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RESEARCH ARTICLE

STUDIES ON THE EFFECT OF NAPHTHALENE BALLS ON BIOCHEMICAL AND HISTOLOGICAL CHANGES IN COCONUT PEST RHINOCEROS BEETLE (*ORYCTES RHINOCEROS*)

Settu, K., *Subramanian, N. and Muthu, M.

PG and Research Department of Zoology, Aringar Anna Govt. Arts Collage, Cheyyar – 604407

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ABSTRACT

To understand the effect of Naphthalene balls on Brain and Muscle of coconut pest Rhinoceros beetle was selected. The biochemical parameters like Protein, Glucose and Glycogen on Brain and Muscle of both control and Naphthalene balls exposed Rhinoceros beetle have been carried out. The beetles were divided into two groups, one is control group (N=6). Another one is Experimental groups (N=6). The experimental group beetles were exposed with Naphthalene balls (l ball/beetle). After 96 hours of exposure the beetles in experimental group were totally dead. The beetle in the control group untreated with any chemical, were showed healthy. After 96 hours of exposure the beetles from both control and experimental group were dissected out for the analysis of biochemical and histological studies. The concentration of protein in Brain and Muscle of control group of beetle showed 11.52±0.3µg/mg in brain, 26.26±0.1µg/mg in muscle. Bat the protein concentration in brain and muscle of experimental group of beetle treated with Naphthalene balls showed decreased level 9.23±0.3 ($p<0.05$) µg/mg in brain and 12.52±0.1 ($p<0.05$) µg/mg in the muscle. The concentration of Glucose in brain and muscle of control group of beetle showed 1.354±0.8 µg/mg in the brain and 0.924±0.1 µg/mg in the muscle. Where as in the experimental group the Glucose concentration was reduced 0.812±0.7 ($p<0.05$) µg/mg in the brain and 0.312±0.1 ($p<0.05$) µg/mg in muscle, were noticed. ($p<0.05$) statistically Significant. The concentration of glycogen in the brain of control group showed much higher level 2.866±0.9 µg/mg and muscle showed 1.473±0.1 µg/mg. but in the case of Experimental group the glucose cotent was lesser when compared to control group 1.256 ±0.8 µg/mg in Brain and 0.711±0.1 µg/mg in muscle. The brain of control group of beetle showed normal histology outer nerve fibers inner granular layers and neural bundles were arranged uniformly, in the case of experimental group of beetle treated with Naphthalene balls showed broken of neural bundles, scaterly arranged glial cells and disturbance of glandular layers were seen. On the other hand the muscle of control group beetle showed uniformly arranged dark and light bands with connective tissue. The sarcoplasm and sarcolemma are clearly arranged, but in the case of experimental group of beetle the muscle showed broken of dark and light bands, unevenly arranged sarcolemma of sarcoplasm. This may be due to exposure of naphthalene balls.

INTRODUCTION

The Rhinoceros beetle, *Oryctes rhinoceros* L. (Scarabaeidae: Dynastinae) is one of the serious pests of coconut in all coconut growing countries including India. The adult beetles damage palms by boring into the center of the crown, where they injure the young, growing tissues and feed on the exuded sap (Brdford, 1980). The practice of application of insecticide / sand mixtures (1:1) once in three months in the top-most three leaf axil interspaces (Jayaraman, 1985), or application of never insecticides will create environmental pollution and will not be effective for the control of adult beetles due to its hard exoskeleton. Moreover the insecticidal control may lead to the outbreaks of leaf eating caterpillars. Alothough recommendations are available for the insertion of naphthalene balls into the frond axil, significant effect could not be obtained during monsoon periods.

Corresponding author: Subramanian, N.,
PG and Research Department of Zoology, Aringar Anna Govt. Arts Collage, Cheyyar – 604407.

