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Biological Science

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Highlights

Effect of Populus Nigra Bark

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Discovering Thoughts, Inventing Future

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Comparative Studies of the Effect of Diazepam and Magnesium Sulphate on Motor Coordination and Seizures in 4-Aminopyridine Induced Swiss Mice

By Kebe, E. Obeten, Charles, C. Mfem, Usun, O. Usun & Gabriel, Udo-Affah
University of Calabar, Nigeria

Abstract- The comparative effect of Diazepam and magnesium sulphate on motor coordination and seizures in mice induced with 4-Aminopyridine (4-AP) was studied. Thirty six (36) Swiss white male and female mice were randomly assigned into three (3) groups of six (6) animals each for seizure and beam walking test. Group one animals served as control group and were given normal saline intreperitoneally. Group two animals were given Magnesium sulphate (M.S) (4.5mg/kg) intraperitoneally. Group three (DZP) animals receieved Diazepam (DZP) (2mg/kg) intraperitoneally for the beam walking test. While for the seizure tests, Group one (4-AP) served as control and were given 4-Aminopyridine (4-AP) (13.3mg/kg) only, intraperitoneally. Group two (M.S) animals were given 4-Aminopyridine and Magnesium sulphate (M.S) intraperitoneally 15 minutes before inducing epilepsy with 4-AP. Group three (DZP) animals received 4-Aminopyridine and Diazepam intraperitoneally 15 minutes before inducing epilepsy with 4-AP. Anti-epileptic effect was assessed by scoring the onset of both tonic and clonic seizures.

Keywords: diazepam, magnesium sulphate, motor coordination, seizures, 4-aminophyridine, swiss white mice.

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Comparative Studies of the Effect of Diazepam and Magnesium Sulphate on Motor Coordination and Seizures in 4-Aminopyridine Induced Swiss Mice

Kebe, E. Obeten [α], Charles, C. Mfem [σ], Usun, O. Usun [ρ] & Gabriel, Udo-Affah [ω]

Abstract- The comparative effect of Diazepam and magnesium sulphate on motor coordination and seizures in mice induced with 4-Aminopyridine (4-AP) was studied. Thirty six (36) Swiss white male and female mice were randomly assigned into three (3) groups of six (6) animals each for seizure and beam walking test. Group one animals served as control group and were given normal saline intreperitoneally. Group two animals were given Magnesium sulphate (M.S) (4.5mg/kg) intraperitoneally. Group three (DZP) animals receieved Diazepam (DZP) (2mg/kg) intraperitoneally for the beam walking test. While for the seizure tests, Group one (4-AP) served as control and were given 4-Aminopyridine (4-AP) (13.3mg/kg) only, intraperitoneally. Group two (M.S) animals were given 4-Aminopyridine and Magnesium sulphate (M.S) intraperitoneally 15 minutes before inducing epilepsy with 4-AP. Group three (DZP) animals received 4-Aminopyridine and Diazepam intraperitoneally 15 minutes before inducing epilepsy with 4-AP. Anti-epileptic effect was assessed by scoring the onset of both tonic and clonic seizures. The results showed the early onset of tonic and clonic seizures in both magnesium sulphate and diazepam group capered to control group. ($p<0.001$) the delay in the onset of seizures was significantly higher ($p<0.001$) in the diazepam group compared to magnesium sulphate group. In beam walking test, the frequency of line crossing was significantly higher in the group treated with magnesium sulphate ($p<0.001$) and also frequency of reversals were significantly higher ($p<0.01$) compared with control. Diazepam treated group was significantly lower ($p<0.001$) in line crossing and significantly lower ($p<0.01$) in reversals when compared with control. Number of foot slips were significantly lower ($p<0.01$) in diazepam group when compared to control group, but significantly higher ($p<0.05$) compared to magnesium sulphate. Magnesium sulphate group showed significantly lower ($p<0.001$) number of foot slips when compared with control and also significantly lower ($p<0.05$) when compared with diazepam group. Thus diazepam and magnesium sulphate have anti-epileptic effects but does not reverse the seizures caused by 4-AP though magnesium sulphate was more potent. Diazepam reduces motor coordination in mice but magnesium sulphate improves motor coordination in mice.

Keywords: diazepam, magnesium sulphate, motor coordination, seizures, 4-aminophyridine, swiss white mice.

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I. Introduction

Magnesium sulfate (or magnesium sulphate) is an inorganic salt (chemical compound) containing magnesium, sulfur and oxygen, with the formula $MgSO_4$. It is often encountered as the heptahydrate sulfate mineral epsomite ($MgSO_4 7H2O$), commonly called Epsom salt, taking its name from a bitter saline spring in Epsom in Surrey, England, where the salt was produced from the springs that arise where the porous chalk of the North Downs meets non-porous London clay. The monohydrate, $MgSO_4 \bullet H_2O$ is found as the mineral kieserite. The overall global annual usage in the mid-1970s of the monohydrate was 2.3 million tons, of which the majority was used in agriculture (Quick Cures/Quack cures, 2012).

Anhydrous magnesium sulfate is used as a drying agent. The anhydrous form is hygroscopic (readily absorbs water from the air) and is therefore difficult to weigh accurately; the hydrate is often preferred when preparing solutions (for example, in medical preparations). Epsom salt has been traditionally used as a component of bath salts. Epsom salt can also be used as a beauty product. Athletes use it to soothe sore muscles, while gardeners use it to improve crops. It has a variety of other uses: for example, Epsom salt is also effective in the removal of splinters. (Quick Cures/Quack cures, 2012).

It is on the WHO Model List of Essential Medicines, the most important medications needed in a basic health system. (WHO, 2014). Magnesium sulfate is a common mineral pharmaceutical preparation of magnesium, commonly known as Epsom salt, used both externally and internally. Epsom salt is used as bath salts and for isolation tanks. Oral magnesium sulfate is commonly used as asaline laxative or osmotic purgative. Magnesium sulfate is the main preparation of intravenous magnesium.

Diazepam is a Benzodiazepine drug commonly used to treat anxiety, panic attack, insomnia, seizures (including status epilepticus), muscle spasms, restless leg syndrome, alcohol withdrawal syndrome (National Institute of health, 2006). It possesses anxiolytic,

anticonvulsant, hypnotic, sedative skeletal; muscle relaxant, and amnestic properties (Mandrioli, *et al*., 2008). Diazepam enhances the effect of the neurotransmitter gamma aminobutyric acid (GABA) by binding to benzodiazepine site on the $GABA_A$ receptor leading to central nervous system depression (Riss, *et al*., 2008). Diazepam has no effect on GABA levels and glutamate decarboxylase activity but has a slight effect on gamma-aminobutyric acid transaminase activity. Anticonvulsants are used to control immediate fits and to prevent further seizures, but the choice of anticonvulsant preferable is debatable. (Gifford, *et al*., 1990) reported, that magnesium sulphate has been the drug of choice in the United State. (Hutton, *et al*., 1992) reported, only 2% of obstetricians in the United Kingdom use magnesium sulphate. There has been little adequately controlled evidence to support the use of any of the options of anticonvulsant.

Anticonvulsant drugs act by blocking sodium channels, calcium channels or enhancing gamma aminobutyric acid (GABA) function. Phenytoin and carbamazepine are sodium channel blockers. They bind to the active state of the channel and reduce high frequency firing while allowing normal action potential to occur (Rogawski&Loscher, 2004). Lamotrigineblosks high voltage activated calcium channels and Zonisamidehas activate T-type calcium channels (Matar, *et al*., 2009). Rogawski (2004), states that GABA enhancers are the benzodiazepines and Phenobarbital which act by increasing the open time or opening frequency of the $GABA_A$ receptor-mediated chlorine channel.

4-Aminopyridine is an organic compound. The molecule is one of three isomeric amines of pyridine. It is used primarily as a research tool, characterizing subtype of potassium channel, and has also been used to manage some of the symptoms of multiple sclerosis (Solari, *et al*., 2001). It acts by blocking potassium channels, prolonging action potentials and thereby increasing neuromuscular junction (Judge &Bever, 2006). This k+ antagonist is a powerful convulsant in animals and in man. The drug readily penetrates the blood-brain barrier and is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter release. In mice, parenterally administered 4-AP induces clonic-tonic convulsions, wild running, tonic hind limb extension and lethality. Eclampsia, is an acute but life threatening complication of pregnancy characterized by the appearance of tonic clonic seizures (convulsions), usually in women who have developed pre-eclamsia (Ghulmiyyah&Sibai, 2012). Pre-eclampsia is a disorder of pregnancy characterized by high blood pressure and large amount of protein in the urine. Pre-eclampsia and eclampsia are collectively called “hypertensive disorder of pregnancy” and “toxemia of pregnancy”. Eclampsia is the occurance of generalized convulsion(s) associated with signs of pre-eclampsia during pregnancy, labour or within 7 days of delivery and not caused by epilepsy or other convulsion disorder (Davey &MacGillivray, 1988). In developed countries, this incident occurs in one in 2000 deliveries and one in 100 in developing countries (Mushambi, *et, al*., 1996).

Fine motor control is the coordination of muscles, bones, and nerves to produce a small precise movements. An example of fine motor control is picking up a small item with the index finger and thumb (Kimmel & Ratliff-Schaub, 2011) problems of the brain, spinal cord, peripheral nerves, muscles, or joint may all decrease fine motor control. Balance is the ability to maintain a controlled body position during task performance, whether it is sitting at a table, walking the balance beam or stepping up unto a kerb. To function effectively across the environments and tasks, we need the ability to maintain controlled positions during both static (still) and dynamic (moving) activities.

II. 4-Aminopyridine

4-Aminopyridine is an organic compound. The molecule is one of three isomeric amines of pyridine. It is used primarily as a research tool, characterizing subtype of potassium channel, and has also been used to manage some of the symptoms of multiple sclerosis (Solari*et al*, 2001). In laboratory, 4-AP is a useful pharmacological tool in studying various potassium conductances in physiology and biophysics. It is a relatively selective blocker of members of kv1 family of voltage-activated k+ channels. At concentration of 1 Mm it selectively and reversibly inhibits shaka channels without significant effect on other sodium, calcium and potassium conductances. It acts by blocking potassium channels, prolonging action potentials and thereby increasing neuromuscular junction (Judge and Bever, 2006). This k+ antagonist is a powerful convulsant in animal and in man. The drug readily penetrates the blood-brain barrier and is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter release. Although both excitatory and inhibitory synaptic transmission is facilitated by 4-AP, the epileptform activity induced by the drug is predominantly mediated by non-N-methyl-D-aspartate type excitatory amino acid receptors (non NMDA). In mice, parentarally administered 4-AP induces clonic-tonic convulsions, wild running, tonic hind limb extension and lethality. Drugs with phenytoin-like profile of activity are more effective anticonvulsants with 4-AP. Phenobarbital and valproate are also effective.

III. Motor Coordination

Motor coordination is defined as the harmonious functioning of the body parts that involves movements including fine motor movement, gross motor movement and motor planning. It is also defined

as the combination of body movements created with kinematics (spatial direction) and kinetic (force) parameters that result in intended actions. Motor coordination is achieved when subsequent parts of the same movement, or the movements of limbs or body parts are combined in a manner that is well times, smooth and efficient with respect to the intended goal. This involve the integration of proprioceptive information detailing the position and movement of the musculoskeletal system with the neural processes in the brain and spinal cord which plans, relay and control motor command. The cerebellum and basal ganglia play critical roles in this neural control of movement and damage to these parts of the brain mainly the cerebellum or its connecting structures and pathways results in impairment of coordination known as Ataxia.

IV. Materials and Method

a) The Materials

The following materials/apparatus were used for the experiment, most of which were obtained from the Department of Physiology, University of Calabar, Calabar.

1. Cages
2. Cotton wool
3. 70% ethyl alcohol
4. Disposable gloves
5. Conical flask
6. Distilled water
7. Feed (Vital Feeds)
8. Marker
9. Plastic container
10. Electronic weighing balance
11. Stopwatch
12. Spatula
13. Stirrer
14. Water bottle
15. Disposable syringes
16. Izal (disinfectant)
17. Rubber basin
18. Tissue paper
19. Normal saline
20. Sample bottle

b) Experimental animal

The animals used for this study were thirty (36) healthy male and female Swiss white mice purchased from the animal house of the Department of Physiology, Faculty of Basic Medical Science, University of Calabar, Calabar. The mice were kept in a well ventilated cage at room temperature 25 ± 2^0C and 12/12 hour light dark cycle. The animals were fed with pellet feed and had access to clean water every morning. The animals were kept in hygienic environment with their bedding changed every morning until the date of the experiment.

c) Experimental protocol

Thirty (36) Swiss white male and female mice weighing between 20-25g were randomly assigned into three (3) groups of six (6) animals each for both seizure and beam walking tests. Group one animals served as control group and were administered with normal saline intraperitoneally, according to their body weight. Group two (M.S) animals were administered with magnesium sulphate (M.S) (4.5mg/kg of body weight) which was administered intraperitoneally. Group three (DZP) animals received Diazepam (DZP) (2mg/kg of body weight) administered intraperitoneally for the beam walking test. While for the seizure tests, group one (4-AP) (13.3mg/kg of body weight) intraperitoneally. Group two (M.S) animals were administered with 4-Aminopyridine (13.3mg/kg of body weight) and magnesium sulphate (M.S) (4.5mg/kg of body weight) which was administered intraperitoneally. Group three (DZP) animals received 4-Aminopyridine (13.3mg/kg of body weight) and the Diazepam (DZP) (2mg/kg of body weight) administered intraperitoneally.

0.8ml of Diazepam was mixed with 19.2ml of normal saline, 100mg of Magnesium sulphate and dissolved in 111ml of normal saline and 100mg of 4-Aminopyridine was dissolved in 37ml of normal saline before administration. This dilution was done before administration to prevent venous damaging.

V. Apparatus and Behavioural Score

a) The beam walking (balance) apparatus

The beam has a length of 100cm, a width of 2cm and is elevated to a height of 40cm. the beam is marked at 5cm and 1cm intervals. It is composed of metal and is coated with black paint. The animal was carried to the test room in the home cage. The mouse was removed from its home cage and placed at one end of the balance beam. Each trial was done after the mouse had secured its grip on the beam. The maximum length of the trial was two minutes. The mouse was tested under white light, during the dark phase. The beam was cleaned with 70% ethanol and allowed to dry between each trial.

Behavior scores on the beam walking apparatus include:

Distance travelled: The number of line crosses.

Foot slips: Number of times one of the mouse's back feet slips from the beam.

Number of turns: Frequency that the animal reversed direction

Fall: Time at which the animal fell off the beam.

If a fall occurred the animal was not placed back on the beam but was returned to the home cage. The trial was not repeated.

VI. Results

a) Beam walking test results

The beam walking test was used to assess the test substances on motor coordination and their results are shown below.

b) Comparison of frequency of line crossing during beam walking test

The mean values for the frequency of line crossing from the Control, Magnesium sulphate (M.S) and Diazepam (DZP) were; 207.17±5.36, 256.33±9.65 and 113.50±4.09 per 2 minutes respectively.The result showed that the group treated with M.S was significantly higher in line crossing ($p<0.001$) compared to the control group. Also, the group treated with DZP had a significant decrease ($p<0.001$) when compared with M.S group. The DZP group was significantly lower ($p<0.001$) compared with the control group. This is shown in figure 1.

c) Comparison of reversal of line crossing during beam walking test

Figure 2 shows the frequency of reversals in beam walking test. The mean value for the frequency of reversals for the control, magnesium sulphate (M.S) and Diazepam (DZP) were, 6.83±1.08, 12.00±1.15 and 2.17±0.60 per 2 minutes respectively.The result showed that the group treated with M.S was significantly higher ($p<0.01$) when compared to control. Also the DZP group was significantly lower ($p<0.01$) when compared with M.S and significantly lower ($p<0.01$) when compared to control. Figure 2

d) Comparison of frequency of foot slip during Beam walking test

Figure 3 shows the frequency of foot slips in beam walking test. The mean values for the frequency of foot slips for the control, magnesium sulphate (M.S) and diazepam (DZP) were; 4.17±0.31, 1.67±0.21 and 2.67±0.33 per 2 minutes respectively.The result showed that the M.S group was significantly lower ($p<0.001$) when compared with the control group. The DZP group was significantly higher ($p<0.05$) when compared with M.S group. It is also significantly lower ($p<0.01$) compared to control group. Figure 3

VII. Seizures

a) Comparison of onset of trembling

The comparison of onset of trembling in mice treated with 4-Aminopyridine and magnesium sulphate (4-AP +M.S) as M.S group and mice treated with 4-Aminopyridine and diazepam (4-AP + DZP) as DZP group. The mean values for control, M.S and DZP were; 81.50±2.29, 282.17±17.30 and 207.83±3.68 per 5 minutes respectively.The result showed that the group treated with M.S was significantly higher ($p<0.001$) compared with control. Also the group treated with DZP was significantly lower ($p<0.001$) when compared with the M.S group. DZP group was also significantly higher ($p<0.001$) compared to control. This is shown in figure 4.

b) Comparison of onset of wild running

Figure 5 shows the comparison of onset of wild running with mean values for control, M.S and DZP as; 300.50±5.49, 502.00±4.75 and 425.83±17.56 per 15 minutes respectively.

