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With the increase in population, third world countries today are facing many problems, supply of sufficient food being one of them. In animal sciences we have to understand and preserve the vast diversity of species on our planet. Losing them would be a huge shame and almost a crime of humanity. We have caused a continuous trouble that leads to species extinction. Just because we are the "dominant" species on Earth, it doesn't mean that we can do whatever we want without suffering consequences. We do not have to protect endangered species only, but we also have to protect species essential for the continuation of Earth's life. Believe it or not, without animals, humans would die out pretty quickly. First of all, there would be no more meat. But we can't all become vegetarians either if there are no insects to pollinate the plants. From animals, we can also learn about our anatomy and can understand the function of our bodies in a better way, which help us combat human diseases. In termination, animal's science is an important field that applies to many real-world situations.

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The University of Sindh Journal of Animal Sciences (USJAS) with modernized and cost effectiveness will light the tools for numerous directions and problems related to improve identification of pest species, conservation of wild animals, diversity of animals including animal breeding, environmental impact of animal, agriculture, diseases, nutrition and animal products. When animals grow well and stay healthy, farmers can produce more meat, milk or eggs for our consumption. They check meat quality or screen milk for pathogens. Advances in food safety keep humans healthy and increase the world's supply of nutritious food. Beside this, articles regarding entomological science contribute to the betterment of humanity by detecting the role of insects in the spread of disease and discovering ways of protecting food and fiber crops, and livestock from being damaged. Journal provides the way how beneficial insects contribute to the well being of humans, animals, and plants. This journal will also defend and assess the application of well proven research activities in natural science particularly, Zoology, Physiology, Fresh Water Biology & Fisheries, Biochemistry and Biotechnology of host universities; neighboring and sister universities which are performing research activities on any area of animal's sciences. They have necessity of proper platform for their research exposure around the country as well as in world.

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BODY SIZE DECREASES IN ALTITUDE GRADIENT BUT SEXUAL SIZE DIMORPHISM DOES NOT IN GROUND BEETLE *CARABUS ODORATUS* SHIL.

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RAS and TLA designed the study and wrote the paper. TLA collected the data and performed the morphometric analysis. AAS and TAG performed statisticalanalysis and illustrations.

Key words:
Body size variation, ground beetles, altitude gradient, sexual size dimorphism.

ABSTRACT

Attributing biological explanation to observed ecogeographical patterns requires intra-specific studies. Body size variation in latitude/altitude gradient and sexual size dimorphism variation reflect adaptation of the organisms to the varying environment and future climate impact. Investigations took place at Barguzinsky Ridge (North-East part of Baikal Lake, N 54° 20'; E 109° 30', Russia). Beetles of the Ground Beetle *Carabus odoratus* Shil. were sampled in 30 -km transect, divided into four plots – the coast, low-, middle- and high mountains (455-460, 500-720, 721-1300, 1301-1700 m above sea-level, respectively). In total 968 individuals were measured by six traits – the length and the width of elytra, pronotum and head. Our results showed that altitude and sex but not their interaction affected body size in *C. odoratus.* The values of all morphometric characters decreased towards the highlands in females and males. Sexual size dimorphism (SSD) varied in different traits: the highest values of SSD were recorded for the elytra length and the pronotum width (at all altitudes), and the head length (at the coastal and high mountains populations). For the other traits values of SSD at different altitudes did not differ significantly. The mean values of SSD for all the traits were similar at the coastal, low- and high mountains populations but in the middle mountains populations SSD was significantly lower.

1. INTRODUCTION

Body size is a vital trait which affects behavior, physiology and fitness in insects [1]. Large in size specimen can overcome difficulties more easily (food limitation, overwintering etc.) [2, 3, 4, 5]. Frequently larger sized insects cope better with stressful environments. Larger males mate more successfully [6, 7]. Larger females are more fertile [8, 9].

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But benefits of large body size are not absolute always: they are limited under certain suboptimal conditions [10], large size requires more food, the longer development leads to increased risk of predation [11]. Thus, intra-species variation in body size is observed in environmental gradients. The most well-known ecogeographical Begrmann rule is devoted to interspecific variation in body size in latitudinal gradients: larger animals have the lower surface-to-volume ratio and then lose less heat in cold environment. However, at the intraspecific level in ectotherms, the validity of this rule is not entirely unambiguous: species can follow the rule, convert, or have some other image [12]. Several explanations for Bergmann rule exist: temperature variation during larval stages [3], food resources [10], season length and voltinism [13, 14].

Body size variation in insects in relation to Bergmann rule accordance is studied successfully in mountain ecosystems. The latter have unique biodiversity as a result of geologic history and specific environmental factors. Furthermore, adaptation of species to changing environment parameters varies along the altitudinal gradients. All this highlights the need for ecological investigations to assess the state of the mountain ecosystems. Insects body size variation in altitude gradient in various species differs: body size can decrease [15, 16, 17], increase [18] or be stable [19].

The females frequently are larger than males (SSD) in insects [20, 2, 21, 22]. Different net selection pressures on sexes affects SSD [23] and different response to abiotic factors in sexes leads to different body size clines in males and females [24, 25]. Generally, it is believed that males produce steeper geographical clines than females [26].

In our study we turned to Ground Beetles – the excellent bioindicators and model species [27]. Their body size variation relatively widely discussed, showing different types of this trait clines in latitude and altitude gradients as well [28, 29, 30, 31]. We put emphasis on sexual size dimorphism (SSD) in studied species of Ground Beetles *Carabus odoratus* and its variation in altitude gradient. So, we tested the following hypotheses: (i) female – biased SSD in *C. odoratus* is similar to the majority of other carabid species; (ii) the trends of body size variation in altitude gradient are similar in females and males; (iii) the value of SSD varies in altitude gradient.

2. MATERIALS AND METHODS

Sites and design: The study was performed in the Barguzinsky State Natural Biosphere Reserve (Republic of Buryatia, Russian Federation). The research area is located on the North-Eastern coast of Baikal Lake in the central part of the Barguzinsky Ridge. We sampled Ground Beetles at 30 km long transect in Davsha river valley. It crosses all high-rise belts from the shore of Lake Baikal to the watershed Davsha-Tarkulik rivers (second-order spur of the Barguzinsky Ridge).The study area is characterized by a relatively gentle rise from the shore of Lake Baikal (455m above sea level) to the low – mountain part of the ridge (at 535 m), steeper – to the upper border of the forest (1407 m), and a sharp rise to the highest point of the watershed – the pass (1700 m). A landscape features were designated as: the coast – 458-500 m above sea level, the low (the lower part of the mountain forest zone – 501-720 m), middle (upper part of the mountain forest zone – 721-1004 m), high (bald belt of vegetation – 1005-1700 m). Coast included biotopes with Bilberry cedar and Grass birch, the bottom part of the mountain forest zone (Low Mountain) – Blueberry larch and Red bilberry pine, middle mountains – Bergenia aspen and Bilberry abies, High mountains – Sparse birch woodland and Lichen tundra (Fig. 1).The climate of the studied region is sharply continental, with sea features. It is characterized by frosty long winters and cool short summers. Humid Baikal type of altitudinal zones, associated with temperature inversions, is formed on the western slopes of the Barguzinsky Ridge. The so-called "false-bald" vegetation belt, consisting of larch forests (*Larix czekanowskii*Szaf.), sparse thickets of cedar dwarf (*Pinus pumila* Reg.), golden rhododendron shrub (*Rododendron aureum* L.) developed from the coastline to 100 m above the lake level. These species grow both on the coast and in the high mountains but are absent in the low- and middle mountain vegetation belts. This fact testifies to the similarity of environmental conditions on the Baikal coast and the high mountains (Tyulina, 1954). Close analogs are noted on the Okhotsk sea coast [32] (Tyulina, 1967). *Carabus odoratus barguzinicus* Shil, 1996 was chosen as a model species for our research. This is the largest ground beetle that dwells here, convenient in measurement. *C. odoratus* is abundant (17.6 % of the total population) in the entire gradient of the Barguzin range. It is endemic there. According to the classification of life forms, *C. odoratus* belongs to walking epigebionts and zoophages with extra-intestinal digestion. The body is convex, the integument is strongly sclerotized. The head is narrower than the pronotum, and there are large compound eyes on the sides. Beetles hunt on the surface of the soil, eating sedentary prey. *C. odoratus* has a two or three-year life cycle with a summer development period and a winter diapause at the imago and larval stages in the study area. Two or three peaks of population growth during seasonal activity are recorded at different altitude levels. The first early peak associated with the emergence from hibernation and the beginning of sexual activity (in the third decade of June) is observed in the low mountains, later (in the first and second decades of July) – in the high mountains [33]. Quantitative counts of beetles were carried out on stationary sites of the altitude transect in 1988-2014 by means of pitfall traps [34] (Barber, 1931). We used glass jars with of 70 mm diameter and a volume of 0.5 liters, and used 4% formalin as a fixative. Pitfall traps were placed in a straight line at 5 m interval. The captured insects were selected every decade from the third decade of May to the second decade of September. The following measurements were made: elytra length and width, pronotum length and width, head length and distance between eyes (Fig. 2).