Since frequent application is required for these methods, the reduced a availability and increased cost of labor for climbing charges for plant protection operations is a serious lacuna in IPM practices in coconut. Therefore an ecofriendly approach should be developed, such as use of bio-control agents like Metrhizium anisopliae (Bedford, 2013), which can be easily applied in the Coconut basins and manure pits. The notorious rhinoceros beetle, *Oryctes rhinoceros* L., (Coleopteran : Scarabaeidae) is considered to be the most destructive insect pest attacking coconut palms (Cocusmucifera L.) all over the coastal region of Dhofar province in the southern region of Sultanate of Oman. The adult beetles of *O.rhinoceros* fly to the central crown of the palm, craw 1 down the axle of the young frond and then bore through the heart of the palm into the unopened fronds. Thus, the fronds destroyed unfold later to reveal tattering and V-shaped cutting of the leaflets. The coconut palms may be killed by repeated or heavy beetle attacks In Sultanate of Oman (Dhofar province), the infestation of rhinoceros beetle was increased from 30% in 1983 to 68% in 1986, (kinawy 1987). The previous rapidly increasing of

infestation must be due to increase and widespread of the breeding sites along Dhofar province. From 1982, after opening the banana area under cultivation was increased five times than before. The cattle manure was used in huge amounts, which is considered as preferable breeding stage for the immature stages of rhinoceros beetle. Strategy of the short term control program based only on the chemical and mechanical methods was conducted during 1985 and 1986. Kinawy (1987) reported that the previous control program did not give sufficient control against the rhinoceros beetle. In addition, it was expensive and difficult to apply especially with the tall coconut palms (more than 8 meter tall), which are common in Dhofar plain. The African rhinoceros beetle, Oryctes mono-ceros (Oliver) (Coleoptera : scarabaeidae), is one of the most destructive pests of commercial coconut, oil and date palm in Africa (Hill, 1983). While the larvae develop in decomposing organic matter, adults feed inside of unopened fronds and meristem of palms. Beetle attacks kill young palms, provide entry holes for lethal diseases and other destructive insects, damage inflorescences and reduce photo synthetically active foliage. Thereby diminishing revenue of oil and coconut production (Mariau *et al.,* 1981). Introduction of pathogenic baculovirus, Rhabdionvirus oryctes, suppressed population of the rhinoceros beetle, O. rhinoceros, in parts of Asia (Bedford, 1986; Zelazny and Alfiler, 1987, 1991; Young, 1986) but did not effect. Monoceros in Africa (Julia and Mariau, 1976). *O.monoceros* is currently controlled by silvicultural methods (Hinckley, 1973; Ouvrier, 1980) and removal of adults from young palms and larvae from decomposing logs.

Pheromone-based trapping would be an ideal alternative and/or additional strategy to manage rhinoceros beetle in Africa the coconuts Rhinoceros beetle, *O. rhinoceros* (L.), has been a pest of coconuts and other palms in the south pacific since its accidental introduction into Samoa from Sri Lankan in 1909. Rhinoceros beetle is mainly a pest of Coconut and oil palms; but it also attacks other palm species coconut rhinoceros beetle adults damage palms by boring into the center of the crown, where they injure the young, growing tissues and feed on the exuded sap. As they bore into the crown, they cut through the developing leaves. When the leaves grow out and unfold, the damage appears as V- shaped cuts in the fronds or holes through the midrib. Eggs are laid and larvae develop indicating logs or stumps, piles of decomposing vegetation or other organic matter. Eggs hatch in 8-12 days, and larvae feed and grow for another 82-207 days before entering an 8-13 days nonfreezing pre pupa stage. Pupae are formed in a cell made in the wood or in the soil beneath where the larvae feed. The pupa stage lasts 17-28 day before emerging and flying to palm crowns to feed. The beetles are active at night and hide in feeding sites during the day. Most mating takes place at the breeding sites. Adult may live 4-9 months and each female lays 50-100 eggs during her lifetime.