The result showed that the group treated with M.S was significantly higher ($p<0.001$) when compared with control. Also the group treated with DZP was significantly lower ($p<0.001$) when compared with M.S group bur significantly higher ($p<0.001$) when compared with control.

c) Comparison of onset of jerking

Figure 6 shows the comparison of onset of jerking with mean values for control, M.S and DZP as; 32.17±2.74, 130.50±4.55 and 79.67±2.86 per 15 minutes respectively.

The result showed that the group treated with M.S was significantly higher ($p<0.001$) compared with control. Also the group treated with DZP was significantly lower ($p<0.001$) compared to M.S group but significantly higher ($p<0.001$) compared with control.

d) Comparison of onset of tonic clonic seizure

Figure 7 shows the onset of tonic clonic seizures with mean values for control, M.S and DZP as; 608.67±11.46, 1624.33±20.17 and 1001.17±30.58 per 15 minutes respectively. The result showed that the group treated with M.S was significantly higher ($p<0.001$) compared with control. Also the group treated with DZP was significantly lower ($p<0.001$) when compared to M.S group but significantly higher ($p<0.001$) compared with control.

e) Comparison of time of death

Figure 8 shows the time of death with mean values for the control, M.S and DZP as; 626.83±25.15, 2148.33±130.31 and 1404.17±160.36 per 15 minutes respectively.The result showed that the group treated with M.S was significantly higher ($p<0.001$) compared to control. Also the group treated with DZP was significantly lower ($p<0.001$) compared to M.S group but significantly higher ($p<0.001$) when compared with control.

VIII. Discussion

The comparative effect of diazepam and magnesium sulphate on motor coordination and its anti-epileptic effect on 4-Aminopyridine (4-AP) induced epileptic mice were investigated. The anti-epileptic effect of magnesium sulphate and diazepam were investigated after treatment with 4-AP to induce

epilepsy. Records of onset of trembling, onset of wild running, onset of jerking, onset of tonic clonic seizure and time of death were studied. The potency of both anti-epileptic agents were studied, noting the frequency and duration of the seizures.

a) *Comparison of onset of trembling, wild running, jerking, tonic clonic seizures and time of death in all the groups of mice tested for anti-epileptic effect*

The onset of trembling, wild running and jerking was administered. Onset of tonic clonic seizures is the time it takes the mice to undergo radical seizures when 4-AP was administered. Time od death is the time it takes for the mouse to die after administration of 4-AP.

The onset of trembling, wild running, jerking, tonic clonic seizures and time of death decreased significantly in the group of mice treated with only 4-AP. This shows that 4-AP induces epilepsy, as stated by Judge and Bever (2006) that 4-AP which is a strong potassium ion antagonist is a powerful convulsant in animals and man.

Rogawski (2004) states that sodium channel blockers, drugs with phenytoin-like profile of action which reduce high frequency firing while allowing normal action potential to occur are more effective anticonvulsants with 4-ap. This is probable to attain sodium and potassium balance within cells in order to provide a suitable environment for proper nervous transmissions. The result also showed that magnesium sulphate improves motor coordination while diazepam reduces motor coordination.

Magnesium sulphate and diazepam have anti-epileptic effect to a certain extent but magnesium sulphate was more potent compared with diazepam. Engbaek (1948)argued that magnesium sulphate prevents the contracture caused by potassium more readily than that produced by acetylcholine and 4-AP acts by blocking potassium channels thereby prolonging action potentials (Judge and Bever, 2006). Probably this is why magnesium sulphate is more potent than diazepam in 4-AP induced seizures.

IX. Motor Coordination Behaviour

The beam walking test used to access motor coordination. Behavior in the beam walking test such as frequency of line crosses, reversals and foot slips were used in the assessment of motor coordination. In this test, increased frequency of line crosses and reversals indicate greater ability to maneuver on the beam walking apparatus and hence better motor coordination. Also increased frequency of foot slip indicates decreased motor coordination.

The frequency of line crossing for the group treated with diazepam (DZP) was significantly lower compared to control group and also lower compared to magnesium sulphate group (M.S), which shows the mice had poor maneuvering abilities. As stated by (Tasman &Liberman, 2006), the most common side effect of diazepam are related to their sedating and muscle relaxing actions which include drowsiness, dizziness, decreased alertness, concentration and lack of coordination. Although diazepam was used to reduce epileptic seizures, it impairs motor coordination. Probably due to the enhanced effect of GABA which is a major inhibitory neurotransmitter which causes central nervous system depression (Risse*t al*, 2008). Those treated with magnesium sulphate (M.S) were significantly higher compared with the control group and also higher compared with DZP thus showed improved motor coordination. Magnesium participates in muscle contraction, plays a key role in the excitation-contraction coupling in the skeletal muscle, also essential for the neurotransmission that orchestrates mood, cognitive functions, memory, sleep, relaxation and emotions in general (Szewczyk, *et al*., 2008). Probably for this reason M.S improves motor coordination.

The frequency of reversals were seen to be significantly lower in DZP group compared with both control and M.S group, a sign of poor motor coordination. Also the group treated with M.S was significantly higher compared with both control and DZP group, showing improved motor coordination.

The frequency of fool slips were seen to be significantly higher in DZP group compared with M.S group but significantly lower compared with control. M.S group had reduced number of foot slips when compared with both control and DZP group.

X. Conclusion

From the result obtained, 4-Aminopyridine (4-AP) induced epileptic seizures were delayed by both diazepam and magnesium sulphate although magnesium sulphate was more potent than diazepam.

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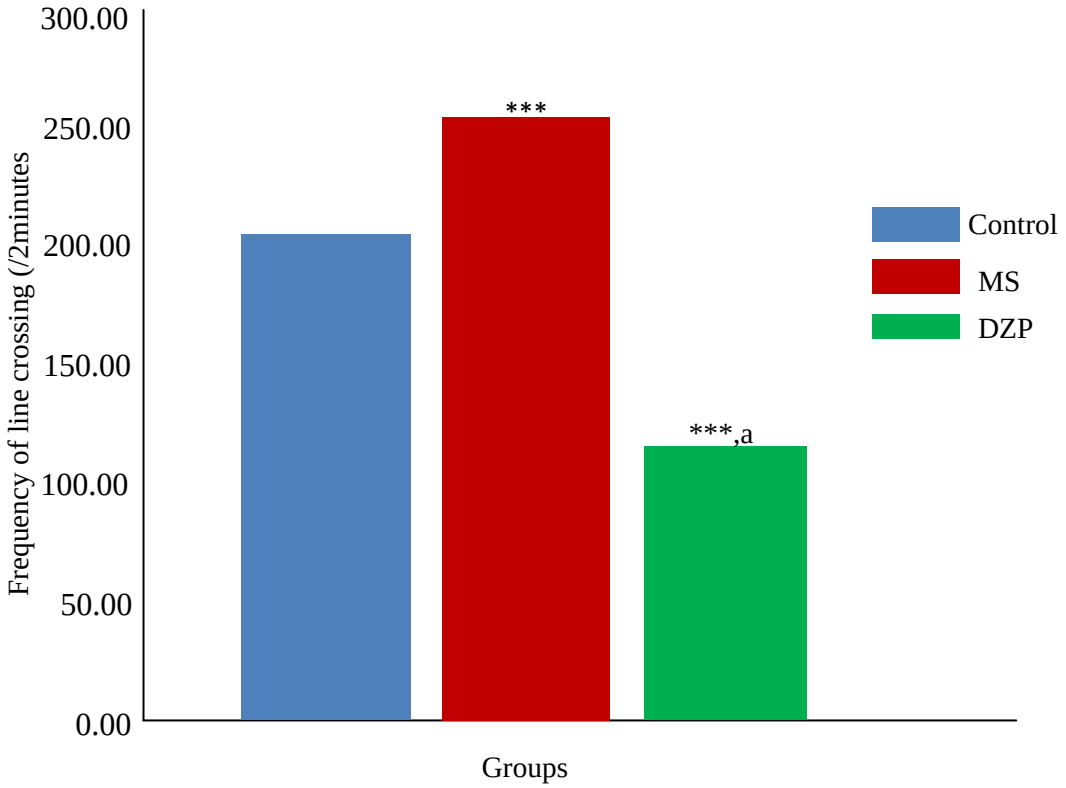

Figure 1 : Comparison of frequency of line crossing during beam walking in the different experimental groups.
Values are mean ± SEM, n=6
***p<0.001 vs Control; a = p<0.001 vs MS

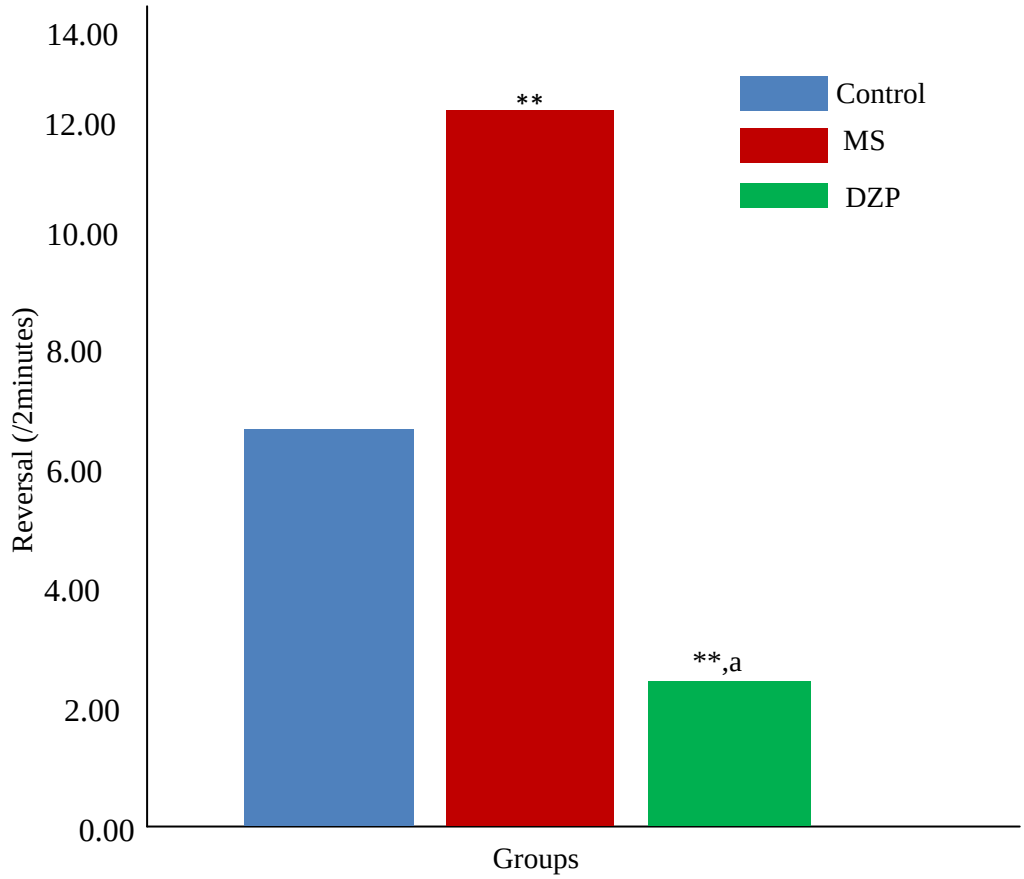

Figure 2 : Comparison of reversal of line crossing during beam walking in the different experimental groups. Values are mean ± SEM, n=6

**p,0.01vs control; a = p<0.001 vs MS

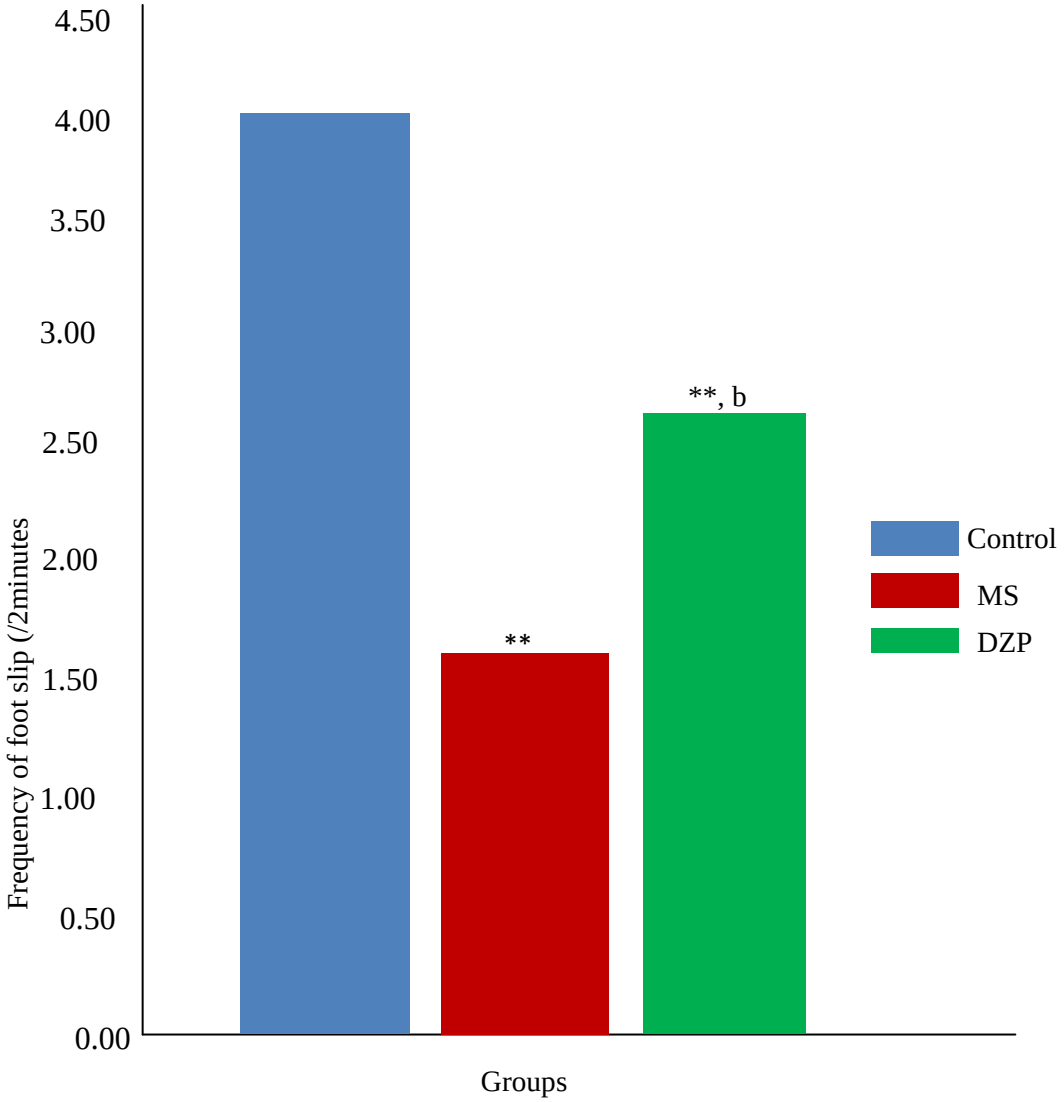

Figure 3 : Comparison of frequency of foot slip during mean walking in the different experimental groups.
Values are mean ±SEM, n=6
**p<0.01, ***p<0.001 vs Control; b = p<0.05 vs MS