A –length of the elytra, B –length of the pronotum V– length of the head, G– width of the elytra, D – width of the pronotum, E – the distance between eyes.

We selected undamaged specimens for habitat analysis, but without fixing the selection time (year, month, decade). A total of 883 specimens of ground beetles were selected from 8 biotopes for the period 1988-2014.The sex of beetles was determined by the shape of the segments on the front legs - the segments in males are wide, and in females are narrow.

***Data analysis**:* In the analyses, body size was used as a proxy for describing environmental quality (temperature drops, humidity and as a consequence food availability, food quality): a larger final size was considered to indicate more favorable conditions during the juvenile development (a common practice in insect ecology) [35]. To study variation of sexual size dimorphism (SSD) we calculated the size dimorphism index (SDI) [36] by dividing the trait size of the females by the trait size of males and subtracting one, resulting in negative SDI when male's trait is larger, and positive values of SDI when female's trait is larger. In R environment we used ANOVA to detect effect $\alpha_{\text{coast\_high}}$ of altitude (coast_high), effect of Sex a_{Sex}, and effect of their interaction $\alpha_{Sex,\ coast_high}$the beetles traits variation. The models were as follows:

$$\text{Trait} = a_0+a_{Sex}+a_{coast\_high}+a_{Sex,\ coast\_high}+\varepsilon$$

If the interaction was significant, both variables were considered significant also. If the interaction was not significant, we excluded interaction and conducted the type-II ANOVA to detect the significance of the variables:

$$\text{Trait} = a_0+a_{Sex}+a_{coast\_high}+\varepsilon$$

3. RESULTS

Beetles body size monotonically decreased from the coast to the high mountains (Fig. 3 – 8) in females and males as well. The highest values of SSD were recorded for elytra length (at all altitudes) and for the pronotum width and the head length (at the coastal and high mountains populations). For the other traits values of SSD at different altitudes did not differ significantly (Fig. 9).

We calculated the mean value of SSD for all the traits at the certain altitudes: SSD were similar at the coastal and low mountains populations, then significantly decreased at the middle mountains and then increased again in the high mountains population (Fig. 10).

Sex ratio in all populations were female-biased with significant prevalence of females (Table 1).

Table 1. Sex ratio (females/males) in *C. odoratus* populations at different altitudes populations

	coast	low	middle	high
SR	2,50	1,53	1,51	1,56
χ^2	2,57	13,98	9,36	15,02

ANOVA showed that sampling elevation and sex are significant but their interaction - not in effect on beetles body size. Tables 2, 3 demonstrate elytra length variation.

Table 2. Results of elevation and sex interaction effect on elytra length variation in *C. odoratus* ($A = a_0+a_{Sex}+a_{coast\_high}+a_{Sex,\ coast\_high}+\varepsilon$)

	Df	F value	p-value
$a_{Sex,coast\_high}$	3	0.6391	0.5932

Hereafter: a_0 is constant, a_{sex} – sex effect, a_{coast_high}– altitude effect, and $a_{Sex,coast\_high}$– interaction between the sex and altitude effect, and ε - random error. Since the interaction was not significant, we performed the next model.

Table 3. Results of altitude and sex effects on elytra length variation in *C. odoratus* ($A = a_0+a_{Sex}+a_{coast\_high} +\varepsilon$)

	Df	F value	p-value
a_{Sex}	1	170.098	< 2.2e-16
A_{coast_high}	3	54.439	< 2.2e-16

4. DISCUSSION

In invertebrates, changes in body size with altitude often do not follow Bergmann rule. Rather, it has been shown to decrease with altitude in beetles and butterfly [37, 38], and a number of other studies also indicated converse-Bergmann rule or lack of pattern [39, 40]. Body size often correlates with development time, resulting in a converse-Bergmann cline, i.e., decreasing body size with shorter growing season at higher altitude, and this is conforming to our observations on *C. odoratus*[29]. The size of every 6 treated traits monotonically decreased towards the high altitudes. Smaller body size at high altitude in this study were hypothesized to be linked to high metabolic costs due to low temperature at high altitude which cannot be compensated for by increased feeding rate. At the family level, a negative relationship between altitude and insect (Carabid beetles) body length was found; this was predicted because of a decrease in the diversity of resources, habitat area and primary productivity, and the increase in the unfavorable environment observed at high altitudes [37, 41]. On community level mean individual biomass also decreased in ground beetle communities [42].We analyzed the variation in average body size with height in the studied ground beetle populations. The analysis showed a decrease in average body size with increasing height. However, at low altitudes there are both "large" and "small" individuals, and at high altitudes - mostly "small". This fact indicates a stronger selection pressure in the high mountain areas. Individuals living at high altitudes are probably unable to grow to large size. The adaptability of "small" beetles to difficult mountain conditions is much lower than the adaptability of "large" ones.

However, SSD did not change similarly. We did not investigate males and females sensitivity in the present paper, but in our earlier studies there had been shown that sensitivity in both sexes might be different in relation to different traits at the altitudes studied. Ground beetle *Pterostichus montanus* Motsch. is another dominant ground beetle species inhabiting all biotopes of the Barguzinsky Ridge (19.7% of the total population). It belongs to the group of litter-soil stratobionts, has a one-year life cycle [33]. On the contrary, according to the RMAII, the sensitivity of males was very high in the midlands [42]. Males *Pt. montanus* in the midlands reached larger sizes than females, and the SSD values were lower. A favorable habitat can be determined, in particular, by lower intraspecific competition, since the population density of *C. odoratus* is lower in middle mountains than in low and high mountains [43, 44]. In addition, there were significant differences in the interpopulation morphometric structure. The latter, apparently, reflects the height difference. Two relatively different environments often exist on the mountains: the 'upper mountain' environment, treeless and the subject to more extreme cold temperatures or different rainfall patterns (and often above the tree line), and the 'lower mountain' environment, which is covered with forest. There is a transitional zone between them - "middle mountain". In the Barguzinsky Ridge, the mid-mountain belt is steeper and colder than the low-mountain belt [45]. Another explanation for mid elevation diversity maxima is the ‘mid-domain effect’ (MDE). It argues that if all species ranges are scattered randomly between the limits of the top and bottom of a mountain, there will be a ‘bulge’ of maximum numbers of overlapping species in the mid elevations. A recent advance of MDE theory has been to include a midpoint attractor – a unimodal gradient of environmental favorability, using a Bayesian simulation model to estimate the location and strength of the attractor from empirical species distribution data along the elevations, within geometric constraints [46]. It has been suggested that gradients of environmental favorability, together with the geometric constraints imposed by the base of a mountain and its summit, will more parsimoniously explain elevational species richness patterns.

