cinnamomum verum* J. Presl	Daru chini	Lauraceae	Stem Bark	Bark is used to treat eczema
23.	*Cleome viscosa* L.	Hurhuria	Capparidaceae	Leaf	Leaf paste is used to treat skin disease
24.	*Clerodendrum infortunatum* L.	Ghetu	Verbenaceae	Leaf & Root	Leaves & roots are used to treat skin disease
25.	*Coccinia grandis* (L.) Voigt.	Telakucha	Cucurbitaceae	Flower	Flower is used to treat skin disease
26.	*Combretum decandrum* Roxb.	Alang	Combretaceae	Seed	Seed oil is used to treat skin disease
27.	*Crataeva roxburghii* R.Br.	Barun	Capparidaceae	Stem-bark	Decoction of stem-bark is used to treat skin disease

Sl. No.	Botanical name	Local name	Family	Parts used	Mode of applications
28	*Curcuma amada* Roxb.	Am ada	Amaryllidaceae	Rhizome	Rhizome paste is used to treat skin disease
29	*Eucalyptus globulus* Labill.	Eucalyptus	Myrtaceae	Leaf	Leaf decoction is used to treat skin disease
30	*Euphorbia pulcherrima* Willd.	Lalpata	Euphorbiaceae	Leaf & Flower	Useful to treat skin disease
31	*Glycosmis pentaphylla* (Retz.) Correa	Ban jami	Rutaceae	Leaf	Leaf paste is used to treat skin disease
32	*Ipomoea batatas* (L.) L.	Ranga alu	Convovulaceae	Whole plant	Used to treat skin disease
33	*Ipomoea pes-caprae* Sweet	Chhagalkuri	Convovulaceae	Whole plant	Used to treat skin disease
34	*Jatropha multifida* L.	Tortora	Euphorbiaceae	Fruits	Used to treat skin disease
35	*Leonotis nepetifolia* (L.) R.Br.	-	Lamiaceae	Flower	Ashes of flower head is used to treat ringworm
36	*Leucas aspera* (Willd.) Link	-	Lamiaceae	Leaf	Juice of leaf is used to treat skin disease
37	*Lindnbergia indica* (L.) Kuntze	Halud Basanta	Scrophulariaceae	Whole plant	Used to treat skin disease
38	*Nerium oleander* L.	Karabi	Apocynaceae	Leaf	Decoction of leaf is used to treat skin disease
39	*Nicotiana plumbaginifolia* Viv.	-	Solanaceae	Leaf	Decoction of leaf is used to treat skin disease
40	*Ocimum sanctum* L.	Tulsi	Lamiaceae	Leaf	Decoction of fresh leaves are used to treat skin disease
41	*Piper nigrum* L.	Golmirich	Piperaceae	Leaf	Leaf paste is used externally to ringworm
42	*Plumbago zeylanica* L.	Chitrak	Plumbaginaceae	Leaf	Leaf extract is used at infected skin disease
43	*Psoralea coryfolia* L.	Hakuchi	Fabaceae	Fruits & seeds	Paste is used to treat skin disease
44	*Schleichera oleosa* (Lour.) Oken	Kusum	Sapindaceae	Stem Bark	To treats skin diseases
45	*Senna tora* (L.) Roxb.	Chakunda	Fabaceae	Leaf & seeds	Paste is used to treat skin disease
46	*Sida rhombifolia* L.	Peetbala	Malvaceae	Stem	To treat skin disease
47	*Solanum lycopersicon* L.	Bilatibegun	Solanaceae	Fruit	Fruit juice is used to treat scabies externally.
48	*Solanum nigrum* L.	Kakmachi	Solanaceae	Young shoot	Young shoot paste is used to treat skin disease
49	*Solanum surattense* Burm.f.	Kantakari	Solanaceae	Root	Paste is used to treat scabies
50	*Solanum torvum* Sw.	Titabegun	Solanaceae	Root	To heal cracks in feet
51	*Tectona grandis* L.f.	Segun	Verbenaceae	Wood	Timber oil is used to treat eczema
52	*Tephrosia purpurea* (L.) Pers.	Ban-neel	Fabaceae	Whole plant	Used to treat the eczema
53	*Terminalia chebula* Retz.	Haritaki	Combretaceae	Fruits	Used to treat skin disease
54	*Tinospora sinensis* (Lour.) Merr.	Gulancha	Menispermaceae	Stem	Used to treat skin disease
55	*Tribulus terrestris* L.	Kantagokhur	Zygophyllaceae	Root & fruit	Used to treat skin disease
56	*Tridax procumbens* L.	Targanda	Asteraceae	Leaf	Fresh leaf paste is used to cure scabies
57	*Triumfetta rhomboidea* Jacq.	Banokra	Tiliaceae	Root	Paste is used to treat pimples
58	*Ventilago denticulata* Willd.	Raktapita	Rhamnaceae	Stem bark	Used to treat skin disease
59	*Vitex negundo* L.	Nishinda	Verbenaceae	Seed	Used to treat skin disease
60	*Wedelia chinensis* (Osbeck) Merr.	Bhingaraj	Asteraceae	Leaf	Leaf juice is used to treat scabies
61	*Withania somnifera* (L.) Dunal	Aswagandha	Solanaceae	Root & Leaf	Used to treat scabies
62	*Zingiber zerumbet* (L.) Roscoe *ex* Sm.	Kulango	Zingiberaceae	Rhizome	Paste is used to treat skin disease