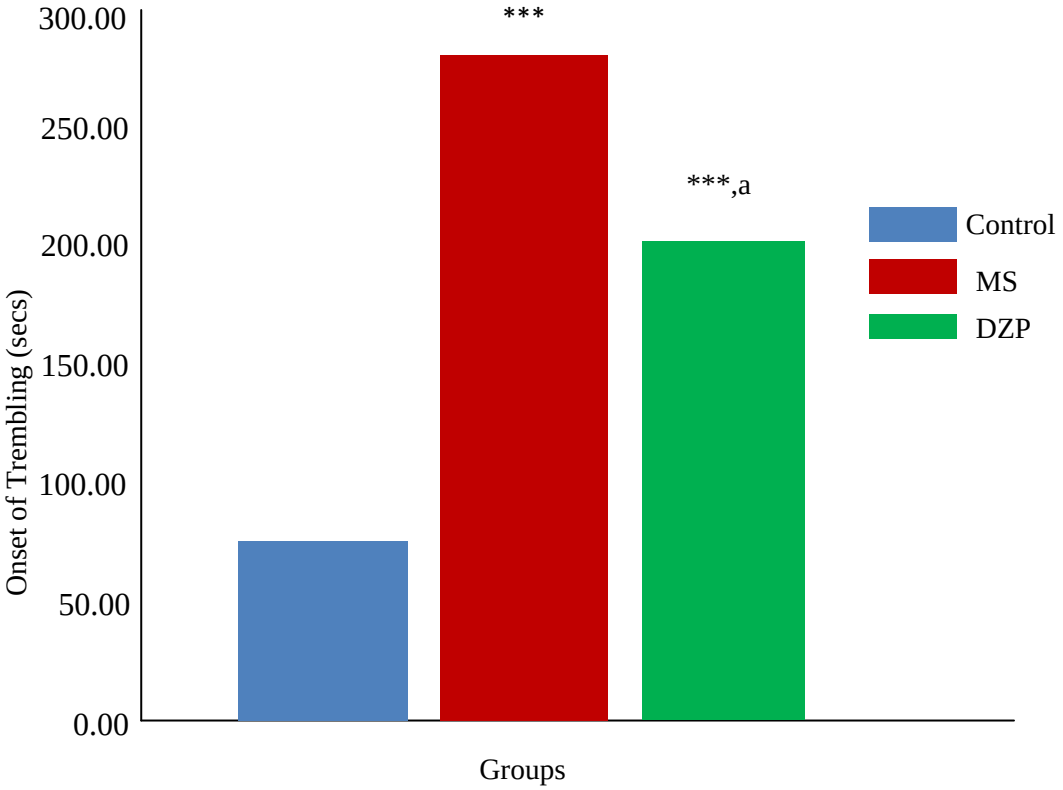

Figure 4 : Comparison of onset of trembling in the different experimental groups. Values are mean ±SEM, n=6
***p<0.001 vs Control; a = p<0.001 vs MS

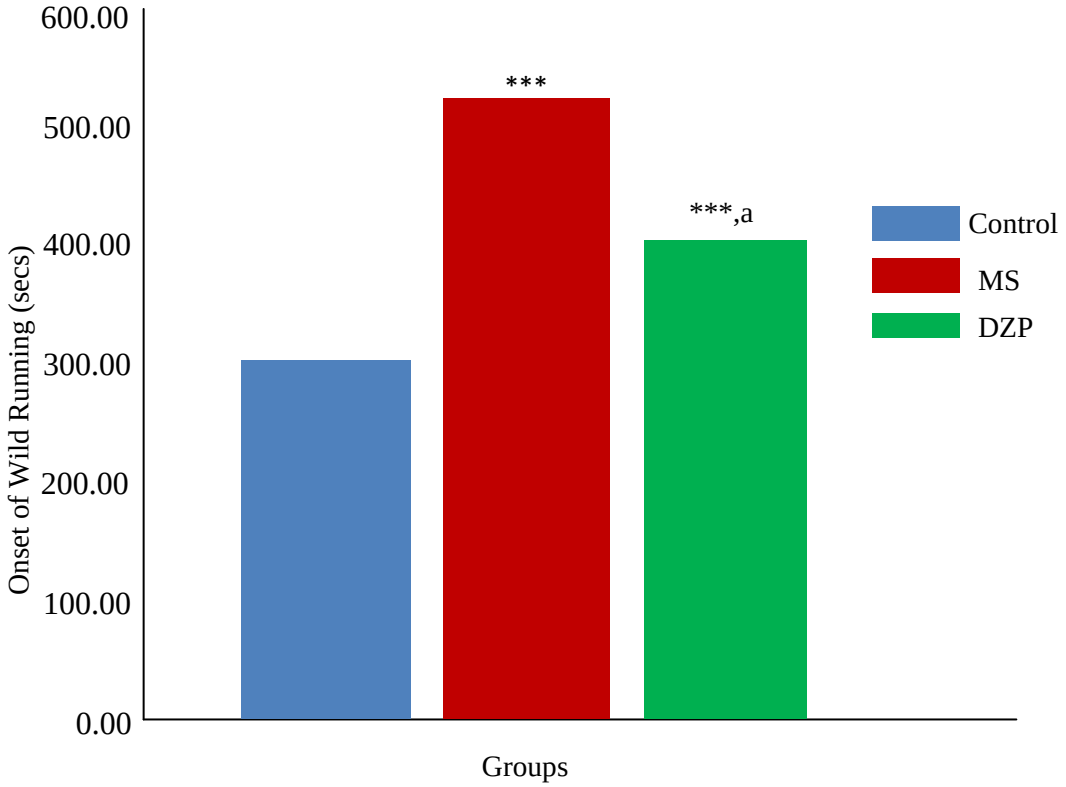

Figure 5 : Comparison of onset of wild running in the different experimental groups.
Values are mean ± SEM, n =6
***p<0.001 vs Control; a = p<0.001 vs MS.

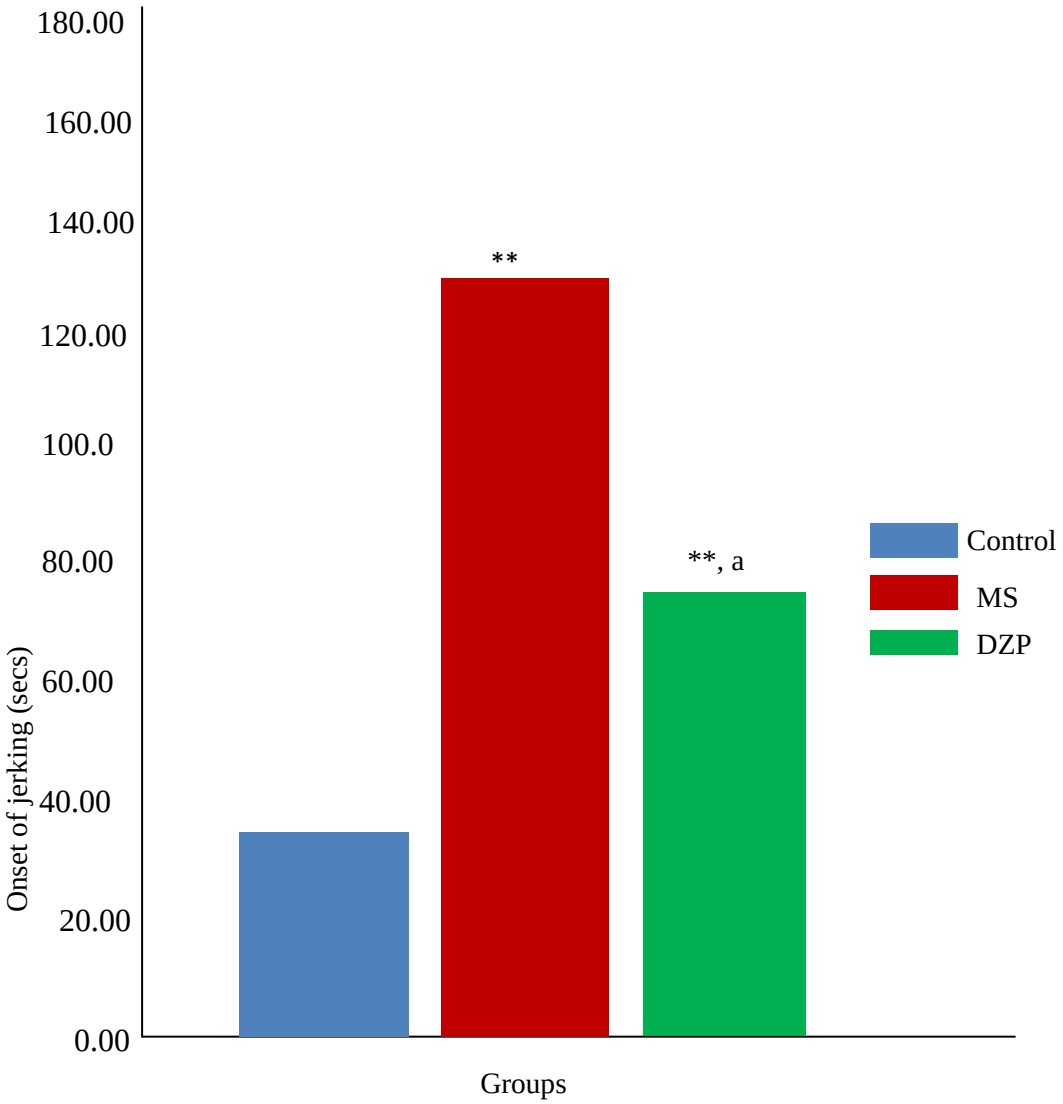

Figure 6 : Comparison of onset of jerking in the different experimental groups.
Values are mean ± SEM, n = 6
***p<0.001 vs Control; a = p<0.001 vs MS.

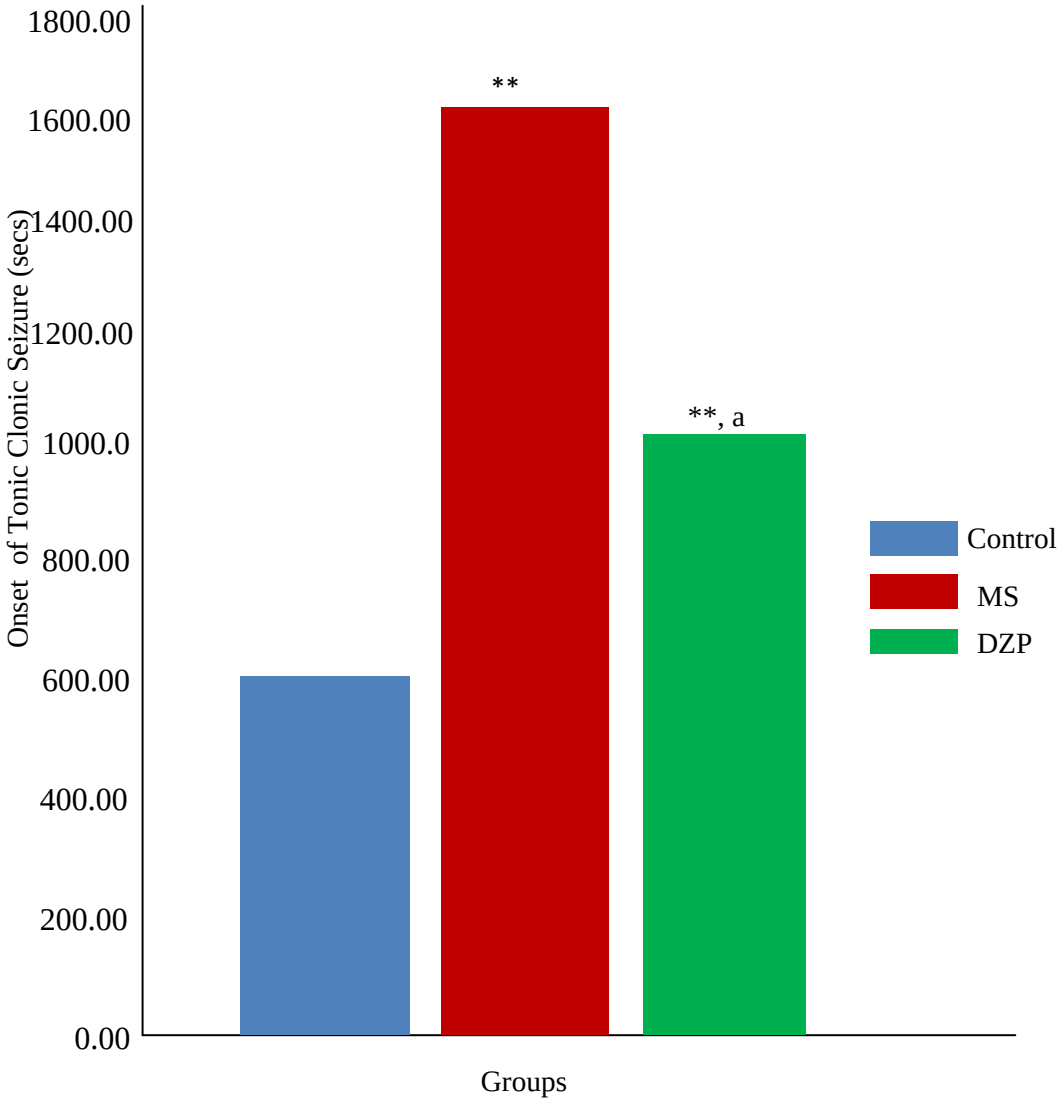

Figure 7 : Comparison of onset to tonic clonic seizure in the different experimental groups.
Values are mean ± SEM, n =6
***p<0.001 vs Control; a =p<0.001 vs MS

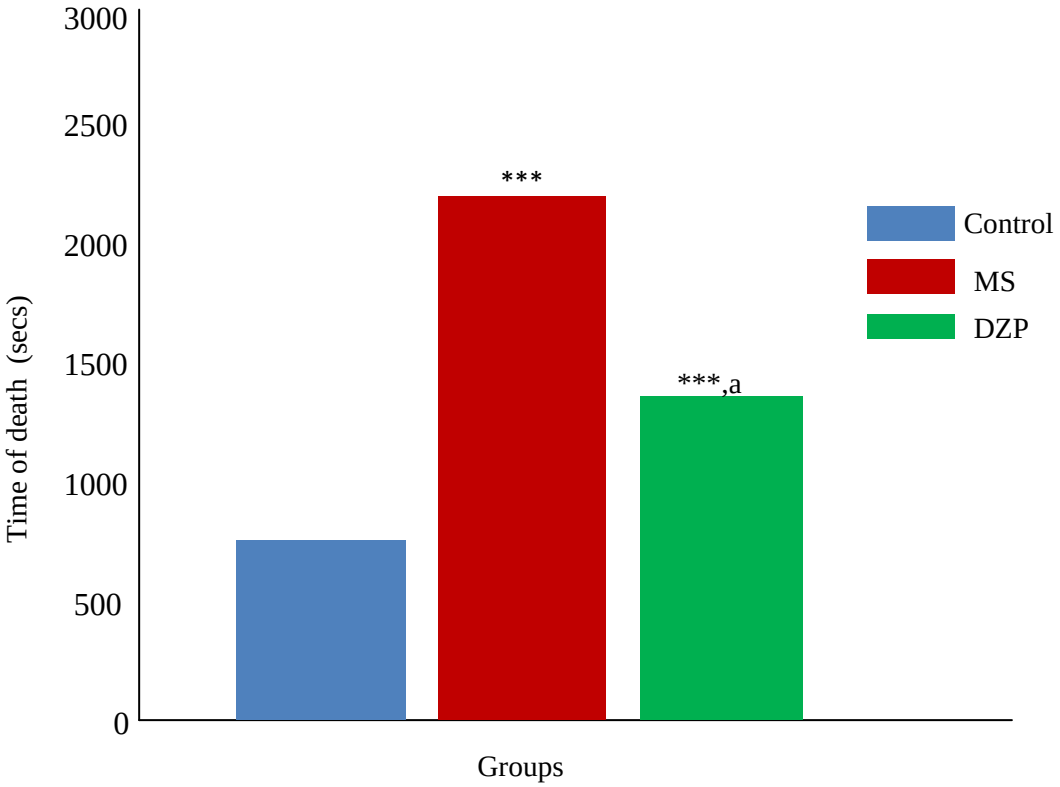

Figure 8 : Comparison of time of death in the different experimental groups.
Values are mean ± SEM, n =6
***p<0.001 vs Control; a = p<0.001 vs MS

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New Records of Five Species of Scleractinian Corals to Indian Waters from Andaman and Nicobar Islands

By Tamal Mondal & C. Raghunathan
National Coral Reef Research Institute, India

Abstract- Oligotropic waters of Andaman and Nicobar archipelago harbor a rich diversity of faunal communities. Scleractinian corals are recorded from this group of islands are highly diverse in comparison with the other reef areas of India. Five species of hermatypic corals viz. *Acroporalovelli* Veron and Wallace, 1984 and *Acropora willisae* Veron and Wallace, 1984 belonging to Acroporidae family, *Myceto- phyllia lamarckiana* Milne Edwards and Haime, 1848 and *Isophyllia sinuosa* (Ellis and Solander, 1786)under Mussidae family and *Porites cumulates* Nemenzo, 1955 of Poritidae family are recorded for the first time in Indian waters from Andaman group of islands. The range distribution of this five species increases the Indian scleractinian database upto 611 species, of which 579 species from Andaman and Nicobar Islands. The present paper dealt with the morphological characteristics of these four newly recorded species of scleractinians with their distribution and conservational status.