Information on sex-specific within-population variation along an altitudinal gradient could provide insight into mechanisms generating altitudinal clines in sexual size dimorphism, for example, by revealing that phenotype canalization in females is increased under harsh high-altitude conditions, that is, within population variability in female body size decrease. In general, patterns observed at scales within a population can provide useful additional information to patterns observed at scales between populations. Nevertheless, the sex bias in all the studied populations of *C. odoratus* indicates that the ecological conditions for this species are quite favorable at all altitudes.

5. CONCLUSION

Overall, considering that body size is a master trait driving fundamental characteristics of organisms, its study along altitudinal gradients under different bioclimates may allow better understanding of the factors driving elevational patterns in the populations’ structure of Carabidae and ecogeographical rules as well. The proposition that Carabidae generally follow Bergmann rule or any common pattern is clearly challenged by available studies. The results suggest that to improve understanding of the drivers of the observed patterns further investigations on changes in ground beetles communities along altitudinal gradients should consider different species and bioclimatic contexts and use similar sampling designs.

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7. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFRENCES

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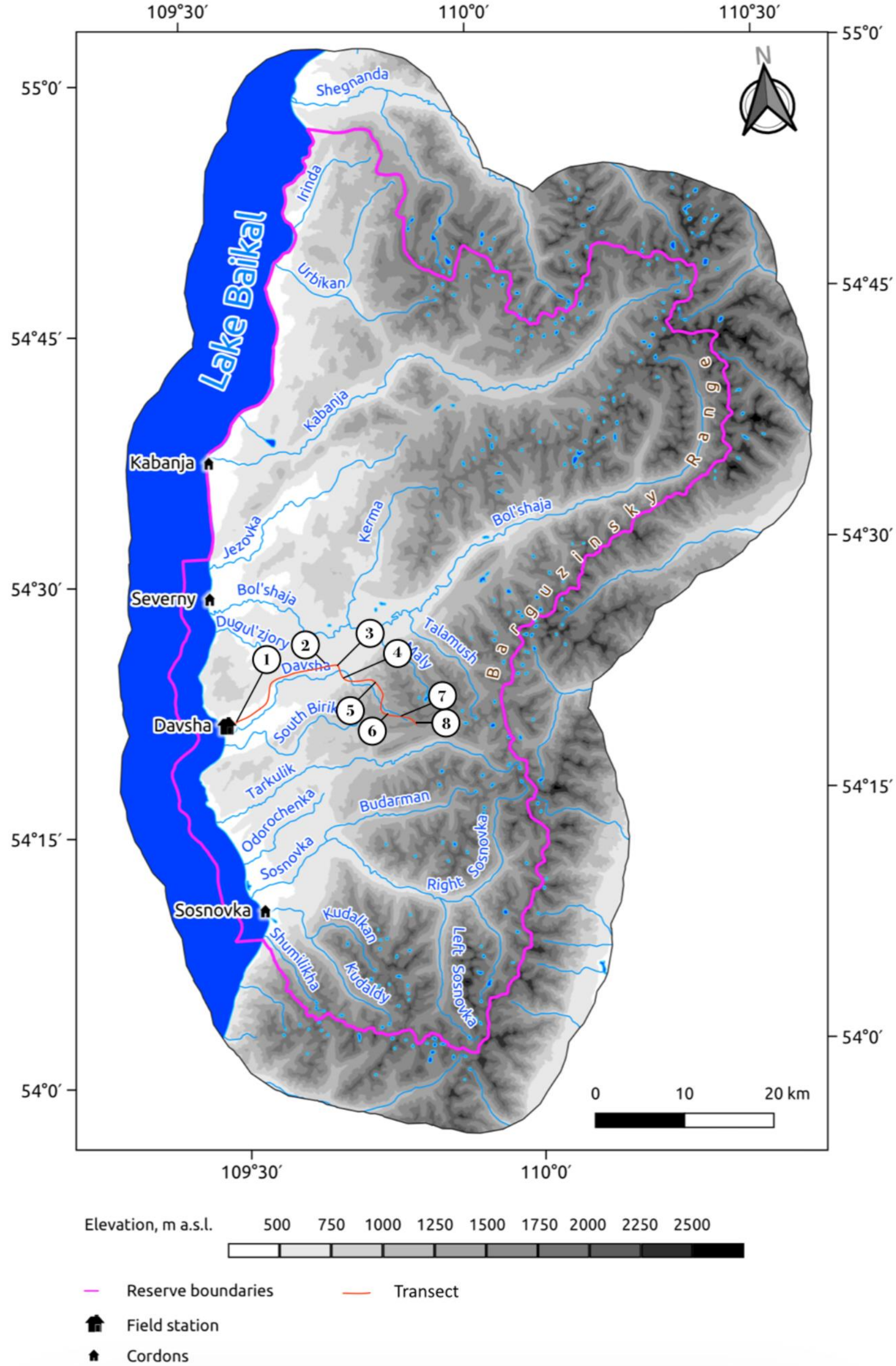

Fig.1. Location of entomological sites on the high-altitude transect of the Barguzinsky ridge:
1 – Bilberry cedar, 2 – Grass birch, 3 – Blueberry larch, 4 – Red bilberry pine, 5 – Bergenia aspen, 6 – Bilberry abies, 7 – Sparse birch woodland, 8 – Lichen tundra.

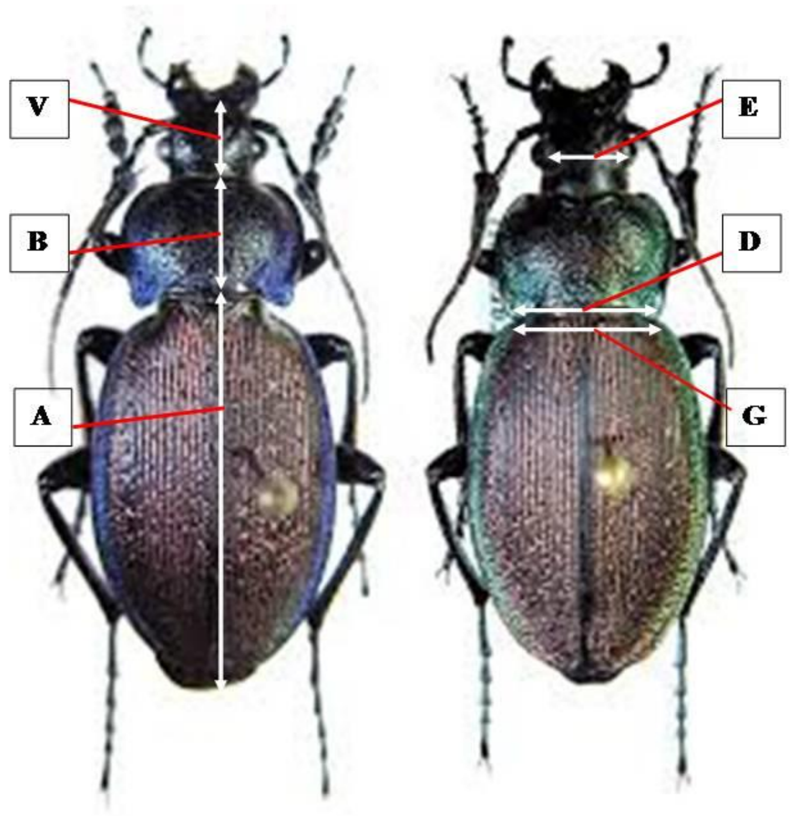

Fig. 2. Measured morphometric features *C. odoratus:*
A –length of the elytra, B –length of the pronotum V– length of the head, G– width of the elytra, D – width of the pronotum, E – the distance between eyes.