Natural Enemies

Rhinoceros beetle eggs, larvae, pupae, and adults may be attacked by various predators, including pigs, rats, ants, and beetles. They may also be killed by two important diseases: the fungus smetarhizium anisopliae and the Oryctes virus disease. The female coconut rhinoceros beetle (coleoptea: scarabaeidae) burrows into rotting stumps standing palms and rubbish piles to lay their eggs. The eggs hatch in 8-12 days into whitish grubs, called curl grubs because of their shape. There are four larval stages lasting 12-16 days and a pupa period lasting three to four months to six months, during which time a female can lay up to 100 eggs. The Adult stage the damaging stages of this insect. They fly at night and feed by tunneling into the young coconut leaves. This damage causes a typical V- shaped notch. If they reach the growing tip, the palm may cause decreased nut set. The beetles attack many species of palms including coconut, beetles nut, sago palm and dates. They can also feed on pandanus and other fleshy plants. This beetle is present in American Samoa and palau, as well as S.E. Asia and many other islands in the pacific and Indian ocean.

The Rhinoceros Beetle was introduced into the pacific area from South East Asia, where it was endemic (Jepson, 1912). Palm culture is a long established industry throughout the endemic area so that one could reasonably expect that solutions to the problem of control might be found there. For this reason, India and South East Asia became an early focus of attention in the search for biological control agents and cultural methods that might reduce the impact of the pest. Scientists in India and South East Asia were experimenting with various control possibilities. In India, Nirula *et al,* (1995) worked with the fungus metarrhizium anisopliae. A second focus of excellent research developed in Malaysia, where Wood (1969) began to experiment with cultural methods of reducing beetle breeding. The adult beetle spends intervals of several too many days alternately at feeding in the palm crown and breeding on the ground these two living sites during its life time (Zelazny, 1975). In when an individual of the same species comes in contact with pheromone, it elicits a response, depending on the type of pheromone. In this way, specific information is conveyed. Pheromones that cause clumping or clustering behavior in a species, which bring individuals into a closer proximity, are referred to as aggregation pheromones (Figueiras and Lazzari, 1998). In general, male-produced sex attractants have been called aggregations pheromones, because they usually result in the arrival of both sexes at a calling site, and increase the density of conspecifics surrounding the pheromone source (karlson and luscher, 1959). Aggregation pheromone functions in defense against predators, mate selection, finding shelter and to overcoming host resistance by mass attack (Fifueiras and Lazzchari, 1998). Cockroaches produce a specific pheromone with their experiment when they find safe shelter, which attracts other members of their species. As a result, aggregation pheromones function such that individuals aggregate around a **"**good position**"** with some rate. This prevents other individuals to aggregate to a local position (Tsutsui, 2004).

Coconut (Cocosnucifera Linn.) is a major, commercially grown plantation crop in 93 countries of the world. India and Sri Lanka together account for 78% of total coconut world production. India contributes 15.53% area and 22.34% of the global coconut production. It contributes more than US $ 1400million to the country's gross domestic product apart from an export earning of US $ 80 million. It also provides livelihood securities to more than 10 million people (Rethinam and Singh, 2007). The coconut palm is susceptible to attack by a large number of pests, of which red palm weeil (Faleiro, 2005) and rhinoceros beetle (Valentine et al 2007) are serious and cause enormous damage to coconut palms.

The rhinoceros beetle, Oryctes rhinoceros (colepotera: Dyastidae), is one of the most damaging insect to coconut palm. The adult feed on the growing portion of the palm leading to ragged appearance. A severely attacked palm will die or gets exposed to damage by secondary pests (Molet, 2013). The life cycle of this pest lasts from 4 to 9 months allowing more than one generation (Giblin-Davis, 2001) in a year. The beetle breeds in dead standing coconut palms killed by pest, disease, lightening, decaying organic materials like and sawdust heaps. Floating logs containing larvae in tunnels might spread the pest to new areas (Howard *et al.,* 2001). The rhinoceros beetle into the base of cluster of spears, causing wedge shaped cuts in the unfolded fronds. In younger palms the effect of damage can be more severe. Attack by adults may reduce yield and kill seedlings. They may provide entry points for lethal secondary attacks by the red palm weevil or pathogens (Molet, 2013).