Keywords: *Scleractinian corals, New record, Mycetophyllia, Andaman and Nicobar Islands.*

GJSFR-C Classification : *FOR Code: 279999*

Strictly as per the compliance and regulations of :

New Records of Five Species of Scleractinian Corals to Indian Waters from Andaman and Nicobar Islands

Tamal Mondal [α] & C. Raghunathan [σ]

Abstract- Oligotropic waters of Andaman and Nicobar archipelago harbor a rich diversity of faunal communities. Scleractinian corals are recorded from this group of islands are highly diverse in comparison with the other reef areas of India. Five species of hermatypic corals viz. *Acroporalovelli* Veron and Wallace, 1984 and *Acropora willisae* Veron and Wallace, 1984 belonging to Acroporidae family, *Myceto- phyllia lamarckiana* Milne Edwards and Haime, 1848 and *Isophyllia sinuosa* (Ellis and Solander, 1786)under Mussidae family and *Porites cumulates* Nemenzo, 1955 of Poritidae family are recorded for the first time in Indian waters from Andaman group of islands. The range distribution of this five species increases the Indian scleractinian database upto 611 species, of which 579 species from Andaman and Nicobar Islands. The present paper dealt with the morphological characteristics of these four newly recorded species of scleractinians with their distribution and conservational status.

Keywords: Scleractinian corals, New record, Mycetophyllia, Andaman and Nicobar Islands.

I. Introduction

Coral reefs are one of the most imperative biological creatures of marine ecosystem in shallow tropical seas and nourish several numbers of imperious ecosystem services [1, 2]. The structural organization, gradual developmental pattern along with accumulation and secretion of calcareous aragonite skeleton of the coral reefs can be seen under main three categories such as fringing reefs, barrier reefs and atolls [3] while a number of different types of other small reefs like, patchy reef, ribbon reef, table reef etc. can be seen throughout the world's ocean. Geographical ranges along with ecological parameters attributes the most for the settlement of corals. The diverse species content, complex structure, inter-linking correlation with a wide number of other associated species demonstrates the immortal role of coral reefs for the sustenance marine biodiversity. Along with the ecological services, it also takes active part for the coastal and marine protection, conservation, fishery and aquaculture, industrialization of ornamentation, biomedical applications, climatic restoration, chemical balance in world's ocean and so on [4]. Nearly 500 million people depend directly and indirectly on coral reefs for their livelihoods, food and other resources [5], while 30 million of the poorest human populations in the world depend entirely on coral reefs for their food [5]. Andaman and Nicobar Islands are the biologically diverse islands of Indian Ocean due to its geographic location with the presence of sustainable biogenic marine habitat. This paper deals with new record of five species of scleractinian corals from Andaman and Nicobar Islands to Indian waters along with their previous distribution.

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II. Material and Methods

Surveys were conducted from February 2013 to April 2015 to explore the scleractinian coral species at Andaman group of islands by employing Self-Contained Underwater Breathing Apparatus (SCUBA) diving. The underwater species recording was done for detailed identification with the help of Canon Power Shot G15 while small portions/colony of the three species was sampled for detailed morphological studies. Corallites of the specimen were studied in details under stereo zoom microscope (Leica, M 205 A). Identification of species was made in conjunction with Veron and Pichon [6], Veron and Wallace [7], Veron [3] and Wallace [8]. The global status of the species was recognized by IUCN Redlist category and criteria [9]. On completion of detailed taxonomical characters, the specimens were registered in National Zoological Collections and deposited at Zoological Survey of India, ANRC, Port Blair.

III. Results

Five species of scleractinian corals were recorded as new to Indian waters from Andaman and Nicobar Islands on the basis of their taxonomical studies.

Family: ACROPORIDAE Verrill, 1902
Genus: *Acropora* Oken, 1815

*a) Acroporalovelli*Veron and Wallace, 1984(Fig. 1)

i. *Material examined*

Three colonies were observed at Ray Island (Lat. 12°57.454'N; Long. 92°54.452'E) of North and Middle Andaman at the depth of 8 m on 21.ii.2013. One

small portion of the colony was sampled for taxonomical analysis (Reg. No.: ZSI/ANRC-12429).

ii. *Description*

Colonies are caespitose with terete or slightly tapered main branches or hispidose with compacted, elongate, tapering branches. Radial corallites are immersed on the lower branches. These are tubular and appressed on upper branches. Upper branch corallites have dimidiate openings. Axial corallites are dome shaped. Coenosteum is reticulate or finely costate.

Colour: Colony is pale brown or blue.

Habitat: The colonies are seen on the shallow and tropical reef environments up to the depth of 10 m.

Occurrence in A and N Islands: Rare.

IUCN Red List Category and Criterion: Vulnerable, 2014.

iii. *Distribution*

In India: Andaman and Nicobar Islands; *Elsewhere*: Australia, Eritrea, Fiji, French Polynesia, Guam, Indonesia, Kiribati, Marshall Islands, Mauritius, Micronesia, Federated States of Myanmar, Nauru, New Caledonia, Norfolk Island, Northern Mariana Islands, Palau, Papua New Guinea, Pitcairn, Réunion, Saudi Arabia, Solomon Islands, Sri Lanka, Thailand, Tuvalu, Vanuatu, Wallis and Futuna, and Yemen.

Fig. 1 : Acropora lovelli Veron and Wallace, 1984

*b) Acroporawillisae*Veron and Wallace, 1984 (Fig. 2)

i. *Material examined*

Two colonies were observed at Cinque Island (Lat. 11°19.703'N; Long. 92°43.037'E) of South Andaman at the depth of 27 m on 30.iv.2015.

ii. *Description*

Coralla is corymbose in structure with short branchlets. Axial corallites are incipient and proliferous. Radial corallites are tubular and appressed near branchlet tips with nariform openings. Septa are bilaterally symmetrically arranged. The coenosteum between corallites is spongy with fine spinules.

Colour: Colony is grey, cream, blue or brown.

Habitat: The colonies are seen on the shallow and tropical reef environments up to the depth of 30 m.

Occurrence in A and N Islands: Rare.

IUCN Red List Category and Criterion: Vulnerable, 2014.

iii. *Distribution*

In India: Andaman and Nicobar Islands; *Elsewhere*: Australia, Cambodia, Comoros, Indonesia, Japan, Kenya, Madagascar, Malaysia, Mauritius, Mayotte, Micronesia, Federated States of Mozambique, Palau, Papua New Guinea, Philippines, Réunion, Seychelles, Singapore, Solomon Islands, Somalia, South Africa, Taiwan, Province of China, Tanzania, United Republic of Thailand and Viet Nam.

Family: MUSSIDAE Ortmann, 1890

Genus: Mycetophyllia Milne Edwards and Haime, 1848

Fig.2 : Acropora willisae Veron and Wallace, 1984

c) *Mycetophyllialamarckiana*Milne Edwards and Haime, 1848 (Fig. 3)

i. *Material examined*

Five colonies were observed at Trilby Island (Lat. 13°25.636'N; Long. 93°04.273'E) of North and Middle Andaman at the depth of 24 m on 05.iii.2015. A small colony was sampled for taxonomical studies (Reg. No.: ZSI/ANRC-12203).

ii. *Description*

Colonies are solid in structural confirmation, rounded or circular plates like. Valleys are shallow and continuous. Those are radiating from the original point of growth. Vaguely concentric corallite centres to plate margins. One row of mouths are visible in valleys Columellae are rudimentary.

Colour: Colony is grey, cream, blue or brown.

Habitat: The colonies are seen on the deep for e reef environment up to depth of 75 m.

Occurrence in A and N Islands: Rare.

IUCN Red List Category and Criterion: Least Concern, 2014.

iii. *Distribution*

In India: Andaman and Nicobar Islands; *Elsewhere*: Anguilla, Antigua and Barbuda, Bahamas, Barbados, Belize, Bonaire, Sint Eustatius and Saba, Cayman Islands, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Honduras, Jamaica, Mexico, Montserrat, Nicaragua, Panama, Saint Barthélemy, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Sint Maarten, Trinidad and Tobago, Turks and Caicos Islands, United States, United States Minor Outlying Islands, Venezuela and Bolivarian Republic of Virgin Islands.

Genus: Isophyllia Milne Edwards and Haime, 1851

Fig. 3 : Mycetophyllia lamarckiana Milne Edwards and Haime, 1848

d) Isophylliasinuosa(Ellis and Solander, 1786) (Fig. 4)

i. *Material examined*

Three colonies were observed at North Bay (Lat. 11°41.962'N; Long. 092°45.219'E) of South Andaman at the depth of 5 m on 6.i.2014.

ii. *Description*

Colonies are massive in structure. The development pattern of this species is meandroid in nature. The valleys of the colony are short and sinuous. Septal are categorized in arrangements. They are thin. The septa are well ornamented with pointed fine teeth. Columella centres are extended and difficult to distinguish.

Colour: Colonies are green, lavender or yellow while the colour of the valleys and walls are contrasting.

Habitat: The colonies are seen on the shallow and protected reef environments up to the depth of 20 m.
Occurrence in A and N Islands: Rare.

IUCN Red List Category and Criterion: Least Concern, 2015-4.

iii. *Distribution*

In India: Andaman and Nicobar Islands; *Elsewhere*: Anguilla, Antigua and Barbuda, Bahamas, Barbados, Belize, Bermuda, Bonaire, Saint Eustatius and Saba, Cayman Islands, Colombia, Costa Rica, Cuba,Curaçao, Dominica, Dominican Republic, Grenada, Guadeloupe, Haiti, Honduras, Jamaica, Mexico, Montserrat, Nicaragua, Panama, Saint Barthélemy, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Saint Maarten, Trinidad and Tobago, Turks and Caicos Islands, United States, United States Minor Outlying Islands, Venezuela, Bolivarian Republic of Virgin Islands and British.

Family: PORITIDAE Gray, 1842

Genus: Porites Link, 1807

Fig. 4 : Isophyllia sinuosa (Ellis and Solander, 1786)

e) Porites cumulates Nemenzo, 1955 (Fig. 5)

i. *Material examined*

Twelve colonies were observed at Oliver Island (Lat. 12°59.585'N; Long. 92°58.154'E) of North and Middle Andaman at the depth of 6 m on 16.v.2014. One small portion of the colony was sampled for taxonomical studies (Reg. No.: ZSI/ANRC-12354).

ii. *Description*

Colonies are highly fused flattened and developed branches. Corallites are angular and superficial with a diameter of 0.8 mm. Branch surfaces are smooth. Corallite walls are thin. Triplet is fused. 5 tall pali are present. One dentricle is present. Columella is tall.

Colour: Colony is cream or pale brown.

Habitat: The colonies are seen on the shallow and protected reef environments up to the depth of 20 m.

Occurrence in A and N Islands: Rare.

IUCN Red List Category and Criterion: Vulnerable, 2014.

iii. *Distribution*

In India: Andaman and Nicobar Islands; *Elsewhere*: Australia, Cambodia, Indonesia, Malaysia, Papua New Guinea, Philippines, Singapore, Solomon Islands, Taiwan, Province of China, Thailand and Viet Nam.

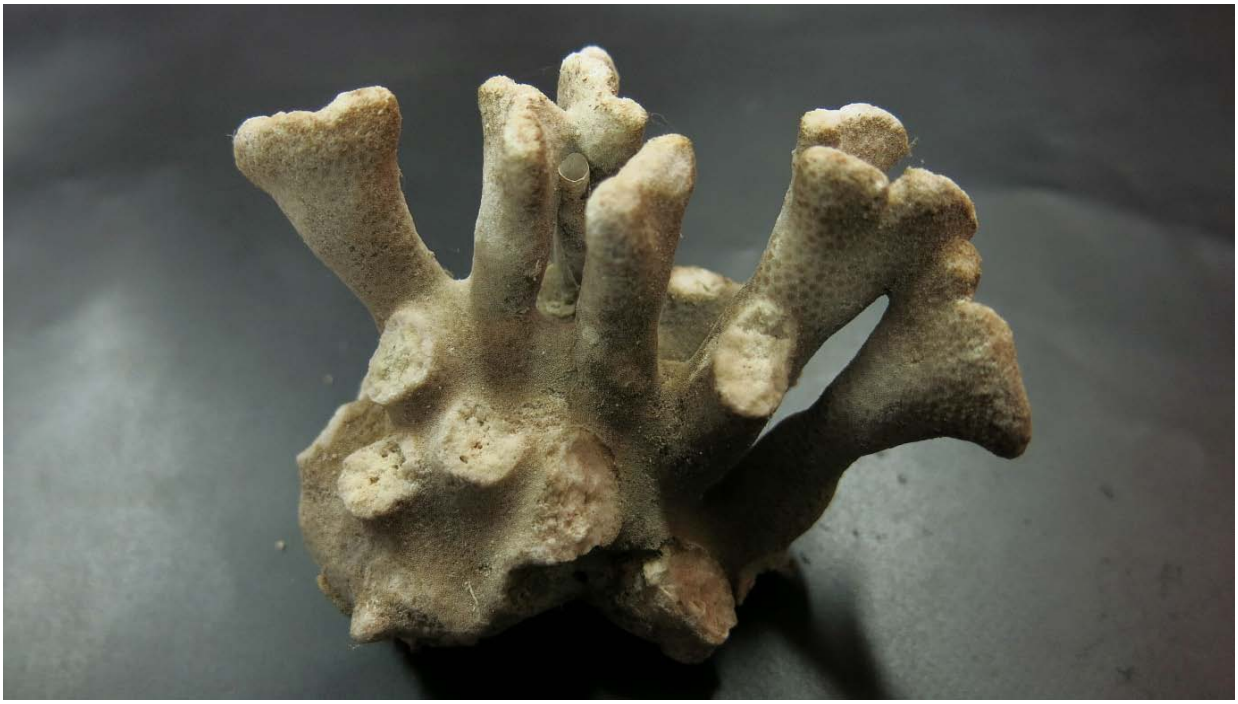

Fig. 5 : *Porites cumulates* Nemenzo, 1955

IV. Discussion

The corals are one of the primitive faunal communities but the gathering of knowledge on them was initiated during the period of mid-19th[10, 11]. The studies of corals of India as well as Andaman and Nicobar Islands were pioneered by Alcock during 1900 on deep sea corals [12]. Since then, contributions from various authors enhanced the scleractinian coral species database of India up to 199 species [13-22]. In a detailed checklist and descriptive manual, Venkataraman *et al.* [23] mentioned a total of 177 species of scleractinian corals belong to 57 genera and 15 families from Andaman and Nicobar Islands while India was with 208 species under 60 genera 15 families including other three reef areas such as Gulf of Mannar and Palk Bay, Lakshadweep and Gulf of Kachchh. Since the year 2009, the taxonomic exploration of scleractinian lives have been priotorised in these islands by Zoological Survey of India resulted with the reporting of 575 species from Andaman and Nicobar Islands and 607 species from India [24]. Identification of five species of scleractinian corals such as *Acropora lovelli*, *Acropora willisae*, *Mycetophyllia lamarckiana*, *Isophyllia sinuosa* and *Porites cumulates* increase the species database of Andaman and Nicobar Islands and as well as India. Recording of a species under the genera *Mycetophyllia* is also made for the first time from Indian waters under the family Mussidae and included under 8th one with the existing genera of same family [24]. Three species of scleractinian corals among the four under Acroporidae and Poritidae family were listed as vulnerable (VU) and other two species belong to Mussidae family was listed under least concern (LC)species according to IUCN 2015.4 Red List categorization [9]. The record of threatened species along with the other species from Indian waters signifies the stable ecological ambience for scleractinian corals as well as accelerating our ideas for the construction of progressive conservatory and managing measures.