Table-2: Taxonomic breakup of the medicinal plants from the southern parts of West Bengal

Plant groups	Families	Genera	Species
Dicots	27	50	56
Monocots	5	6	6
TOTAL	**32**	**56**	**62**

Table-3: Types of skin diseases and their number of species

Types of diseases	Number of species
Crack	1
Eczema	4
Pimple	1
Ringworm	2
Scabies	8
Skin disease	45
Urticaria	1
TOTAL	**62**

Table-4: Types of parts and their number of uses

Parts	Number of uses
1. Bark & leaf	2
2. Bud & flower	1
3. Bulb	1
4. Flower	2
5. Fruit	4
6. Fruits & seeds	1
7. Leaf	21
8. Leaf & flower	1
9. Leaf & root	2
10. Leaf & seed	1
11. Leaf Latex	2
12. Rhizome	2
13. Root	5
14. Root & fruit	1
15. Seed	3
16. Stem	2
17. Stem bark	4
18. Whole plant	5
19. Wood	1
20. Young shoot	1
TOTAL	**62**

Discussion

Present investigation recorded 62 species [Table-1] of ethno-medicinal plants from the southern parts of West Bengal for treating 7 different types of skin disease and its related diseases such as crack, eczema, pimples, ringworm, scabies and urticaria [Table-3]. To fight against the above mentioned skin related diseases, anti-dots (medicines) were extracted basically from the different parts [bark and leaf (2); Bud & flower (1); Bulb (1); Flower (2); Fruit (4); Fruits & seeds ((4); Leaf (21); Leaf & flower (1); Leaf & root (2); Leaf & seed (1); Leaf Latex (2); Rhizome (2); Root (5); Root & fruit (1); Seed (3); Stem (2); Stem bark (4); Whole plant (5); Wood (1) and Young shoot (1)] of 62 medicinal plant species [Table-4]. With keen investigation it was observed that skin disease (45) is very much predominant followed by scabies (8), eczema (4), ringworm (2), cracks (1), pimple (1) and urticaria (1) [Table-3].

Conclusion

Our mother earth is the treasure house of enormous number of medicinal plants. Unfortunately we are losing these medicinal plants gradually due to progressive urbanisation, indiscriminate forest destruction, pollution, ecological fragmentation, disruption of food web, habitat destruction, pollinator reduction, elimination of keystone species and over exploitation of important species like *Abrus precatorius*, *Aloe vera*, *Alstonia scholaris*, *Azadirachta indica*, *Caesalpinia bonduc*, *Cinnamomum verum*, *Ocimum sanctum*, *Piper nigrum*, *Solanum nigrum*,*Terminalia chebula*, *Tinospora sinensis*, *Withania somnifera* etc from their natural habitats. Recently the attitude of our present generation towards the biodiversity conservation is changing rapidly in a large scale; as a result the acceptance of biodiversity is also losing its importance. So with a view to protect our biodiversity and for the interest of the sustainable future generation, these bio-resources (phyto-resources) can be protected not only by the implementation of conservational measures (*ex-situ* & *in-situ*) but also the mass involvement of the local people. As a result we can conserve these potential medicinal plants from their extinction to a certain extent.

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