MATERIALS AND METHODS

Adult beetles of *Oryctes rhinoceros* irrespective of sexes were collected by hand in the field during day time from the infected coconut palms or after sunshine from the breeding sites in and around cheyyar Taluk, Thiruvannamalai District, Tamil Nadu, India. These beetles were brought to the PG and Research Department of Zoology, Arignar Anna Government Arts College, Cheyyar. The Rhinoceros beetles were examined properly for any damage and free from any disease, starved for one night and placed in aerated plastic Indies dual Jars in the laboratory

EXPERIMENTAL ANIMALS

Healthy *Oryctes rhinoceros* beetles weighing about 10-15gm were used in the present study. The animals were housed in plastic jars with free aeration the animals were feed with coconut young leaves. Care and supervision were provided throught the period of study.

EXPERIMENTAL DESIGN

The animals were divided into two groups Group I, and Group II with 6 beetles in each group, Group I is called control group, Group II is called Experimental group.

Group I: Control group of beetle which received normal coconut leaf feed and no exposure of any chemicals.

Group II: Experimental group of beetle which received normal coconut leaf feed and they were exposed to Naphthalene balls 6 (30grm) (5grm/beetle) for a period of 96 hours.

The animals were acclimatized to laboratory conditions for two days with normal feed and before the starting of the experiment. Initial and final body weight were recorded before and at the end of experiment period the experimental group of beetles (6 beetles) were exposed to Nahthalene balls (6 No's) placed in the plastic jar. The animals were sacrificed after morbiant stage of exposure. (12 hrs. morbiant stage 96 hrs death stage). The brain and muscle tissues were distracted out washed with 0.9N saline solution blotted on a filter paper weighed.

The weighed tissues were homogenized in 1ml of 0.1M. Tris buffer (p^H 7.2) for estimation of protein, glucose and glycogen the homogenate of the tissues were centrifuged at 3000 rpm for 15 minute and the clear supernatants were used for biochemical analysis.

BIOCHEMICAL ESTIMATION

The quantitative determination of biochemical constituents like Protein, Glucose and Glycogen in Brain and muscle were performed by spectrophotometer using the following method. Total protein was estimated by the method of Lowry *et al* (1951). Total Glucose was estimated by the method of Cooper and McDanial (1970). And the total glycogen content was estimated by the method of Carrol *et al* (1956).

HISTOLOGICAL STUDY

The brain and muscle tissues of both control and Experimental group of beetles (Exposed to Naphthalene balls) were dissected out (carefully removed). The tissues were immediately washed in 0.9% NaoH. It was kept on the blotting paper to drain the moisture. The tissue samples were processed for histological observation. The brain and muscle of both control and Experimental group of beetle were fixed in physiological saline solution for 24 hrs. Using tetra hydrofuron as a dehydrating and clearing agent. The sections of 6µm thickness were selected to observe the changes in the brain and muscle by adding Haematoxylin and Eosin are counter stain, (Humason 1972).

RESULTS

BIOCHEMICAL STUDY

The biochemical analysis such as Protein, Glucose and Glycogen contents in the Brain and muscle tissues of both control and Experimental (exposed to Naphthalene balls) group of *O.rhinoceros* beetles are given in Table 1 and 2.

Protein content

The protein content in the brain of control group of beetles showed (11.52 ± 0.3 µg/mg) at the end of the experiment. But in the case of experimental group of beetle treated with Naphthalene balls showed lowest level of protein content in the brain (9.23 ± 0.3µg/mg) tissue at the end of the exposure period. On the other hand the protein content in the muscle of untreated control group of beetle showed much higher level (26.26 ± 0.1µg/mg) of tissue. After the exposure period with Naphthalene balls the experimental group of beetles showed low level of protein content in the muscle (12.52± 0.1µg/mg) of tissue, the results are given in Table 1.