V. Acknowledgements

Authors are grateful to the Director, Zoological Survey of India, Kolkata for providing facilities. The logistic support provided by the Department of Environment and Forest, Andaman and Nicobar Administration to conduct surveys is duly acknowledged.

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Allelopathic Effect of *Populus Nigra* Bark on *Zea Mays* in Agroforestry Ecosystems

By Musharaf Khan, Muhammad Zakaria, Fawad Ali, Farrukh Hussain, Imdadullah & Shahana Musharaf

Federal Government College Mardan, Pakistan

Abstract- The study was designed to explore the allelopathic effect of *Populus nigra* bark on Zea mays under labourtary condition during 2014-2015. The allelopathic influence of aqueous extracts of *P. nigra* bark have determined on the germination, seedling growth, fresh weight and dry weight of Zea mays. ANOVA (RCBD) showed no significant effects of concentration and duration on germination between group as well as within group. On plumule length the significant effects of concentration (F=28.1457) was found within group while the effect of duration (F=2.4125) showed significant effects within group and between group i.e. concentration and duration, no significant was found. On plumule length significant effects of concentration was found within group (F=17.2154) and between group (F= 12.8457) while the effect of 48h duration showed significant effects within group (F=4.8654). On fresh weight significant effects of high concentration was found within group (F=37.3254) and between group (F=18.5241) while the effect of duration (F=4.6584) showed significant effects within group. On dry weight significant effects of concentration (F=27.5684) was found within group. The effect of duration (F=412.8457) showed significant effects within group while between the group (F=7.76352) significant effect was present. These findings indicate that *P. nigra* bark sown in fields which had leaf and stem litter of test plant will be adversely affected regarding germination, growth and ultimately resulting in lower yield

Keywords: concentration effect, duration effect, germination, seedling growth, fresh weight and dry weight.

GJSFR-C Classification : FOR Code: 069999

Strictly as per the compliance and regulations of :

Allelopathic Effect of *Populus Nigra* Bark on *Zea Mays* in Agroforestry Ecosystems

Musharaf Khan [α], Muhammad Zakaria [σ], Fawad Ali [ρ], Farrukh Hussain [ω], Imdadullah [¥] & Shahana Musharaf [§]

Abstract- The study was designed to explore the allelopathic effect of *Populus nigra* bark on Zea mays under labourtary condition during 2014-2015. The allelopathic influence of aqueous extracts of *P. nigra* bark have determined on the germination, seedling growth, fresh weight and dry weight of Zea mays. ANOVA (RCBD) showed no significant effects of concentration and duration on germination between group as well as within group. On plumule length the significant effects of concentration (F=28.1457) was found within group while the effect of duration (F=2.4125) showed significant effects within group and between group i.e. concentration and duration, no significant was found. On plumule length significant effects of concentration was found within group (F=17.2154) and between group (F= 12.8457) while the effect of 48h duration showed significant effects within group (F=4.8654). On fresh weight significant effects of high concentration was found within group (F=37.3254) and between group (F=18.5241) while the effect of duration (F=4.6584) showed significant effects within group. On dry weight significant effects of concentration (F=27.5684) was found within group. The effect of duration (F=412.8457) showed significant effects within group while between the group (F=7.76352) significant effect was present. These findings indicate that *P. nigra* bark sown in fields which had leaf and stem litter of test plant will be adversely affected regarding germination, growth and ultimately resulting in lower yield

Keywords: concentration effect, duration effect, germination, seedling growth, fresh weight and dry weight.

I. Introduction

The phenomenons of allelopathy were explained where one plant exerts a negative effect on another through the production of germination and growth inhibiting substances. Agroforestry, which involves connecting woody plants with annual or perennial crops or livestock, increases the biophysical and/or socio-economic productivity of an agricultural enterprise (Bansal, 1988). However, farmers have expressed alarm about the harmful effects of trees on cultivated lands and standing crops. Although allelopathy the direct or indirect toxic effect of one plant upon another through the production of chemical inhibitors. Thus, Baker (1966) reported that the root and hypocotyl growth of cucumber seedlings were inhibited by *Eucalyptus globulus* which produces volatile materials. *Eucalyptus* however a potential industrial crop is not being recommended as an intercrop in agroforestry systems (Bansal, 1988), apparently due to the release of inhibitory compounds from the trees (Lisanework and Michelson, 1993). *Eucalyptus* reduces the growth of neighboring crops through the release of allelochemicals (May and Ash, 1990). The release of phenolic compounds adversely affects the germination and growth of plants through their interference in energy metabolism, cell division, mineral uptake and biosynthetic processes (Rice, 1984). Leachates from stemflow and litterfall are responsible for such an effect (Molina *et al.*, 1991). Lisanework and Michelson (1993) reported the the effects of leaf extracts of three Eucalyptus species on four Ethiopian crops. A number of trees do, however, negatively affect performance of crops through allelopathy. These include *Leucaena leucocephala, Populus deltoides, Eucalyptus* and *Acacia* species (Bansal *et al*., 1988; Ralhan *et al*., 1992; Bora *et al*., 1999; Singh *et al*., 1999a,b). Moradshahi *et al*., (2003) found that aqueous extracts of *Eucalyptus camaldulensis* has the potential to suppress growth of *Echinochloa crus-galli, Avena fatua*, and *Rumex acetosella.* Cao and Luo, (2005) reported that aqueous extract from bark and leaf, and volatiles from leaves of *Eucalytus citriodora* showed allelopathic effect on the growth of nine species, including the weeds i.e. *Bidens pilosa*, *Digitarie pertenuis*, *Eragrostics cilianesis*, *Setaria geniculata*, and crops such as corn, rice, cucumber, bean and *Stylosanthes guianensi*. Singh *et al*., (2005) stated that *Eucalyptus citriodora* oil completely inhibited the germination of noxious weed *P. hysterophorus*. Ercisli *et al*., (2005) studied that the Allelopathic effects of *Juglans regia* on yield, growth, chemical and plant nutrient element composition of the *Fragaria ananassa.* Shafique *et al.*, (2007) studied that the effect of aqueous extracts of 8 allelopathic tree species viz., *Accacia nilotica*, *Alstonia scholaris*, *Azadirachta indica*, *Eucalyptus citriodora*, *Ficus bengalensis*, *Mangifera indica*, *Melia azedarach* and *Syzygium cumini* was

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studied on germination of *Triticum aestivum*. Hence, an effort was made to analyze the bark for its allelopathic effect of test crops.

II. Materials and Methods

Plant bark of *Populus nigra* L. 'Italica' was collected from Garden of Government Post Graduate College Mardan, District Mardan, Khyber Pakhtoonkhwa, Pakistan. Plants bark were then washed several time with water and dried in open air and under natural light. Leaf samples were ground and the powdered material were stored in plastic bottles at room temperature. 5g, 10g, 15g and 20g of *Populus nigra* powdered were mixed with 100ml distilled water and left for 24hr, 48hr and 72hr at the room temperature (average during day: 25°C) in dark conditions. Aqueous extract was obtained as filtrate (Figures 1, 2) of the mixture and final volume was adjusted to 100ml; this gave 5g, 10g, 15g and 20g aqueous extract. The extract was considered as stock solution (Figures 3, 4). 05 uniform and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *Z. mays* were kept for germination in sterilized petri-dishes lined double with blotting paper and moistened with 10ml of 5g, 10g, 15g and 20g concentrations of aqueous extracts (Figure 5). Each treatment had 5 replicates (total number of test seeds: 10 x 5 = 50). One treatment was run as control with distilled water only. The petri-dishes were maintained under laboratory conditions (room temperature 25°C at mid day, and diffused light during day). The whole experiment was repeated once (Figure 6). After seven days, the seedling root length (cm), shoot length (cm) were measured (Figures 7, 8) while number of germination percentage, Fresh weight and Dry weight were measured. The data obtained was subjected to three way analysis of variance, Randomized Complete Block Design (RCBD) and the mean values were separated at $P < 0.05$ applying Least Significant Difference Test (LSD).

Figure 1 : Filtration of aqueous extract in lab

Figure 2 : Filtration of aqueous extract

Figure 3 : Stock solution of 5g, 10g, 15g and 20g extract

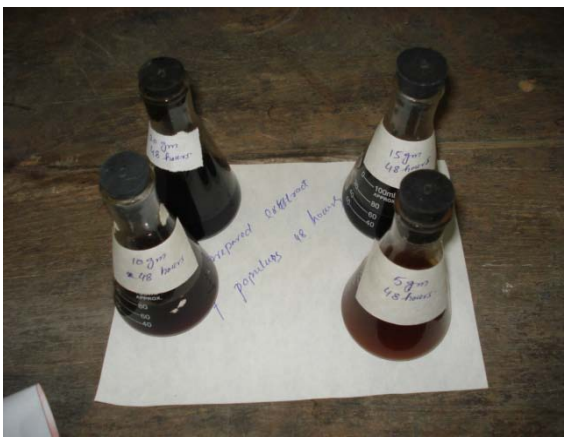

Figure 4 : Stock solution of 5g, 10g, 15g and 20g extract

Figure 5 : Seeds placed in petri dishes

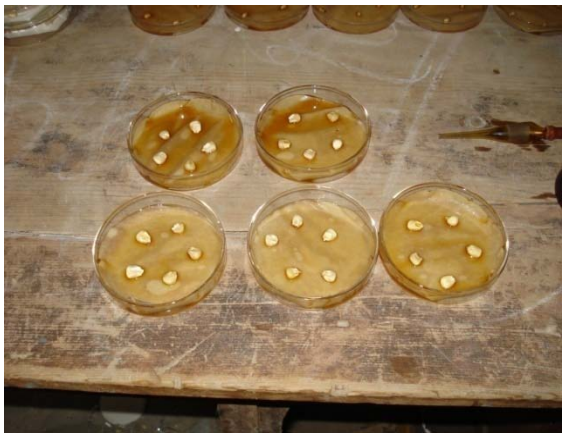

Figure 6 : Seeds soaked in extract

Figure 7 : The germination of seed after seven days

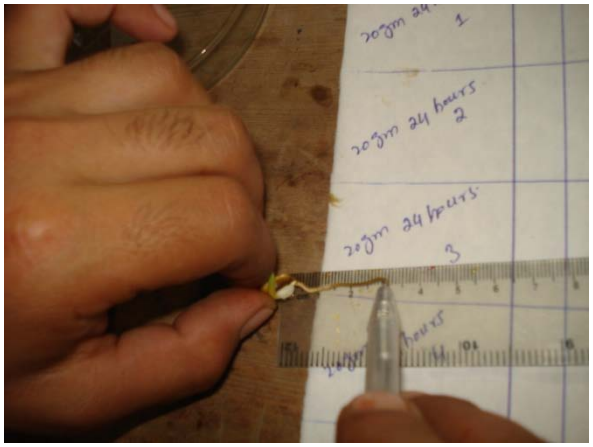

Figure 8 : Measurement of seedling root length (cm) and shoot length (cm)

III. Results

a) Germination

ANOVA (RCBD) (df 1, 56) showed no significant effects of concentration and duration on germination between group as well as within group. The coefficient of variation was found 35.32% for germination (%). (Tables I. a, b).

Table I (a) : Allelopathetic effects of *Populus nigra* bark on germination (%) counts of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	100	100	100	100
5	72	64	52	62.66
10	84	60	60	68
15	88	76	68	77.33
20	68	68	56	64
Mean	82.4	73.6	67.2	74.4

Table I (b) : Analysis of variance on germination (%) counts of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	2425.333	541.333	1.2368	0.2978
2	Factor A	4	3538.667	844.667	1.8743	0.111
4	Factor B	2	2738.667	969.333	2.1563	0.1375
6	AB	8	8321.333	1202.667	2.3617	0.0283
-7	Error	56	13674.667	442.762		
	Total	74	38898.667			

Factor A: Duration Factor B: Concentration

b) Plumule Length

ANOVA (RCBD) (df 1, 56) showed significant effects of concentration (F=28.1457) on Plumule length between group while the effect of duration (F=2.4125) showed significant effects within group and between group no significant was found. The coefficient of variation for plumule length was 46.45%. (Table II. a, b).

Table II (a) : Allelopathetic effects of *Populus nigra* bark on Plumule length of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	4.28	3.89	4.12	4.1
5	1.86	1.56	1.38	1.6
10	1.24	1.12	1.06	1.14
15	1.34	1.21	0.98	1.18
20	0.94	0.82	0.68*	0.81*
Mean	1.93	1.72	1.644	1.766

**: within groups, +: between groups*

Table II (b) : Analysis of variance on plumule length of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	2.609	2.6077	1.6359	0.1661
2	Factor A	4	52.134	11.036	28.1457	0.0000
4	Factor B	2	0.4235	1.1025	2.4125	0.0000
6	AB	8	3.1734	2.1592	1.4245	0.0622
-7	Error	56	22.491	1.0402		
	Total	74	82.578			

c) Radical Length

ANOVA (RCBD) (df 1, 56) showed significant effects of 5g concentration within group (F=17.2154) and between group (F= 12.8457) on radical length while the effect of 48h duration showed significant effects within group (F=4.8654). For radical length the coefficient of variation was 32.34%. (Tables III. a, b).

Table III (a) : Allelopathetic effects of *Populus nigra* bark on radical length of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	17.6	17.6	17.6	17.6
5	3.72	1.14	0.9*	1.92*
10	4.8	3.98	3.24	4.006
15	5.34	3.38	1.54	3.42
20	6.46	3.24	1.68	3.793
Mean	7.584	5.868	4.992+	6.148

Table III (b) : Analysis of variance on radical length of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	25.452	11.348	2.6547	0.0598
2	Factor A	4	2212.2541	634.735	17.2154	0.0000
4	Factor B	2	54.5245	26.977	12.8457	0.0000
6	AB	8	53.2587	12.014	4.8654	0.0000
-7	Error	56	192.2547	5.467		
	Total	74	2856.53			

d) Fresh Weight

ANOVA (RCBD) (df 1, 56) showed significant effects of high concentration within group (F=37.3254) and between group (F=18.5241) on fresh weight while the effect of 24h duration (F=4.6584) showed significant effects within group. The coefficient of variation for fresh weight was 13.21%. (Tables IV a, b).