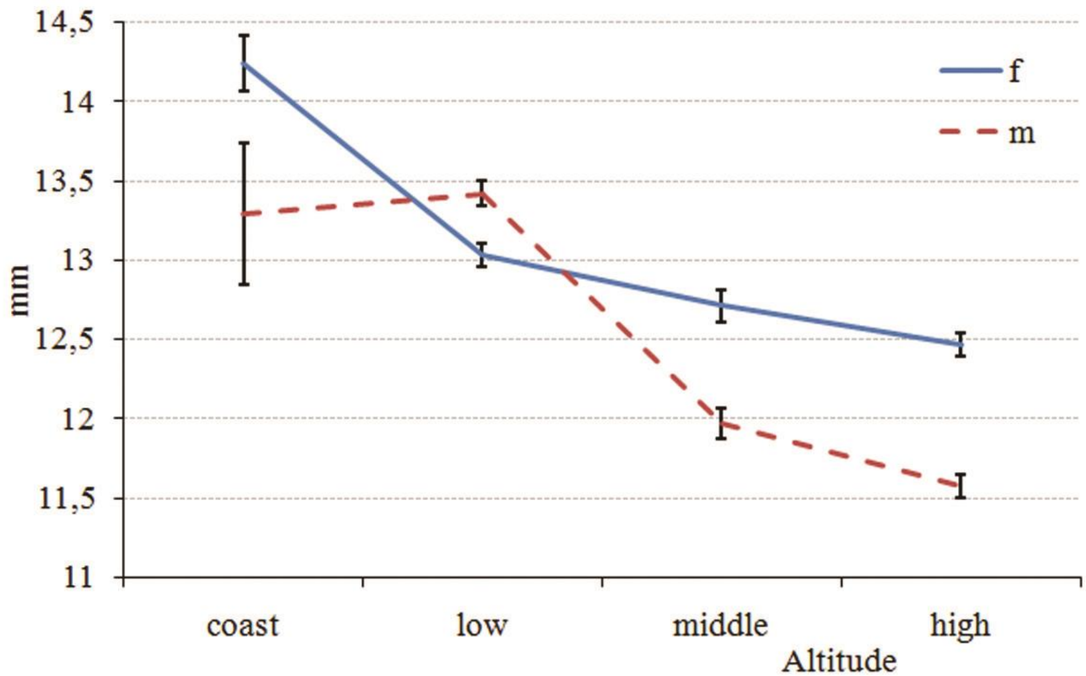

Fig. 3. Elytra length variation in *C. odoratus*

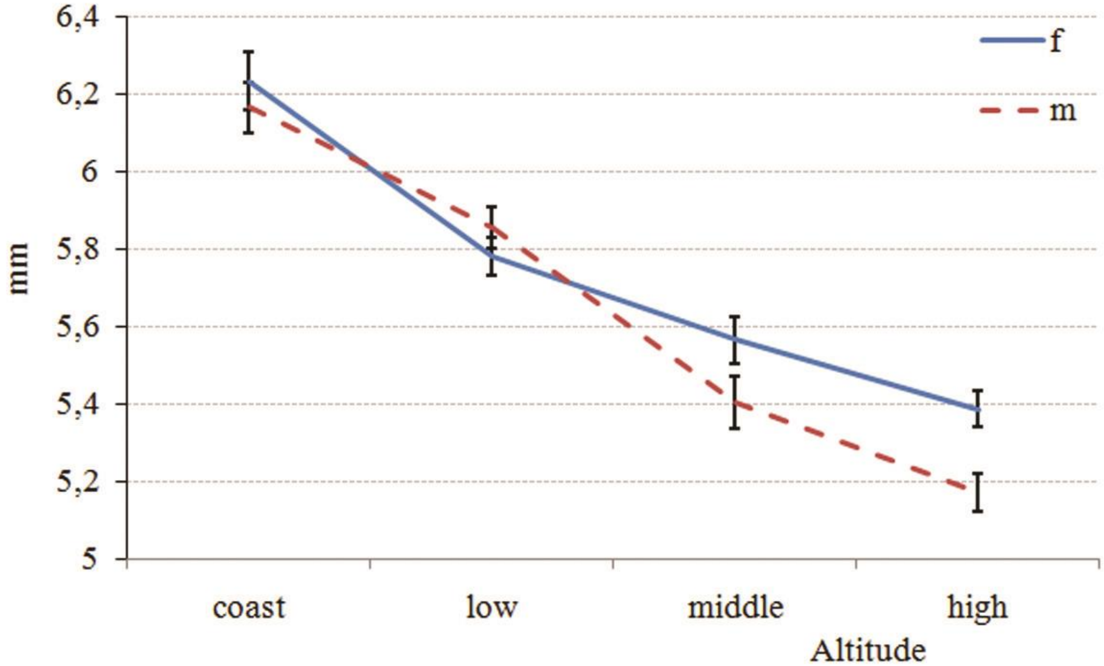

Fig. 4. Elytra width variation in *C. odoratus*

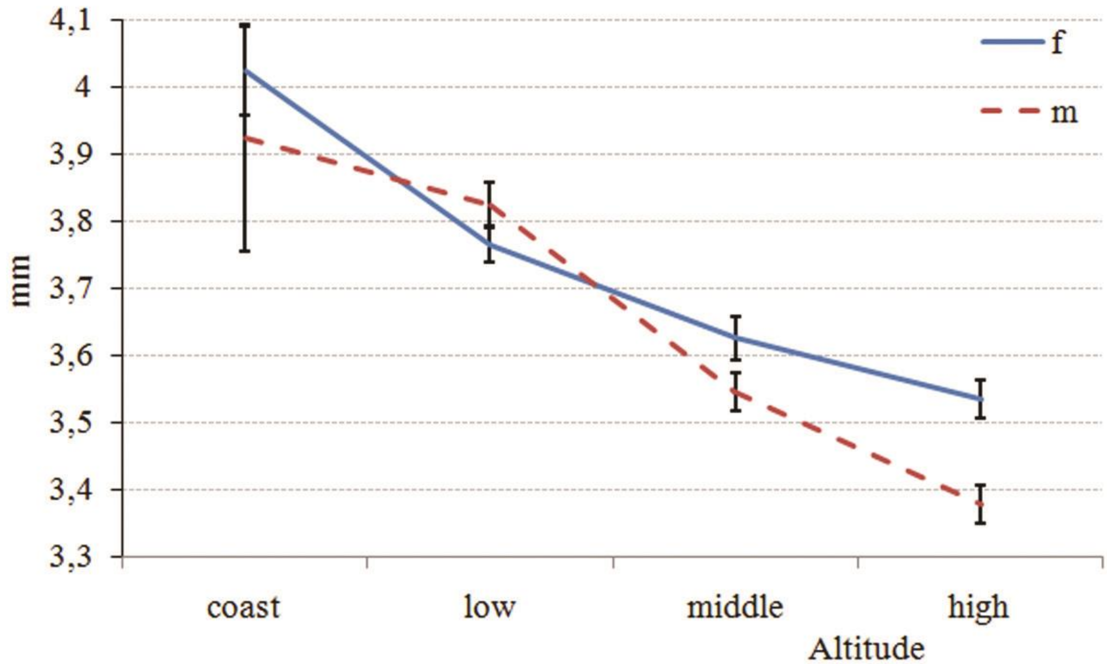

Fig. 5. Pronotum lengh variation in *C. odoratus*

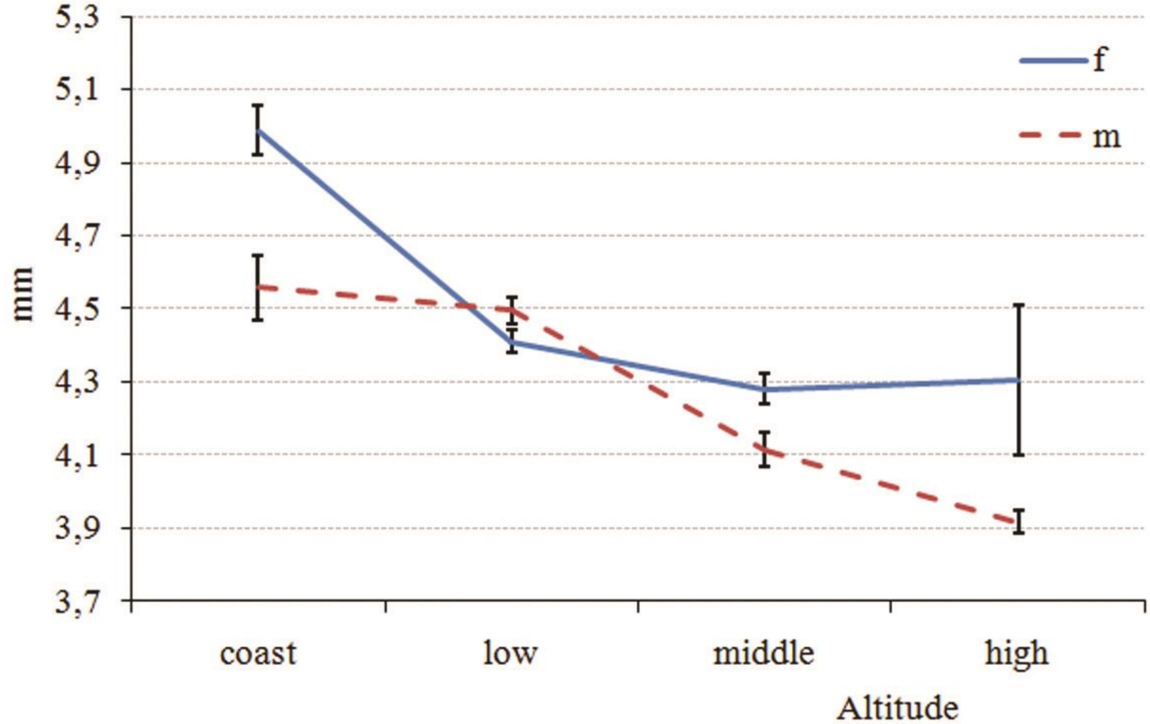

Fig. 6. Pronotum width variation in *C. odoratus*

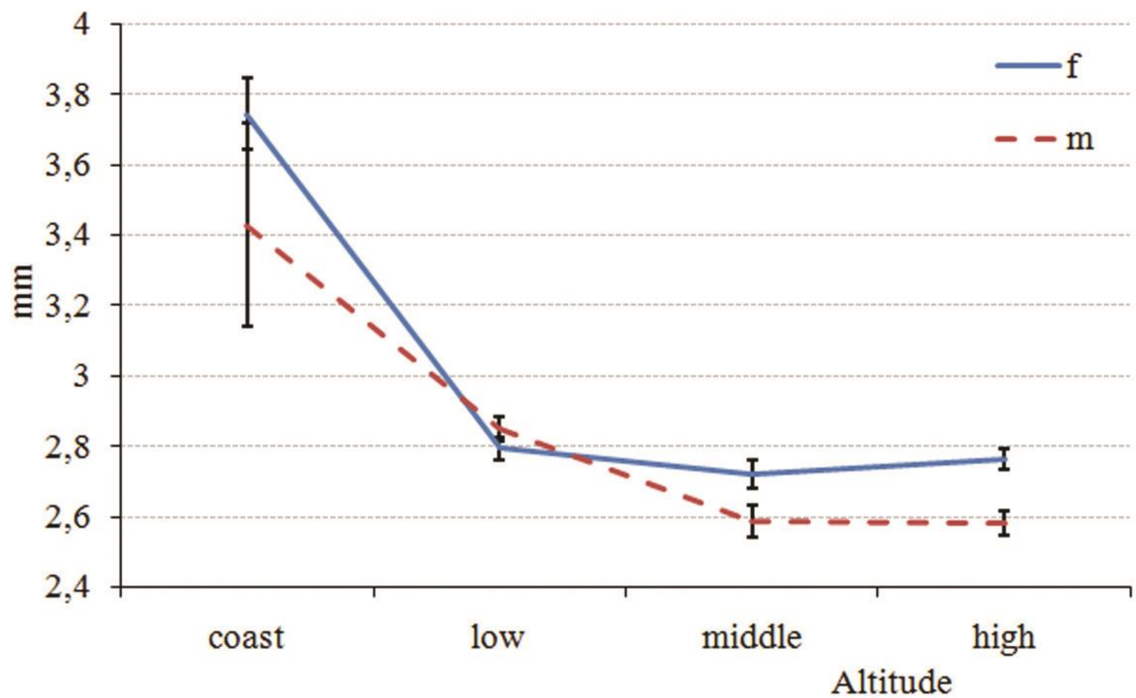

Fig. 7. Head length variation in *C. odoratus*

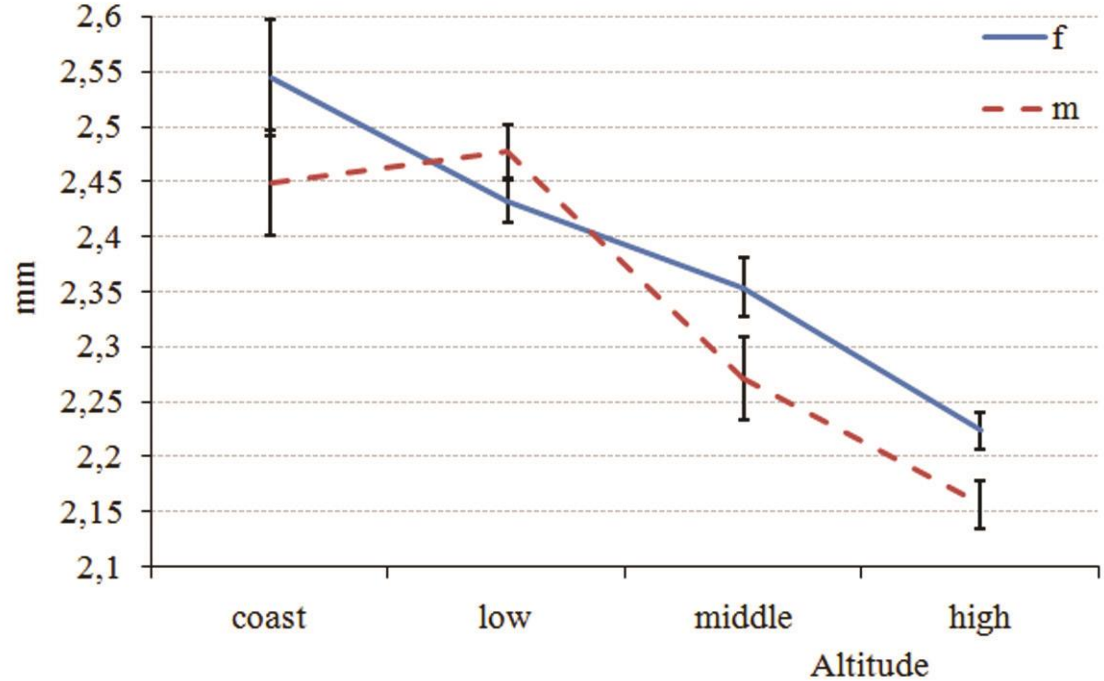

Fig. 8. Distance between eyes variation in *C. odoratus*

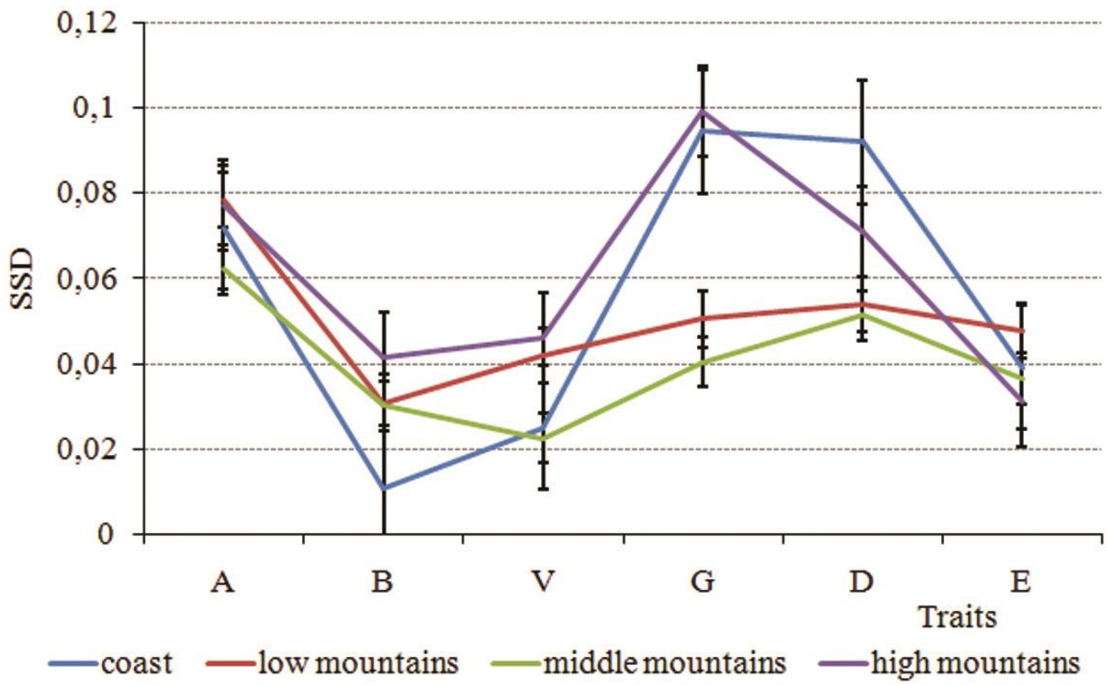

Fig. 9. Sexual Size Dimorphism values in different traits at different altitude in *C. odoratus*

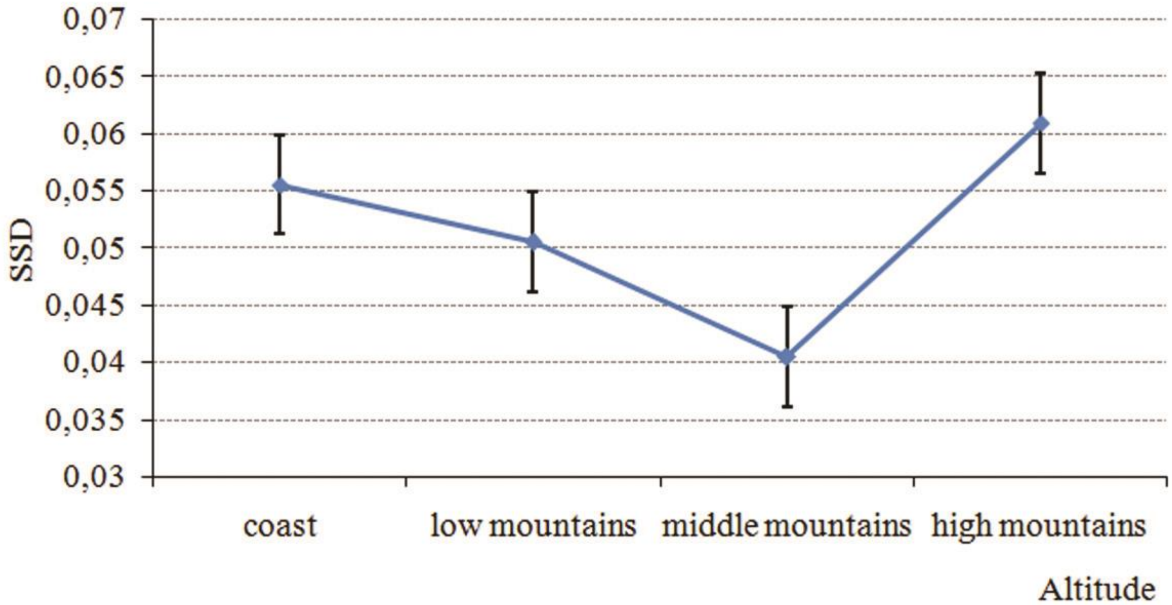

Fig. 10. Mean values of Sexual Size Dimorphism over 6 traits in *C. odoratus*

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DISEASES AND INSECT PESTS PROBLEMS IN NURSERIES STAGE ON SOME FOREST TREE SPECIES OF MYANMAR REFORESTATIONS AND REHABILITATION PROGRAMMEE (MRRP)

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Fungal pathogen, insect pest, obligate parasite, severity

ABSTRACT