Glycogen

Glycogen is the most important biochemical constituent for the metabolism of insect. The results are given in Table 2. The control group of beetles showed, (2.86 ± 0.9 µg/mg) of glycogen in the Brain at the end of the experiment. But the experimental group of beetle showed reduced level of glycogen content in the Brain, (1.256 ± 0.8 µg/mg). Glycogen content in the muscle of both control and Experimental group of beetle are given in Table 2 from the table result were concluded that

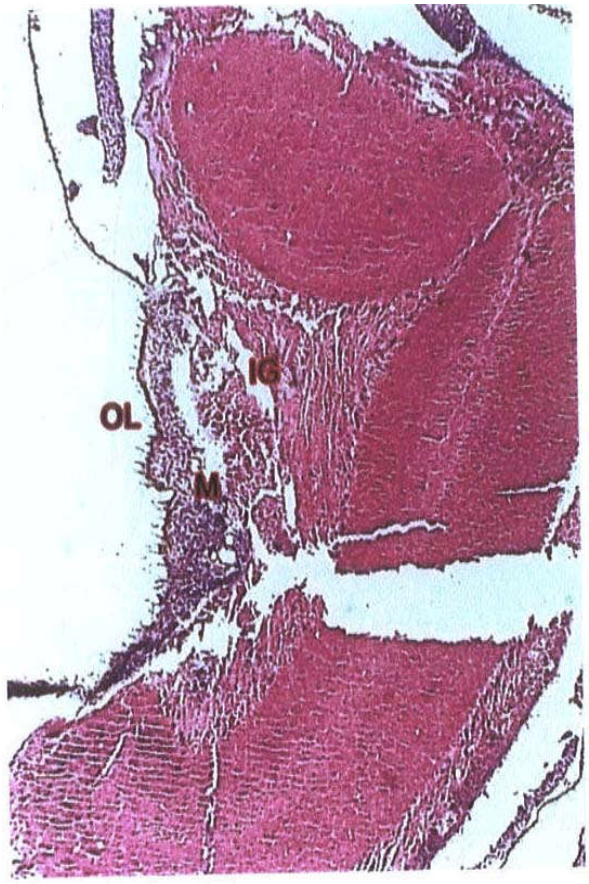

Fig. 1. Control Brain

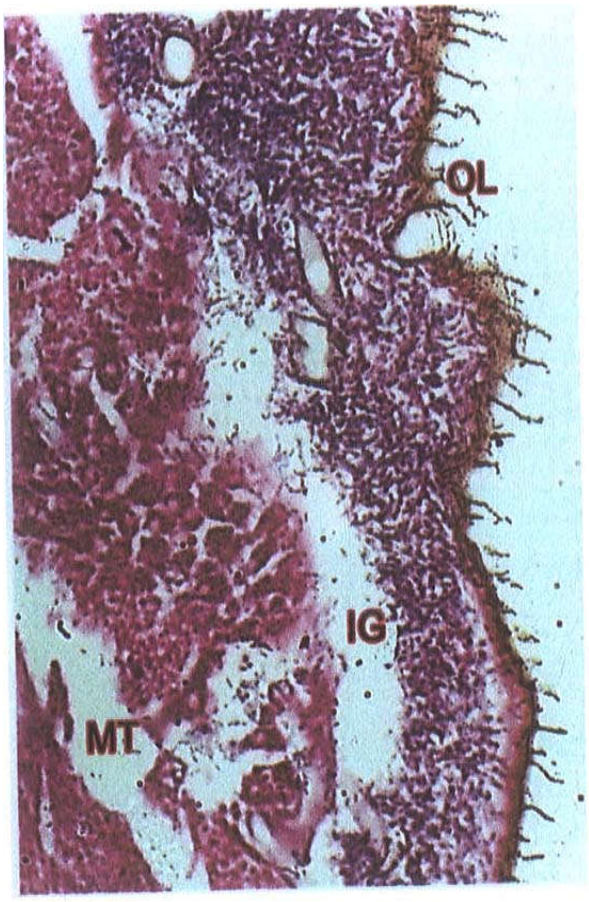

Fig. 2. Experiment Brain

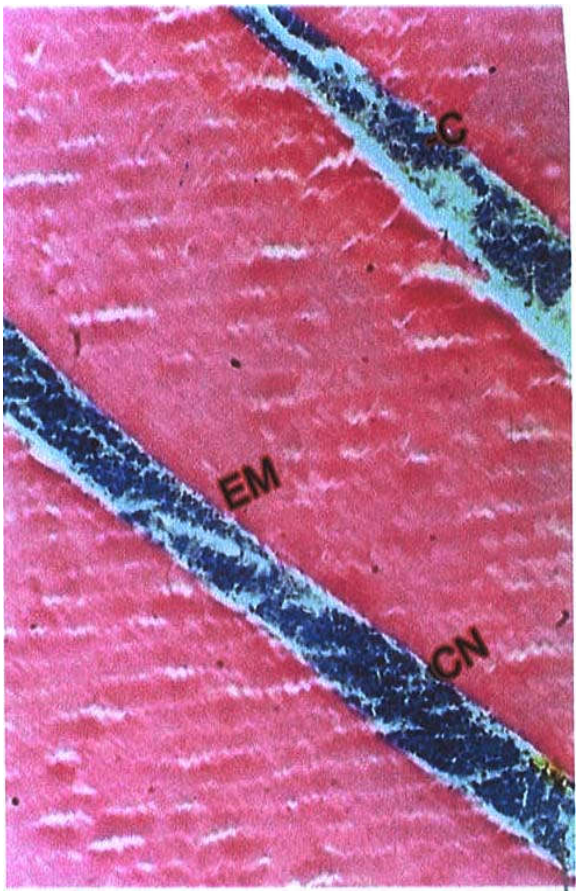

Fig 3. Control Muscle

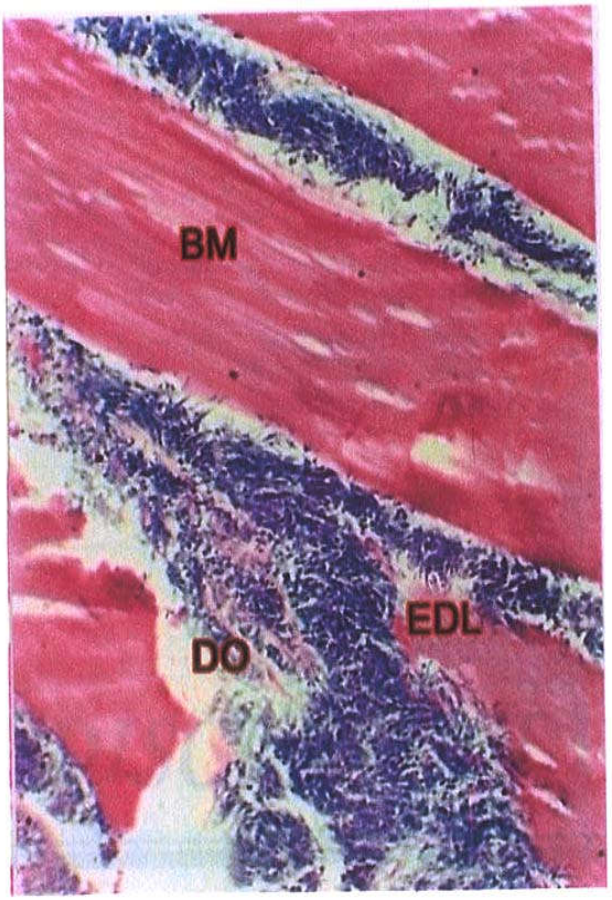

Fig. 4. Experiment Muscle

glycogen content in the control group of beetles showed somewhat high level (1.473 ± 0.1 µg/mg) and comparatively low level of glycogen content was noticed in the muscle of experimental group of beetle (0.711 ± 0.1 µg/mg) exposed to Naphthalene balls.

Table 1. Shows the concentration of protein (µg/mg) present in the brain and muscle of Rhinoceros beetle control and experimental groups (treated with Naphthalene balls

S.No	Tissue	Protein concentration (µg/ mg)	
		Control group	Experimental group Treatment with Naphthalene balls
1.	Brain	11.52 ± 0.3	9.23 ± 0.3
2.	Muscle	26.26 ± 0.1	12.52 ± 0.1

X ± SD

Table 2. Shows the presence of Glycogen concentration (µg/mg) in Brain and muscle of Rhinoceros beetle control and Experimental groups (treatment with Naphthalene balls)