Table V (a) : Allelopathetic effects of *Populus nigra* bark on fresh weight of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	2.954	2.954	2.974	2.961
5	1.785	1.564	1.356	1.568
10	1.654	1.657	1.574	1.628
15	1.745	1.584	1.487	1.605
20	1.324	1.234	1.245	1.268
Mean	1.892	1.799	1.727	1.806

Table IV (b) : Analysis of variance on fresh weight of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	1.685	0.209	2.4325	0.026
2	Factor A	4	8.745	2.462	37.3254	0.0000
4	Factor B	2	2.145	0.558	18.5241	0.0000
6	AB	8	3.548	0.358	4.6584	0.0000
-7	Error	56	3.425	0.163		
	Total	74	18.175			

e) Dry Weight

ANOVA (RCBD) (df 1, 56) showed significant effects of concentration (F=27.5684) within group on dry weight. The effect of duration (F=412.8457) showed significant effects within group while between the group (F=7.76352) significant effect was present. The coefficient of variation of dry weight was 13.31%. (Table V a, b).

Table V (a) : Allelopathetic effects of *Populus nigra* bark on dry weight of Maize

Concentration(g)	Duration			
	24h	48h	72h	Mean
Control	2.448	2.448	2.448	2.448
5	1.985	1.868	1.748	1.867
10	2.157	2.056	1.898	2.037
15	1.85	1.46	1.37	1.56
20	1.716	1.31	1.245*	1.42*
Mean	2.031	1.83	1.741+	1.866

Table V (b) : Analysis of variance on dry weight of Maize

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	0.656	1.164	4.1073	0.1622
2	Factor A	4	6.298	2.574	27.5684	0.0000

4	Factor B	2	0.327	1.163	12.8457	0.0000
6	AB	8	2.02	1.253	7.76352	0.0000
-7	Error	56	3.953	0.1253		
	Total	74	12.254			

IV. Discussion

In the present study allelopathic effects of *Populus nigra* bark was observed on germination, plumule length, radicle length, fresh weight and dry weight of *Z. mays*. Treatment with 5g, 10g and 15g extract has increased the germination with time. It is high in 24h treatment while 20g extract treatment has decreased the germination at 72h treatment. Overall 72h treatment decreased the mean germination in all concentration. At very low concentration increased in time has less effect on germination. The result show that at 24h the germination high with increase in concentration whiles at 48h the germination high with increase in concentration except 20g concentration and high duration the germination rate was low. It is evident from the result that higher aqueous extracts concentration of *P. nigra* bark exhibited more inhibitory effects on germination plumule length, radicle length, fresh weight and dry weight of test specie while higher duration present inhibitory effect on Plumule length and radical length as compare to control (Table I - V). The results of our study showed that the bark extracts of *P. nigra* present inhibitory effect in maize. Similar results have been reported by Ayaz *et al*., (1989); Khan *et al*., 2011a,c) and El-Rokiek and Eid, (2009) while studying the allelopathic effect of different plants. They observed that the foliar leachates have been more phytotoxic in nature. Comparative analysis between extracts and duration showed significant inhibitory effect of 48hr treatment on Plumule and radical length. In addition to it, the comparison of duration and concentration showed significant inhibitory effect of 15g concentration in 24hr treatment on fresh weight. The result shows that the inhibitory effects were increased proportionally with the extract concentration and duration. The present findings corroborate the earlier report by Bora *et al.,* (1999) who found that, the inhibitory effect of *Acacia auriculiformis* on germination of some agricultural crops was proportional to the concentration of the extract. Several reports address the allelopathic effect of various plants that significantly affected seed germination and seedling growth of several crops and weed species (Lisanework and Michelson, (1993); Ercisli *et al*., (2005); Shafique *et al.*, (2007), Akhtar *et al*., 2010) these studies showed that the extract of plant species decreased root growth of the majority of the crops. Similar findings were also reported by (Khan *et al*., 2011a,b; Jabeen and Ahmed, (2009) of different trees in common agricultural crops. Some recent studies indicating the phytotoxic/allelopathic effect of aqueous extracts of plants include *Chrozophora oblique* (Khan *et al*., 2011c) and *Rhazya stricta* (Khan *et al*., 2011a,b). All these studies indicated the release of phototoxic chemicals during the preparation of aqueous extracts. Based on this finding, a study was further extended to explore the impact of *P. nigra* bark as they possessed greater phytotoxicity on the emergence and growth of weed plants.

V. Conclusion

The present investigation revealed that its effectiveness on germination and growth suggests that bark of *P. nigra* may act as a source of allelochemicals after being released into soil or after decomposition. The presence of allelochemicals negatively affects the neighboring or successional plants. Further studies are suggested to clarify the possible physiological mechanism related to allelopathic effect on plants.

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Invitro Antimicrobial Activity And Phytochemical Analysis Of *Murraya Koenigii* (L) Leaf Extracts

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Abstract- Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics. *Murraya koenigii* commonly called curry leaf tree is a multipurpose tree and is a source one of the medicinal products. Different parts of *M. koenigii* are used in folkloric medicine for the treatment of various diseases. It is proved to possess significant wound healing capacity and shows antioxidant activity with high degree of radical-scavenging properties. This article intends to provide an overview of the chemical constituents present in the crude leaf extracts of *M. koenigii* with special emphasis on their pharmacological actions. Qualitative phytochemical screening was carried out using the crude leaf extracts in three different solvents such as water, alcohol and chloroform. Phytochemical analysis of the extracts revealed the presence of glycosides, alkaloids, oils, sapponins and flavanoids.

Keywords: *murraya koenigii, phytochemical analysis, antimicrobial, agar cup method.*

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Keywords: murraya koenigii, phytochemical analysis, antimicrobial, agar cup method.

I. Introduction

Since ancient times, people have been exploring the nature particularly plants in search of new drugs which has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte,1998). According to WHO survey, 80% populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts (Sandhya *et al.,* 2006) The studies of plants continue principally for the discovery of novel secondary metabolites or phytochemicals which are the non essential nutrients derived from plants exhibiting a number of protective functions for human consumers.

Murraya koenigii, belonging to the family *Rutaceae* is a small ever green tree native of India and found in Srilanka and other South Asian countries. Different parts of *M. koenigii* are used in folkloric medicine for the treatment of various diseases. It is proved to possess significant wound healing capacity (Anand *et al .,* 2011). This plant is commonly called curry leaf tree. *Murraya koenigii* shows antioxidant activity with a high degree of radical-scavenging properties (Rao *et al.,* 2006).

Phytochemical screening is a method which exposes or reveals certain components or properties readily available in plants for bio-activity or ethno-medical applications. Plant based antimicrobials has enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu, 1999). Thus it is anticipated that phytochemicals with adequate antibacterial efficiency can be used for the treatment of bacterial infections (Balandrin, 1985). Antioxidants and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones (Deba, 2008). Thus medicinal plants play an important role in the development of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs (Raj ,2011). The present study aims in exploring the phytochemical constituents, antibacterial and antifungal properties of the crude leaf extracts of *Murraya koenigii.*

II. Materials and Methods

a) Collection and extraction of plant materials

The fully matured fresh leaves of *M. koenigii* were collected from Kattakada area in Thiruvananthapuram district, kerala. The leaves were washed thoroughly, shade dried and finely powered. The dried powdered leaves were extracted with three different solvents such as water, acetone and chloroform. For aqueous extraction, ten grams of the powdered leaves were mixed with 100ml distilled water, boiled for about two hours and filtered. Whereas acetone and chloroform extracts were prepared by mixing ten grams of powdered leaf samples with 100ml of each solvent separately in mechanical shaker for 48 hours at room temperature. Extracts were then filtered, concentrated, dried and were stored in the refrigerator at 4°C for future use.

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b) Phytochemical analysis

The prepared plant extracts were analysed for the presence of alkaloids, glycosides, saponins, proteins, aminoacids, fixed oils, phenolic compounds, tannins, flavonoids, gum and mucilage etc (Raaman N,2006).

c) Preparation of plant extract for antimicrobial screening

For antimicrobial screening the concentrated, dried and powdered ethanol leaf extract was dissolved in 10 % dimethyl sulfoxide (DMSO) and were stored at 4^0 C for further use.

d) Test Organisms

Antibacterial activity was carried out against two selected gram negative pathogens (such as *Escherichia coli and Pseudomonas aeroginosa*) whereas antifungal against two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger.* The strains used for the present study were obtained from Biogenix Research centre, Valiyavila, Thiruvananthapuram. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar cup method.

e) Antibacterial activity

Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hr old culture of bacterial strains such as *E.coli and P. aeroginosa* . Wells of approximately 10mm diameter were bored using a well cutter and 25 µl , 50 µl and 100µl of the extracts were added to the wells from a stock concentration of 0.1g/1ml. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone in millimeters formed around the wells (NCCLS, 1993). Gentamycin (standard antibacterial agent, concentration: 20mg / ml) was used as a positive control.

f) Antifungal activity

Antifungal activity was also determined by Agar cup method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100 µl concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole was used as a positive control.

III. Results and Discussion

a) Phytochemical analysis

Table 1 represent the various phytochemical constituents present in the leaf extracts of *M. koenigii*. The phytochemical studies of all the three extracts conclude that acetone and water extracts of leaf samples had more positive results for glycosides, oils, sapponins and flavonoids.

Table 1 : Phytochemical analysis of *Murraya koenigii* leaf extracts

Phytochemicals	Glycosides	Phytosterols	Alkaloids	Oils	Saponins	Phenols	Flavanoids
Water	-	-	+	+	+	+	+
Acetone	+	-	-	+	+	+	+
Chloroform	+	-	-	+	+	+	-

+: Present - : Absent

Preliminary phytochemical analysis revealed the presence of six compounds (Table 1) viz. flavanoids, glycosides, oils, sapponins, phenolics, gum and mucilage. With acetone and chloroform extracts flavanoids, glycosides, oils and sapponins were present .Traditionally sapponins have been extensively used as detergents, pesticides as well as mollucides, in addition to their industrial application such as foaming, surface active agents etc and also found to have beneficial health effects (Arunasalam, 2004). Flavonoids isolated from aqueous extract of *M. koenigii* exhibits antimicrobial activity. The plant is reported to contain glycosides, alkaloids, sapponins, flavonoids, tannins, carbohydrates, phenol compounds and phytosterols by previous workers.

b) Antibacterial activity

Antibacterial activity of *M. koenigii* (leaf ethanol extract with DMSO) was assayed invitro by agar cup method against clinical isolates of *E.coli* and *P.aeroginosa.* The given table shows the microbial growth inhibition of ethanol leaf extracts of *Murraya koenigii.* Among the varying concentration of leaf extracts, higher concentration exhibited maximum antibacterial activity against the two isolates. Table 2 shows the zone of inhibition formed by the extracts against the bacterial strains on Muller Hinton agar.

Table 2 : Zone diameter of inhibition of ethanol leaf extract of *Murraya koenigii*

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
E.Coli	Nil	11	17	20
P.aeroginosa	Nil	Nil	15	20

The sequence of antibacterial activity of leaf extract against *E.coli* exhibited no activity in 25µl but produced a 11mm and 17mm zones of inhibition in 50µl and 100µl concentrations respectively (Table 2). With respect to *P.aeroginosa* the plant extract had shown no activity in both 25µl and 50µl concentrations but produced a 15mm inhibition zone in 100µl concentration (Table 2). Thus antibacterial activity was expressed at varying degrees with the difference in concentration.

Higher concentration of the leaf extract shows highest antibacterial activity. The result obtained might be considered sufficient for further studies for isolation and identification of active principle and for the evaluation of possible antimicrobial activity of other extracts from other parts of *Murraya koenigii.*

Earlier works done by Cosentino *et al*., also states that the extracts from other parts of *M. koenigii* are used against microbial infections due to the presence of secondary metabolites in them such as phenols, essential oils, terpenoids, alkaloids and flavanoids. This was later on supported by Kotkar *et al.,* in 2001 and reported that flavanoids expose strong antibacterial activity.

c) Antifungal activity

In order to access the biological significance and ability of the plant extract, antifungal activity of *M. koenigii* (leaf ethanol extract with DMSO) was assayed invitro by agar cup method against two clinical fungal isolates viz. *Candida albicans* and *Aspergillus niger.* The given table shows antifungal activity of the plant species.

Table 3 : Zone diameter of inhibition of ethanol leaf extract of *Murraya koenigii*

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
C. albicans	Nil	Nil	11	25
A. niger	Nil	Nil	11	25

The sequence of antifungal activity of leaf extract against *C. albicans* and *A. niger* exhibited no activity in both 25µl and 50µl concentrations but produced a 11mm zone of inhibition each in 100µl concentrations respectively (Table 3).

The present study reveals that the ethanol leaf extracts of *M. koenigii* were more active against the clinical bacterial pathogens viz. *E.coli* and *P.aeroginosa*. Anti fungal activity were found to be very negligible when compared to bacterial activity. In literature it has been reported that the antibacterial activity is due to the presence of different chemical agents in the leaf extract including essential oils, flavanoids, terpenoids and other components which are classified as active antimicrobial compounds. The results of the study supports to a certain degree, the use of traditional medicinal plants in human and animal disease therapy and reinforce the concept of ethno botanical approach in screening plants as potential sources of bioactive substances (Valsaraj 1997). The aqueous extract generally exhibits a high degree of antibacterial activity which seems to confirm the traditional therapeutic claims of this plant (Perumalsamy,1998).

IV. Summary and Conclusion

Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities. On the basis of the results obtained, the present work conclude that the leaves of *M. koenigii* are rich in phytochemical constituents even though the phytochemical screening of the leaf extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous extracts of leaves. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, sapponins, phenols and flavanoids were believed to exhibit the antibiotic properties of *M. koenigii* leaves and confirmed their antimicrobial efficacy against selected pathogens.

The present work highlights the possible use of *M. koenigii* leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. The study reveals that the results of extraction yield, total phenol and flavonoid compounds and bioactivity tests varied depending upon the type of solvent being used. The leaves of *M. koenigii* contain a

considerable quantity of phenol - flavonoid compounds which were considered to be the major contributor for their antioxidant and antibacterial activities. Hence it can be concluded that the leaves of *M. koenigii* would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using *M. koenigii* leaves as natural remedied and to protect against infectious diseases.

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Contribution of Three Sedges of *Cyperus* in the Rural Economy of Sundarbans, India

By Sumit Manna, Sudipta Mukherjee & Anirban Roy
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Abstract- Three sedges of *Cyperus* (*C. malaccensis, C. compressus* and *C. iria*) are used for making mats by the forest fringe people of Sunderban mangrove swamp and sale in local markets. The focus of the work is to calculate species wise net economic contribution of these sedges. Fifteen families from each four villages of two islands have been selected for qualitative and quantitative assessment of these resource use pattern through participatory observation and questionnaire method. The annual production, economic valuation of individual species were assessed through quadrat sampling (272 habitat patch) and market survey with cost benefit analysis respectively. Economic contribution of the sedges to the local people in terms of annual income is highest in case of *C. malaccensis (6.638%)* followed by *C. compressus* (1.599%) and *C. iria* (0.690%). The production of the three species is 5-8 times higher than paddy, thus have potentiality as alternative livelihood options.

Keywords: *bioresource, rural economy, sedges, sundarbans, value chain.*

GJSFR-C Classification : *FOR Code: 069999*

Strictly as per the compliance and regulations of :

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I. Introduction

Sundarbans, the world's largest mangrove ecosystem with highest number of mangrove plant species, nurtures significant bioresources that are encouraging to local inhabitants for subsistence use as well as income generation since a long back. This unique estuarine deltaic settings makes possible for co-existence of both immense biodiversity and human settlement and thus as a source of daily household needs to the 4 million aboriginal people (one of the poorer section of India) inhabiting in forest fringes. As 56% people of that area are landless, so they are to depend on the wild floristic resources as food, fodder, medicines, fuel, and house building materials for their daily life support (Singh et al. 2010).