This study was carried out to assess some diseases and insect pests nurseries problems; incidence and severity of diseases and insect pests in four nurseries under the Myanmar Reforestation and Rehabilitation Programmee (MRRP). In this study, three kinds of fungal diseases; seedling blight, teak leaf rust and pine needle blight were found and also three kinds of insect pests: sap sucking, leaf defoliator and shoot borer were collected respectively in seedling Tectona grandis L.f., Pinus kesiya Royal ex Gordon, Xylia xylocarpa Taub., Hopea odorata Roxb. , Bruguiera hainesii C.G.Rogers, Xylocarpus moluccensis (Lam.) M.Roem. and Avicennia officinalis L.. In laboratory examinations, an obligate parasite Olivea tectonae and five facultative parasites Rhizoctonia solani, Fusarium solani, Aspergillus sp., Colletotrichum sp., and Mycosphaerella sp. were identified. The insect pests species, Calliteara sp., Cenopic sp., Hyblaea puera, Hypsipyla robusta and unidentified species (Thripidae) were recorded. According to the results, teak leaf rust, teak blight, pine needle blight and sap sucking insects were high percentage of incidence and severity rate. It can be concluded that the diseases mortality rate were higher than the insect pests except the sap sucking insects. This result showed that nurseries are less resistance than plantations and can spread disease more quickly and increase losses of seedling in the event of fungal infestations. Therefore, nurseries should be more cared according to the nurseries management procedures. This results to be provide the nurseries management information to the MRRP Project as well as other forest nurseries.

1. INTRODUCTION

The diseases and insect pests are invading forests plantations and nurseries of Myanmar in a variety of ways (Mead, 2001). Some micro-organisms are very virulent and can attack and invade healthy plants and some diseases spread and develop quickly within a plant (Cynthia, 2006). Abiotic casual organisms can cause a large number of rapidly growing seedlings to fungal attack and young seedlings are an important first step in establishing a plantation (Landis 1984, 1989).

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Therefore, there is a need to assess the diseases and insect pests' infestations in nurseries. If preventive measures are not taken in nursery, unforeseen losses can occur. This study is the first step to list the spread of diseases and insect pests for these purposes and to record incidence and severity of diseases and insect pests in each nursery within six zones in MRRP Project.