S.NO	Tissue	Glycogen concentration µg/mg	
		Control group	Experimental group (Treatment with Naphthalene balls)
1	Brain	2.866 ± 0.9	1.256 ± 0.8
2	Muscle	1.473 ± 0.1	0.711 ± 0.1

X ± SD

Histology of control beetle (Rhinoceros) Brain

Histology of control beetle (Rhinoceros) Brain is given in Figure 1. The brain showed nerve cells and association of fibers. The outer most layer is molecular layer consists of mainly nerve fibers and occasional horizontal cells or cajal.
The second layer is called external granular layer contains large number of stellar cells and small pyramidal cells next layer is called external pyramidal layer made up of medium sized pyramidal cells and also contains few stellar cells and cells of Martinotti. The other layer is called internal granular layer is composed of closely packed stellar cells and horizontally oriented with fiber band called outer band of bail larger, Internal pyramidal layer or ganglionic layer consist mainly of large pyramidal cells and few stellar cells of martinotti. This layer also contains horizontally arranged fibers that from the inner band of baillarger. The last layer is called multiform layer (layer of polymorphic cells) is the deepest layer. It contains predominantly fusi form cell and also few stellar cells and cells of martinotti intermixed with many nerve fibers entering or leaving the underlying white matter.

Histology of Experimental beetle (Rhinoceros) Brain

The histology of Experimental beetle (Rhinoceros) Brain is given in Figure 2. The experimental beetle brain showed severe damage in the neural cells. The neural bundles were broken and granular cells were scatterly arranged. The glail cells were reduced in to small cells the cell edema was observed. Many areas of brain showed many empty space without any neural cells, at the end 96 hrs of exposure with naptthalene balls, the findings are in agreement with those of USEPA (2000). The report said the exposure of naphthalene leads to damage or enlargement, gastrointestinal bleeding and kidney damage were observed when rats are treated with naphthalene (Fewill 2006).

Histology of control beetle (Rhinoceros) Muscle

The histology of control beetle (Rhinoceros) muscle is given in Figure 3. Each muscle fiber is an elongates, unbranched and cylindrical cell. It as many plat nuclei located just beneath the sarcolemma. It shows cross striations of alternate dark (A) and light (I) bands with Z line intersecting. I band each muscle fiber is made of compactly packed long of cylindrical myofibrils in the sarcoplsm arranged parallel to the long axis. The sarcomere consists ofntwo types of myofilaments arranged parallel to the long axis of myofibril in a symmetrical fashion thick filaments – composed mainly of the protein myosin and occupy the A band. Thin filaments – composed mainly of the protein action and also of tropomyosin and troponin. One end of each thin filament runs between and parallel to the thick ones in the band for some distance. The other end of the filaments is attach to the Z line in the I band.

Histology of Experimental beetle (Rhinoceros) Muscle

The histology of Experimental beetle (Rhinoceros) muscle is given in Figure 4. The muscle fibers are irregularly arranged, the elongation of muscle fibers are disrupted. The dark and light bands showed abnormal arrangements. The space between the bands are much wider. The dark bands are unevenly distributed. Musofibrils and sarcolemma are eroded due to the chemical exposure of naphthalene balls. The naphthalene a chemical compound mainly affected the brain and central nervous system. Though the central nervous system the muscle fiber are also affected. The normal arrangements of muscle fibers are completely disappear with many space between the muscle fibers. The muscle fibers lost their connection tissue fibers for arrangement. The nuclei are disappeared.

DISCUSSION

Application of naphthalene balls at the rate of one ball/beetle was found to be the best treatment and statistically significant ($p<0.05$) between control and experimental group of beetle on both biochemical and histological studies this finding is an agreement with those of Singh (1987). He reported that five naphthalene balls per palm and HCL 10% + fine sand (1:1) was the next best treatment of eradication of rhinoceros beetle. Observation after one week of treatment with naphthalene balls showed 100% mortality of *O. rhinoceros*. The results are in agreement with those of Gopal *et al* (2006) that application of *M. anisopliae* reduced *O. rhinoceros* groups up to an extent of 72% in the field condition. Yamini varma (2013) found out naphthalene balls treatment at 45 days informal at the base of the inter space between leaf sheath of the three top most leaves in the crown was the best method to control of *O. rhinoceros* in the form trails. Moslim *et al* (2002) also found that field application of *M. anisopliae* to rootling debris reduced the *O. rhinoceros* population by up to 80% the farmers practice and recommended practice were toss effective with 83% and 43% pest attack and lower yield of 43 and 62 nuts /palm/year.