Globally around 500 million people directly and one billion people indirectly depend on wild floristic resources (WFRs) (Alexander et al. 2002). Forest fringe people of Africa get their food, fuel, medicine, constructive materials and fodders through collecting wild floristic resources (Byron & Arnold 1999). According to them about 10% of the rural people of Ghana harvest WFRs for their cash income. About 85% household products of the rural people of South Africa are generated from WFRs (Charlie & Sheona 2004). An estimation of US$ 6,800 per hectare from WFRs of Amazonian rain forest which was far higher than the returns from timber harvesting for subsequent plantation or cattle ranching (Peters et al. 1989). Mahapatra & Tewari (2005) estimated that the net present value of revenues from Non-Timber Forest Products (NTFPs) were more as compared to potential timber revenue. Though southern Asia has a long history of human use of forest product (Bawa & Godoy, 1993), the importance of WFRs for rural forest based populations was seldom considered significantly for a long period. A useful & growing body of literature has been established that deals with forest environment valuation including many of the non-market values that were omitted from the calculation in past. But all these literature dealt with NTFPs as a whole or categorized it as food, fodder, medicine, oil yielding plant and very often sub categorized species level contribution to the household level. Manna & Roy, (2013) made an estimation of about Rs. 2068.07 was contributed by wild edible mushrooms to a Santal (Tribal) family per year in the Eastern lateritic part of India which was 10.06% of the total annual income of a tribal family.

The mangrove forest of Sundarbans is valuable as it is a treasure house of rich biodiversity which are commercially exploited, particularly WFRs, which is one of the epitomes for many forest fringe dwellers (Bhattacharya, 2004). The NTFPs of that area such as tannin bark (like *Ceriops decandra, Ceriops tagel, Phoenix paludosa* yield around 30-32% tannin), thatching materials (*Nypa fruticans*), natural honey (*Apis dorsata*), fuel woods, small poles and boles, fish, prawn and crabs attract locals for subsistence or commercial use. An estimation of 79% on an average to the annual income of harvesters' family was generated from these bioresources in Sundarban areas (Singh et al, 2010). Apart from that, many studies related to ecology and taxonomy was performed by many authors (Banerjee 1964; Das 1981; Naskar and GuhaBakshi 1982; Chakrabarti 1986; Mondal and Ghosh 1989; Chaudhuri and Chowdhury 1994; Chattopadhyay 2003, Manna et al. 2012). But the production, utilization and family wise contribution of WFRs of that globally significant geographical terrain was seldom accounted.

Today, sedges are used throughout the tropics for basketry and handcraft weaving, and in parts of Africa and Asia these are cultivated for such purposes. These are also used for thatching, fencing, rope making,

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and perfumery. Several species are recorded as having medicinal properties while others have the potential for use in erosion control and sand stabilization. Local backward aboriginal people of Sundarbans traditionally used three species of Cyperaceae- *Cyperus malaccensis* Lam., *Cyperus compressus* L. and *Cyperus iria* L. for preparation of mat which are either sold in the local markets or used for household purposes. Present study was under taken to highlight the actual and potential annual production, present utilization and contribution per capita of three species of Cyperaceae, widely utilized by the local inhabitants in preparation of ethnic mats in that area, using different models for their value chain analysis. A comparative analysis of the contribution of rice (O. *sativa*, widely accepted and used as a cash crop) and these three species of Cyperus were also made in the present study.

II. Materials and Methods

a) Study area

The Sundarbans (Extends between 21°32" North and 22°40" North Latitude and between 88°05" East and 89°00" East Longitude) forest lies in the vast delta on the Bay of Bengal formed by the super confluence of the Padma, Brahmaputra and Meghna rivers across southern Bangladesh. The Indian part of sundarban is demarcated by the river Hooghly on the west, the Bay of Bengal on the south, the Ichamati-Kalindi-Raimongal rivers on the east and the Dampier-Hodges line on the north. The forest covers 10,000 sq. km of which about 4,200 sq. km is in the South 24 Parganas district of West Bengal, India. The forest became a UNESCO world heritage site in 1987. Out of total 4,200 sq. km about 1,700 Sq. km is occupied by water bodies in the forms of river, canals, creeks and ponds of width varying from a few meters to several kilometers. This Indian land mass is bound on the west by river Muriganga and on the east by rivers Harinbhagha and Raimangal. Other major rivers flowing through this eco-system are Saptamukhi, Thakurain Matla and Gosaba. A land of 54 tiny islands, criss-crossed by innumerable tributaries of the Ganges is now the abode of varied floral & faunal population. The inhabitants of the fringes of Sundarbans are prohibited from entering the core region and need permission for collection of NTFPs from buffer region. The annual temperature of that area ranges from 20°C to 42°C in summer and 9℃ to 32°C in Winter and average annual precipitation of South 24 Parganas stands at 1876.3 mm with total rain days of 81.8 (Alipore Meteorological Department, Kolkata, 2009).

For the present study Gosaba (22°14'32.1" N to 22°06'27.5" N and 88°52'06" E to 88°46'22.0" E) & Sadhupur (22°10'12.5" N to 22°05'08.3" N and 88°55'28.0" E to 88°49'25.5" E) islands of Sundarban were selected on the basis of their dense human settlements. Most of these local communities have little or no land of their own which compelled them to depend on Wild Floristic Resources (WFRs). Average annual income of each of the family with minimum available land of these two islands is Rs. 36,000.00 (SD. Rs. 4,500.00) of which maximum portion comes from working as a daily labourer in agricultural fields in pre-monsoon and post-monsoon seasons. Some people from Below Poverty Level (BPL) families also work on daily wage basis in different government employment schemes. Of a total of 222,822 people living in Gosaba, 143,221 people (64.28%) belongs to scheduled caste (SC) community where as 6992 people living in Sadhupur, 5756 people are under SC community (Census, 2011). Agriculture is the main livelihood option for the people those who have little or adequate land for farming. Those who are landless or little land holders generate their economy through "100 days work" under the Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA), or they work as daily labourer in agricultural field. From these different livelihood options they get their food security for 7-8 months, but for the rest of the year they have to depend on the WFRs.

b) Methods

Two villages from each of the two islands (viz. Pakhirala and Dulki from Gosaba island; Hamilton Abad and Sadhupur from Sadhupur island) were selected randomly and from each of the village fifteen families (total 15×4=60 families in two islands) involved in mat making for generation of partial economy were surveyed. Information like local name of the species used as raw material for preparation of mattress, collection areas, season of harvesting (annual or biannual), amount of collection by the family in a season, used plant part etc. were collected through the group discussions (Goss 1996) which were arranged twice (December and January of 2014-2015 and July and August, of the year 2015) in all villages with backward communities mostly of scheduled caste, scattered in the study area. In each village 15-20 persons were involved of which more or less 70% were female, the principal collectors of sedges Cyperus and mat makers as well.

Dry weight of mat, made from different species were noted down along with the height and breadth. Information related to number of mats prepared from a particular species in a year, number of family members needed to be involved in making of the mat, presence of any value added service, intermediate market supplier (if any) and in case of direct selling to the market, distance of the market from the village and mode of transport were also collected through participatory observation (Marshall & Rossman 1999) and previously made questionnaires method (Grix 2004). A questionnaire was developed (local language translated into English into Table 1) and distributed among the villagers of each

village (25-30 sedge collectors). Seventy to seventy five percent duly filled questionnaires were returned back based on which the analyses were made. The data are used for cost benefit analysis of these species. The participatory observation was performed during the harvesting, mat making procedure in different villages of the study sites.

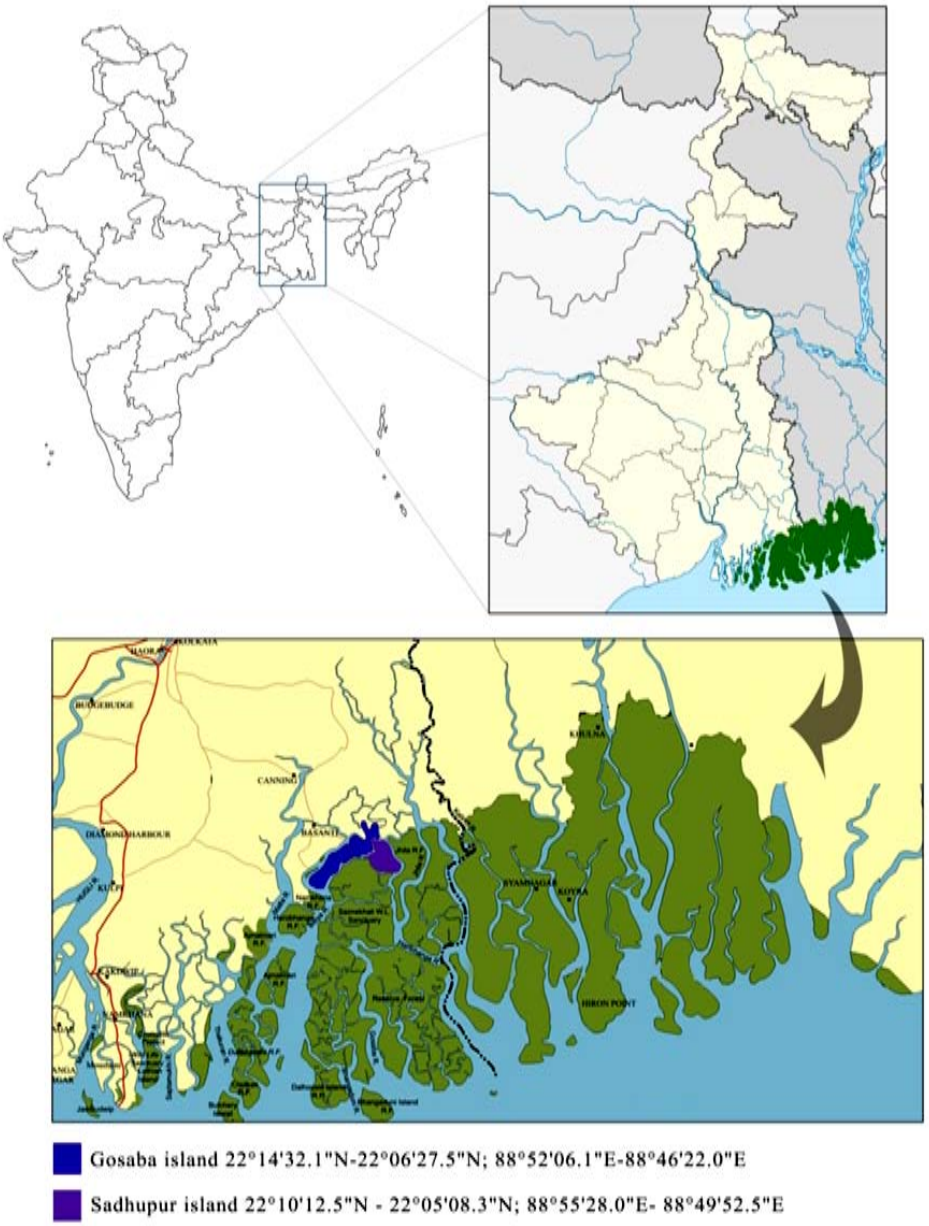

Fig 1 : Map of Study area

Table 1 : Questionnaire distributed among villagers (Translated in English)

Name:.....
Village: .....
Sex: .....
Age: .....
Any comment:.....
.....

Sl. No.	Vernacular name of the species used in mat preparation	Name of the species*	No. of mat prepared from a particular species in a year	Dry wt. of mat	No. of family members involved	Total man hour involved

** To be filled after identification of species*

Value added service (In Rupees)	Intermediate market supplier			In case of direct selling	
	Supplier 1	Supplier 2	Supplier 3	Distance of market from village (km)	Transportation cost (Rs/Q.)

To quantify the annual production of these three species, 272 ponds were visited randomly (136 from each island) and the geographical locations were taken using GPS (Garmin eTrex Vista). Annual production of each species was measured using quadrat {with quadrat size of (0.91 m X 0.91m = 0.83 sq. m)} in the places where the species were found to be growing. From each occurring site, eight quadrats were plotted. Wet biomass of the studied species of each of these sample units (quadrats) were weighed in the field separately, brought to the laboratory, transferred to the hot air oven (for 5 days at 75°C) and dry weight were measured. All the ponds without any direct inundation and having the salinity ranges between 2 ppt to 3.5 ppt were considered the potential habitat of these three species, as these species were not found to occur at higher salinity level (>3.5 ppt). The total potential habitats (bank area of the pond) out of these 136 ponds were measured directly in the field. Out of these 136 ponds, only the pond banks were selected as the actual habitat where these three species were found to be growing. From these, total actual habitat out of these 136 pond bank area were measured from where total actual habitat area of each species in every island was estimated.

c) Valuation of mats

Most of mats prepared from the species of Cyperaceae were used either directly as their house hold use or were sold (by the dwellers) to some local markets. Firstly during the year 2014 to 2015 all of the 3 species were tracked to know their ultimate level of transfer and their form of transfer (either in the form of mat or raw materials of mat) to the consumers from the local collectors to get the highest market price. Different value chain models were used for these species based on their level of transfer to the hand of consumers (diagrammatically represented in the Figure 2). Highest price of a species was considered as the present value of these species.

d) Assessing local markets

To calculate the economic worth of these *Cyperus* species in terms of money, three local markets (Haats), namely *Budhbaarer bazaar, Sukrabaarer bazar* were identified where the maximum portion of these bioresources were brought by the local community to sell from the forest fringe villages. Regular surveys of these local Haats/markets were carried out and the price of the mats were noted. Different value-monitoring factors, e.g., distance of these local markets from the villages, mode of transport of mats, and the number of intermediate market suppliers, if any, were considered for the cost-benefit analysis (Gowdy, 2007). While conducting the market surveys, the products were weighed intermittently to confirm the price per unit weight (kg).

III. Results

a) Actual and potential Production of These three species of Cyperus

A total of three species of *Cyperus* were found to be used in preparation of mat by 200 families of Sadhupur island and 150 families of Gosaba island of Sundarbans. All of these three species were found to be collected from wild. Recently *Cyperus malaccensis* had drawn quite attention by the villagers and sown for a

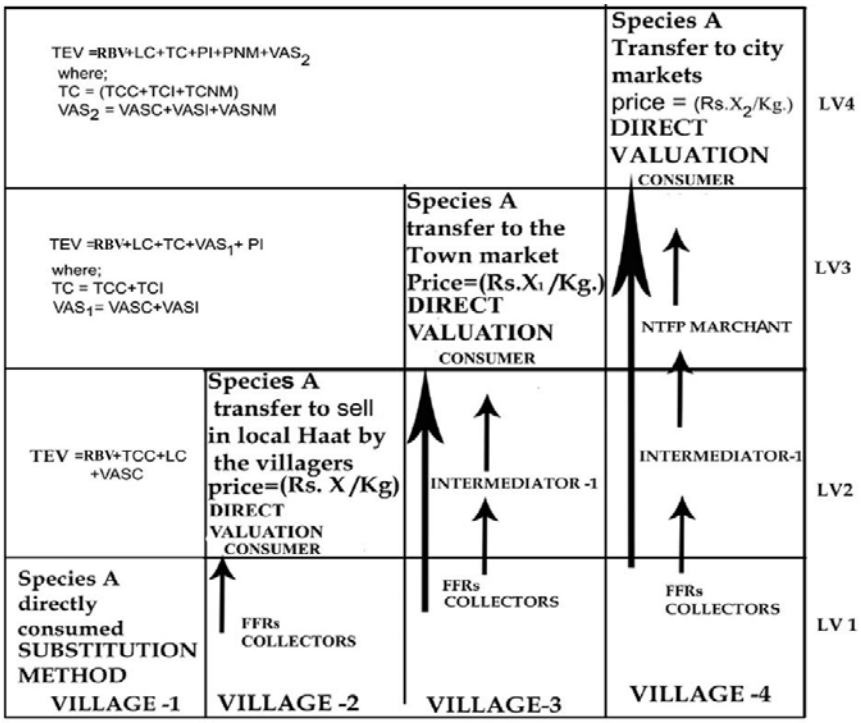

Fig 2 : Value of forest floristic resources (FFRs) always- LV1<LV2<LV3<LV4 or $x_2 > x_1 >$ x LV: level; TEV: Total Economic Value; RBV: Raw Bioresource Value; LC: Labour Cost (The labour used in collection of the resource together with the processing, transport and sale); TCC: Transport Cost of Collector; VASC: Value Added Services by Collector; VAS_1: Value Added services in level 3; PI: Profit of Intermediator-1; VASI: Value Added services of Intermediator-1; TEV_1: Total Economic Value in level 3; TCI: Transport Cost of Intermediator-1: PNM: Profit of NTFPs Merchant; VAS_2: Value Added services in level 4; TCNM: Transport Cost of NTFP Merchant; VASNM: Value Added services by NTFPs Merchant; TEV_2: Total Economic Value in level 4

single time on their pond sides for its superior quality and higher market value of the mats prepared from this species. They collected these species at least twice a year and needed not to be sown after their first plantation. Whereas, other two species (*Cyperus compressus* and *Cyperus iria*) were found to be collected from the side of canal, large water bodies and waterlogged areas. The study revealed that out of the total actual habitat for these three species of *Cyperus*, *Cyperus malaccensis*, *Cyperus compressus*, *Cyperus iria* covered 16622.39 SD. 1933.09 Sq. m, covered 5369.84 SD. 624.12 Sq. m, 10200.73 SD. 1186.09 Sq. m respectively which were 51.63%, 16.68% and 31.68% of their total observed actual habitat. As these species were mostly growing at a salinity range between 2 ppt to 3.5 ppt, the total potential areas of these three species out of 5262 pond bank areas was 1804549.2, 58291.46 and 110733.5 Sq. m. respectively. It was calculated that the total actual production of *Cyperus malaccensis*, *Cyperus compressus* and *Cyperus iria* were 161.90 Q., 84.68 Q. and 100.47 Q whereas on the basis of their potential habitat, these species might contribute their above ground biomass up to 1757.67 Q., 919.25 Q. and 1090.72 Q. respectively **(Table 2).**