2. MATERIALS AND METHODS

Study Sites and Study Periods

The experiment was carried out in the four nurseries within the six zones under the ten-year Master plan of

Myanmar Reforestation and Rehabilitation Programmee (MRRP). The survey was conducted intensively, the number of diseases and insect pests were recorded within two years from 2018 to 2020.

Photography

To study the symptoms of the diseases and insect pests, photographs were taken as far as possible of fresh diseased specimens.

Specimens Collection

Diseases and insect pests specimens were collected in the field using paper bags and plastic bottles for laboratory diagnoses.

Sterilization of Lab Apparatus and Media

Potato, Dextrose Agar (PDA) boiled for the nutrient media about 15 minutes. PDA media and lab instruments were sterilized in the autoclave Pressure 120 lb, for 20 minutes. Then all of cleaning Petri dishes were sterilized in the oven temperature 60 C ˚ for 4 hours.

Isolation and Identification

To avoid any saprophytic growth on the specimens, isolations were made within one week after the collection. Only under unavoidable situations the specimens were stored in a refrigerator. Using the Koch's Postulate, the infected parts of seedlings were washed under the tap water, again sterilized by mercuric chloride ($HgC1_2$) (1.1) g for 1 minute and then washed by double distilled water (DDW) for 1 minute. Then those were isolated on the media at the laminar flow chamber.

Isolated Petri dishes were first examined under a dissecting microscope. To study the detailed structure of fructifications of various pathogenic fungi (conidia, conidiophore, pycnidia, perithecia, rust sori, etc.) were slide preparation stained with Lactophenol Cotton Blue and also the covered glass was sealed with colorless nail polish. Photomicrographs of fungal species were taken using a Nikon Cool Pix B700 digital camera. Fungi were identified to genus level according to various mycological references;

Insect Pests Identification

Rearing the insect pests larvae was done under the laboratory conditions (room temperature about 25 ± 1º C and R.H. (65 ± 5 %) at the Entomology Section of FRI. Collected insect larvae were individually placed in separate glass bottle with specific leaves as food. Fresh leaves were provided every day until the pupate and adults. The adult emerged within fifteen days and then identification were carried out.

Survey Design and Data Formulation

Five nursery plots were selected in each nurseries using the Systematic Sampling Method, 100 sample seedlings were collected from each five selected nursery plots using the Random Sampling Method, to calculate the percentage incidence (PI) of diseases and insect pests. Moreover, 25 sample seedlings were collected again from each of 100 seedling using the Random Sampling Method, to calculate the severity index (SI). Used of the formulas are as follow:

- PI = Nd x 100 /N, where Nd for number of affected and N the total number of trees,
- SI = n L x 1 + n M x 2 +n S x 3 / N, where nL, nM, nS represent total number of plants with Low1-25%, Medium 26-50%and Severe 51–75 or > 25% seedlings dead; 1, 2, 3 severity index (SI) for Low, Medium and Severe and N the total number of trees (Sharma et al. 1985).

3. RESULTS AND DISCUSSION

Recorded Diseases and Insect Pests of Individual Tree Species

In this experiment, three kinds of fungal disease symptoms and three kinds of insects pest symptoms were observed from seven kinds of tree species (Table-**1)**. In this experiment, in insect identification, unidentified species (Thripidae), *Calliteara* sp. (Lymantridae), *Hyplaea puera* (Hyblaeidae), *Cenopic* sp. (Tortricidae) and *Hypsipyla robusta* (Pyralidae) were observed. In fungal diseases, *Collectrichum* sp., *Rhizoctonia solani*, *Fusarium* solani, Aspergillus sp., *Olivea tectonae*, and *Mycosphaerella* sp. were identified respectively. In this table show that the sap sucking insect, teak blight, teak leaf rust and pine needle blight were serious among all infestations. However, some other diseases and insect pests were potential serious in nursery. The percentage of incidence and severity rate of disease and insect pest infestations are showed as following graphs (Fig.1,2). This figure showed that the sap sucking insect pests on *Xylia xylocarpa* seedling were most serious among those insect infestations. The incidence and severity rate of this insect was (61%,33%). Moreover, insect pest infestations were more observed than the diseases in mangrove species in this experiment.

This figure showed that teak blight, teak leaf rust and pine needle blight were serious diseases in nursery. Their incidence and severity rate were (61%,33%), (64%,25%) and (65%,61%) respectively. The seedling blight of seedling *Avicennia officinalis* was potential serious in this experiment.

4. CONCLUSION

In this study, the incidence and severity rate of diseases were higher than the insect pests in nursery except the sap sucking insect pests. This result showed that nurseries are less resistances than plantations and can spread the disease more quickly and increase losses of seedling in the event of fungal infestations. Therefore, nursery in charges needs to care the nurseries according to the nurseries management procedures.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Recorded Tree Species, Diseases/Insect Pests and their Nature of Damage

Sl. No.	Tree species/ Common Name	Disease Symptom	Insect Pest Damage Symptom	Nature of Damage
1.	*Xylia xylocarpa* (Pyinkado)	-	Sap Sucking Insect	Leaf
2.	*Hopea odorata* (Thingan)	-	Leaf Defoliator	Leaf
3.	*Avicennia officinalis* (Thamegyi)	Seedling Blight	Leaf Defoliator	Leaf /Stem
4.	*Bruguiera hainesii* (Myinggyi)	-	Leaf Defoliator	Leaf
5.	*Xylocarpus moluccensis* (Kyana)	-	Shoot Borer	Shoot
6.	*Tectona grandis* (Teak)	1. Teak Blight 2. Teak Leaf Rust	-	1. Leaf/Stem 2. Leaf
7.	*Pinus kesiya* (Pine)	Pine Needle Blight		Needle

Table 2. Recorded Fungal Pathogens, Insect Pests, their Incidence, Severity and Status

Sl. No.	Tree Species and Disease & Insect Pest Name	Fungal Pathogens (Species)	Insect Pests (Species & Family)	PI %	SI %	Disease & Insect Pest (Status)
1.	*Xylia xylocarpa* (Sap Sucking Insect)	-	Unidentified Species (Thripidae)	75	31	Serious
2.	*Hopea odorata* (Leaf Defoliator)	-	*Calliteara* sp. (Lymantridae)	4.8	10.13	Potential Serious
3.	*Avicennia officinalis* (Leaf Defoliator)	-	*Hyplaea puera* (Hyblaeidae)	10.5	16.2	Potential Serious
4.	*Bruguiera hainesii* (Leaf Defoliator)	-	*Cenopic* sp. (Tortricidae)	14.6	27.2	Potential Serious
5.	*Xylocarpus moluccensis* (Shoot Borer)	-	*Hypsipyla robusta* (Pyralidae)	5	12.10	Potential Serious
6.	*Avicennia officinalis* (Seedling Blight)	*Collectrichum* sp. *Rhizoctonia solani*	-	4	8.15	Potential Serious
7.	*Tectona grandis* (Teak Blight)	*Fusarium solani* *Aspergillus* sp.	-	61	33	Serious
8.	*Tectona grandis* (Teak Leaf Rust)	*Olivea tectonae*	-	64	23	Serious
9.	*Pinus kesiya* (Pine Needle Blight)	*Mycosphaerella* sp.	-	65	61	Serious