The changes in the protein content in the control and experimental group (treated with naphthalene balls). A chemical compounds present in the experimental group having the ability to inhibit protein concentration (Khan and Wasim

2001). In afield experiment, ethyl 4-methylloctanoate released at 30mg /day attracted 6 males and 5 females in 9 days whereas the known attractant ethyl chrysanthemate at 30mg/day did not attract on Oryctes lower release rates of the pheromone were not attractive. This experiment clearly showed the effect of naphthalene balls in controlling Rhinoceros beetle altars application of naphthalene balls is quite early and Economical compared to insecticide mixtures and affords effective protection against the rhinoceros beetle. The growers can apply naphthalene balls easily even in the home gardens. Naphthalene is made from crude oil or coal tar. It is also produced when things burn, so naphthalene is found in cigarette smoke, car exhaust and smoke from forest fires. It is used as an insecticide and pest repellent. Naphthalene was first registered as a pesticide in the United States in 1948. Mothballs and other products containing naphthalene are solids that turn into toxic gas. The toxic gas kills insects and may repel animals Naphthalene is classified as Group 2B substance by the International Agency for Research on Cancer (IARC). This means that this agent is possibly carcinogenic (cancer causing) to humans. Naphthalene is a white crystalline, volatile solid with an odour of mothballs. It sublimes at room temperature (transition of a substance directly from the solid to the gas phase). Naphthalene is insoluble in water and is soluble in benzene, absolute alcohol, ether, carbon tetrachloride, carbon disulfide, hydronaphthalenes, and in fixed and volatile oils. Naphthalene is produced from petroleum refining and coal tar distillation. It is used as a chemical intermediate in the production of ophthalmic anhydride, Naphthalene, and chlorinated naphthalene. Naphthalene enters the environment from industrial uses, from its use as a moth repellent, from the burning of wood or tobacco and from accidental spills. Naphthalene at hazardous waste sites and landfilly can dissolve in water and be present in drinking water. Naphthalene can become weakly attached to soil or pass through the soil particles into underground water.

Most of the naphthalene entering the environment is from the burning of woods and fossil fuels in the home. The second greatest release of naphthalene is though the use of moth repellents. Only about 10% of the naphthalene entering the environment is from coal production and distillation. Less than 1% of the naphthalene released to the atmosphere can be attributed to the losses from naphthalene production. Cigarette smoking also releases small amounts of naphthalene into the air. Changes in the biochemical parameters like protein, glucose and glycogen in Brain and muscles tissues of experimental group changes to naphthalene exposure, compared to control group. It is evident that changes in the biochemically can reflect in the histology also. The histological section like brain and muscle in experimental group of beetles exposed to naphthalene balls showed marked verity of altered the cell structure due to naphthalene exposure. This result are in agreecut with those of Feuille (2006). They reported that naphthalene balls cased laryngeal carcinoma in human beings. Persons who work with naphthalene or tar distillation are exposed to rather low concentrations of naphthalene, methylated naphthalene, and naphtha's, but it has been reported that workers exposed to vapors of naphthalene and coal tar can develop laryngeal carcinomas or neoplasm's of the pylorus and cecum (Bieniek, 2002). During the early months of pregnancy of a woman can lead to intentional ingestion of naphthalene with resultant fetal death (caused by hemolytic anemia within days after transplacental diffusion). This experiment clearly shows the efficacy of naphthalene balls in controlling Rhinoceros beetle attacks. Application of naphthalene balls is quite easy and economical, compared to insecticide mixtures and affords effective protection against the rhinoceros beetle. The growers can apply naphthalene balls easily, even in the home gardens.

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