Table 2 : Production of mat sticks. (May be inserted after 1st paragraph of Results)

Sp.	Total actual habitat in these two island (m^2)	Coverage of the total actual areas (%)	Total potential Habitat (m^2)	Actual Gross Production (Kg/Sq. m)		Actual Gross Production (Q.)		Potential Gross Production (Q.)	
				Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
1 Cyperus malaccensis	16622.39 SD. 1933.09	51.635	180459.2	2.557 SD. 0.84	0.974 SD.	425.03	161.90	4614.34	1757.67
2 Cyperus compressus	5369.84 SD. 624.12	16.679	58291.46	3.943 SD. 1.061	1.577 SD.	211.73	84.68	2298.43	919.25
3 Cyperus iria	10200.73 SD. 1186.09	31.684	110733.5	2.957 SD. 0.575	0.985 SD.	301.61	100.47	3274.38	1090.72

b) Outline of utilizing three sedges of Cyperus

In the villages of Pakhirala (405 ha) and Dulki (396 ha) of Gosaba island (7507 ha), 97 out of 520 and 53 out of 142 local families from backward community (Scheduled caste) were involved for 4 (SD. 1.5) months in mat making process. Whereas in case of Sadhupur (374 ha.) and Hamilton Abad (821 ha) villages of Sadhupur Island (5076 ha), 142 out of 1151and 58 out of 647 scheduled caste families were involved for 4 (SD 2) months in preparation of mats. They collected the raw materials mainly from nearby water bodies or in a few cases from their own pond banks where they had planted these sedges of C.*malaccensis* for one time in few years back. They generally collected the sticks of this species twice in a year (in the month of December and mid of June to July). Other two species (*C. compressus* and *C. iria*) were generally collected between October and November. The interactions/group discussions and collected answers of the sets of questionnaires from the local scheduled cast (backward community) of each village in these two island during the year 2014 and 2015, revealed that out of the total harvests, an average of 26% (SD 10.11) and 30 % (SD 8.52) of mat stick collection were utilized directly for their house hold use, while 74% and 70 % of the fresh weight flowed into the local market (Haats) for some cash income in the Gosaba and Sadhupur islands, respectively. Generally from each household, 1 person (80% SD 5 cases female) went to the nearby waterlogged areas to collect the sedges of these three *Cyperus* species. From the two year study it was observed that *C. malaccensis* was harvested maximum by the villagers (122.98 Q.) followed by *C. compressus* (47.72 Q.) and *C. iria* (21.33 Q.). They generally used Nylon thread (Rs. 90 kg-1) to stitch the mat prepared from *C. malaccensis*. This mat is generally made in large (3ft X 6ft) size which is much smooth and soft compared to the mat prepared from *C. compressus* or *C. iria*. *C. iria* was generally used to prepare small size mat of 3.24 sq. ft. (1.8 ft X 1.8ft) having a weight of 0.42 Kg. SD. 0.08 per pcs. mainly for using as 'Aasan' (small size mat).

c) Potentiality of ethnic Mat market

Out of 350 mat maker families of these two islands, about 20% of them found to prepare mat for using their own household needs, rest of the 80% were engaged in this livelihood option for household economy generation. Locally, a (3ft X 6ft) =18 sq. ft. mat of 2.3 kg dry weight of the mat sticks of *C. malaccensis* was purchased from the mat maker by only Rs. 140 SD.5 which was sold in the Gosaba *haat* at the rate of Rs. 202 (SD. 19) per mat and increased to Rs. 350 (SD. 22) in the urban markets. Here the first intermediater secured profit upto 41.42% (SD. 4.2) and the NTFP merchants in between the retail purchaser of Kolkata and the first intermediater of Gosaba market secured upto 73.26% (SD 3.4) of the production price of the mat. So approximately 150% of the mat production value was found to be occupied by different intermediators at different levels. In case of the mat (3.2 kg. dry weights per pcs.) prepared from the species *C. compressus*, which was generally found to be marketed in Gosaba, the gross profit of the intermediator 1 secured 36.75% (SD. 3.2) of the production value of the same. On the other hand, as the producers used to sell the mats prepared from *C. iria* directly to the local *haats* near to their residence, they secured maximum labor charges from that species but not getting more economic worth from this species compared to the other two species as it had only local demand (not flowed to the town or city markets). It was calculated after the cost-benefit and value chain analysis, using different value chain models (Table 3), the present value of *C. malaccensis*, *C.compressus* and *C. iria* is Rupees (Rs.)1673 per Q. of dry weight, Rs. 1125 per Q. of dry weight and Rs. 1303 per Q. of dry weight respectively. Sometime mat makers got the opportunity to sell the mats directly to the tourists visited to Sundarbans, where they received good profit.

Table 3 : Valuation of bioresources

Species	Bioresource valuation model used	Top level Market value (Rs./Q.)	Production +stitching material (Rs./Q.)	Profit of 1st Intermediate er (Gosaba market)	Profit of NTFP marchants (From 1st Intermediate to retail purchaser) (Rs./Q.)	Bioresource value (Rs./Q.)
1 *Cyperus malaccensis*	PV=TEV-(LC+TC+PI+PNM+VAS 2, TC=(TCC+TCI+TCNM), VAS2= VASC+VASI+VASNM	Value of 1 mat @ Rs. 350 of 2.3 Kg i.e. 15217/ Q. mat at different markets of Kolkata). (**Level 4 model**)	5108.69	2260	6177	1673
2 *Cyperus compressus*	PV=TEV-(LC+TC+VAS1+PI), TC=TCC+TCI, VAS1=VASC+VASI	Value of 1 mat @ Rs. 160 of 3.2 Kg i.e. 5007/Q. mat at Gosaba market. (**Level 3 model**)	2718	1156	-	1125

3. *Cyperus iria*	PV=TEV-(TCC+LC+VASC)	Value of 1 mat @ Rs. 20 of 0.42 Kg i.e. Rs. 4849/Q. mat at nearby *hatt* of Sadhupur. (**Level 2 model**)	3545.45	-	-	1303

It was calculated that the mean gross economic revenue generated to each of the mat making family from *C. malaccensis, C. compressus* and *C. iria* was Rs. 3089.96 Yr^{-1} (SD 118.49), Rs. 682.66 Yr^{-1} (SD 27.02) and Rs. 295.51 Yr^{-1} (SD 18.19) respectively. It was revealed from the two year data of the two island of Sundarbans that the mean Net Economic Revenue contributed by *C. malaccensis*, *C. compressus* and *C. iria* to each mat making family was Rs. 2389.96 Yr^{-1}, Rs. 575.66 Yr^{-1} and Rs 248.51 Yr^{-1} respectively which were 6.638%, 1.599% and 0.690% respectively of the total annual income of a family settled any of these two Island. As a whole, an estimation of 8.927% of the total annual income of a family of these two islands of Sundarbans was found to be generated from these three species of *Cyperus*.

Table 4 : Economic value summary in present condition (May be inserted after 4th paragraph of Results)

Species	Mean Actual annual production (Q.) in dry weight	Mean Actual annual utilization (Q.)	Mean market price Per kg	Mean gross revenue/ Family Rs/yr.	Mean cost/Q.	Mean net revenue/ Family. Rs./yr	Gross Economic Contribution/ Fam/Yr. (%)	Net economic Contribution / Fam/Yr. (%)
1 *Cyperus malaccensis*	161.90	61.49 X 2 = 122.98	87.94 Std : 15.01	3089.96	700	2389.96	8.583	6.638
2 *Cyperus compressus*	84.68	47.72	50.07 Std : 4.80	682.66	107	575.66	1.89	1.599
3 *Cyperus iria*	100.47	21.33	48.49 Std : 8.33	295.51	47	248.51	0.820	0.690
							GEC =11.2 9% /Fam/Yr.	NEC=8.927% / Fam/Yr.

IV. Discussion

All of the three species discussed here are either wild or semi wild (*C. malaccensis*) that does not imply any production cost. These species are almost abundant in the waterlogged area with moderately low salinity (3 ppt). Except the cost of stitching materials and labour cost, no other value added service is to be required to prepare the mat. Besides, no economically viable land like agricultural field is necessarily required for bulk production of these species. The two islands studied here, have the potentiality to increase the production of these three species near about 10 times of the present production, if local people allow their spreading or once sow the viable seeds/planted the rhizomes in the bank of ponds or other water bodies for growing and proliferation. The study reveals that these three species presently, as a whole, contributes 8.927% of the total annual income of a mat making family residing any of these two islands. As the villages are in remote places, it is quite difficult for the villagers to bring their prepared mats to the town market for sale where they may get more profit as the mats have well demand in the town markets like Jadavpur market (73.5 km away from Gosaba), Barabazar (87.2 Km away from Gosaba). Thus the local mat makers hand over their mats to some local intermediator at a low price. Actually, the two significant elements influencing bioresource valuation in India are the government policy and marketing infrastructure. Wild floristic resource is a state controlled subject, the pricing mechanism differs between states, which largely influence WFR value at farm gate (Hector, 1992). This supports significantly the present study also. Basically, the trading of mat from these species of *Cyperus* in the local market operates in an imperfect market system. Quantum of extraction of these species is often uneconomical and price was determined by local intermediate stakeholders. The pricing system where in force had no publicity leaving most of the traditional people of these two islands ignorant about the real demand and existing market for the product which results the local mat maker vulnerable to exploitation by unscrupulous intermediators and corrupt traders.

Moreover, Sundarbans, a unique geological and geographical terrain of the world mingled with significant bioresources supports the aboriginal people of that area to live in fragmented rural sets of distantly located islands. Natural calamities like Tsunami, Aila and other storms are the common threats that compel them to be environmental refugee for the time being that hinders traditional livelihood activities like agriculture for a prolonged period until the cultivable condition is restored. To overcome this adverse effect, use of the

sedges of three species of *Cyperus*, as represented in the present study, alternative livelihood options are adapted through which a sum of cash income is to be generated through mat preparation that have the potentiality to contribute a considerable percentage of the total annual income of the people of that areas. From group discussions it was estimated that the average income generated through paddy cultivation per hecter is Rs 17835 SD. 261.72 in case of high yielding variety and Rs. 7354 SD. 206.47 in case of indigenous variety. Whereas, the mean annual turnover from *C. malaccensis, C. compressus* and *C. iria* was calculated to be Rs. 82771.51 SD. 4075.05/ha, Rs. 77792.5 SD. 1651.09/ha and Rs. 94374 SD. 1627.76/ha respectively (Table - 3) with minimum investment of cash and labor compared to agricultural practice. Here in this comparison it should be noted that the actual and potential habitat of these 3 species of *Cyperus* in these two large islands is only 3.21 ha and 34.94 ha respectively as they are very specific to their habitat (only the wetlands and waterlogged areas with moderately low salinity level). Though the comparison does not implies any suggestion of substitution as the paddy is the staple crop and indispensable for the rural livelihood but the mat is not. Here the comparison used only to represent the potentiality of these three species of *Cyperus* to be an alternative livelihood option during the adverse period in those vulnerable remote islands.

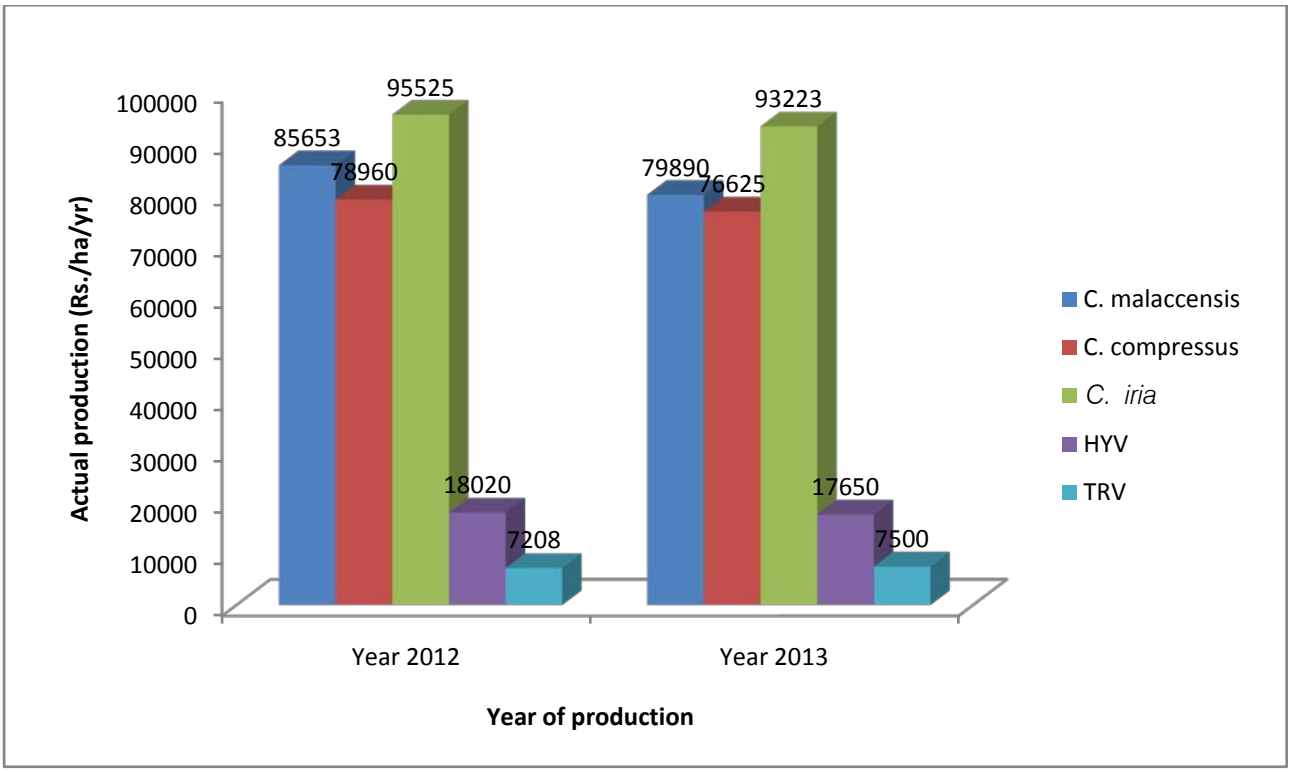

Fig 3 : Comparative study ofannual production of three *Cyperus* sedges with High Yielding Varieties and Traditional Rice Varieties

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Image 1 : Mat prepared from Cyperus species for domestic and commercial use

Image 2 : Collection of *Cyperus* for mat preparation

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The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

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(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

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References

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- To the point depiction of the research
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- Explain materials individually only if the study is so complex that it saves liberty this way.
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What to keep away from

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- Leave out information that is immaterial to a third party.

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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

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- Do not present the similar data more than once.
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Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
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Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- All figure and table must be adequately complete that it could situate on its own, divide from text

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The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

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- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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