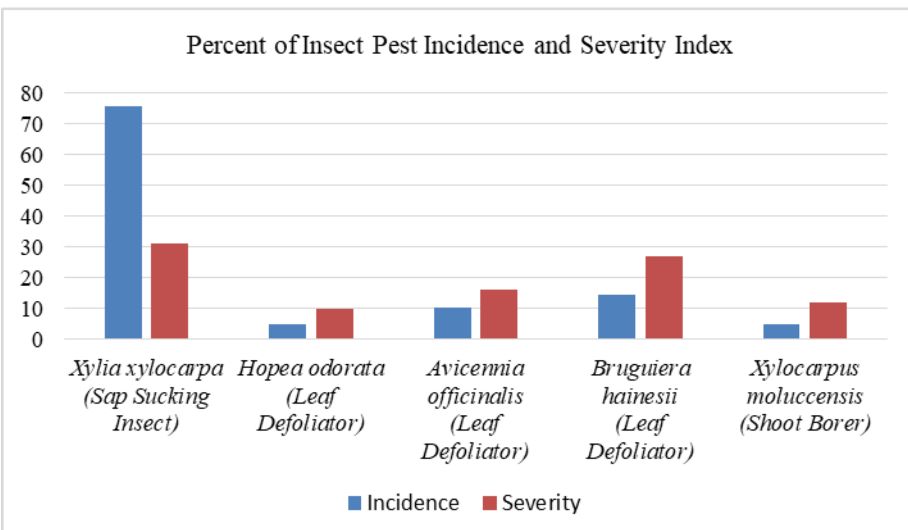

Fig. 1. Incidence and Severity rate of Insect Pests in Different Species of Seedlings

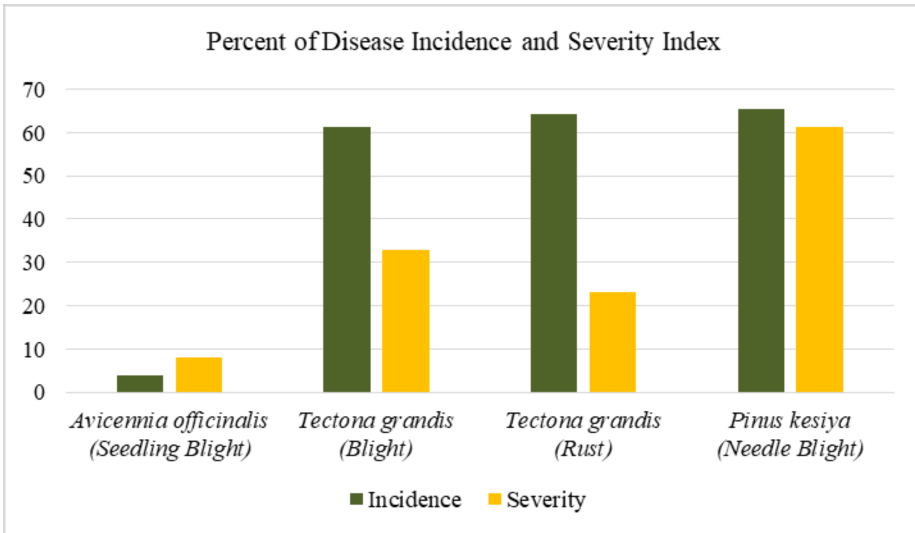

Fig. 2. Incidence and Severity rate of Diseases in Different Species of Seedlings

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EFFECT OF STOCKING DENSITY ON SURVIVAL RATE AND LARVAL DEVELOPMENT OF THE GIANT FRESHWATER PRAWN, *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

A pilot study was carried out to observe the larval development and survival rates of the giant freshwater prawn, *Macrobrachium rosenbergii*, cultured under different culture volume. The study was conducted for 6 weeks in four concrete tanks of size 1.5 ton each at Biotechnological research Department, Yangon. They were fed 3 meals per day with A*rteria naupii* as live food and egg custard as prepared food. The giant freshwater prawn larvae were stocked with the stocking density of 50000 pieces in each of 500 L capacity tanks (100pcs /L stocking rate) as experiment 1 (T-I and T-II) and 1000 L tanks (50pcs/L stocking rate) as experiment 2(T-III and T-IV). The survival rates of the giant freshwater post larvae were obtained as 21% in T-I and 22% in T-II. The survival rates were obtained respectively as 26% in T-III and 25% in T-IV.

1. INTRODUCTION

Freshwater prawn farming is suitable for tropical or subtropical climates. They are reared in a variety of freshwater enclosures, including tanks, irrigation ditches, cages, pens, and reservoirs, although the most common enclosures are earthen ponds, which typically supply the best yields for commercial farming. Farming prawn shares many similarities with marine shrimp operations. The giant freshwater prawn is a valuable aquatic food source, high in protein and commands a good export markets (Brief notes on the giant freshwater prawns as an object of farming by (V.Soesanto 1980). It has become the main target commodity for freshwater aquaculture in Myanmar.

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There are 150 species of *Macrobrachium* in the world (Kumer and Pandy 2003), of which 49 are commercial (Laleh Abbaspour Davassi 2011). Twenty-seven of the commercial species are found in Asia and the Pacific by Holthuis (1980). They are commercially important in terms of capture fisheries and culture industries. It is considered as a suitable candidate for both tropical fresh and brackish water culture (Yutaka, U. and Soo, K.C. 1963). In Myanmar, there are 17 species of native freshwater prawn under the genus *Macrobrachium* (Yixiong Cai and Peter K.L.Ng 2002), they are commercially important in terms of capture fisheries and culture industries. Among them, the giant freshwater prawn (*Macrobrachium rosenbergii* de man) and the Monsoon River Prawn (*Macrobrachium malcomsonii*, H Mile Edward) are the most valuable species. Because of the larger in size and first fast growth they have the main target commody for freshwater aquaculture industries and one of the major earner of foreign exchange.

Culture of giant freshwater prawn culture (*Macrobrachium rosenbergii)* was initiated during the period from 1974 to 1980 in Myanmar. As the pond culture of prawn developed, the demand of the fry increased. Myanmar has a long coastline of nearly 3, 000 km. Myanmar has many brackish water rivers and fresh water rivers, several large estuaries, delta system and numerous off shore islands

Aquatic resource area of the river systems within Myanmar encompasses 8.2 million ha (FAO, 1996) of permanent and seasonal water bodies and there were 29 000 ha of freshwater fishponds and a further 40 716 ha of shrimp ponds in 2001, and 115687 ha of reservoirs. The Department of Fisheries (DOF) in Yangon estimates a figure of six million ha of floodplains, which likely excludes river area and floodplain lakes. In coastal area, so-called unused lands can be used for aquaculture. Shrimp culture ponds can develop in this area. In the year 2004-2005 shrimps ponds area was about 63 000 hectares in the coastal region.

Myanmar tiger prawn culture situations was turns to fail. In the other hands Myanmar tried to substitute, white shrimp in place of tiger prawn in 2004. Nowadays, white shrimp was cultured in both marine and fresh water in the world. Myanmar has more changes than others because of her plenty of verging land and fresh and brackish water area.

In recent years when the higher income are derived from the harvested freshwater prawn, many farmers have converted into freshwater prawn ponds. For the prawn ponds, seed fry are collected from the hatcheries. Therefore ,the expansion of the freshwater prawn farming industries are still restricted due to in sufficient and inconsistent supply of the seed fry for the grow-out culture. For the development of a prawn farming industry, human resource development is essential in Myanmar. The scientists and highly qualified technicians as well as skilled and semi-skilled labors are a must and essential for sustainable production of an aquaculture industry. The result of the present research experiments on the larval rearing of giant freshwater prawn (*Macrobrachium rosenbergii)* in different water volume under laboratory conditions will hoped for the fulfilment in technology transferred to the rural farmers. Also, there may be hoped the development of seed fry production of giant freshwater prawn in rural areas. Finally, there may be hoped for the helpful in aquaculture development in Myanmar.

2. MATERIALS AND METHODS

Matured specimens were collected from estuary of Yangon River, Letkokkon Township. They were carried by placing in Styrofoam box with river water and provided aeration. After reaching the hatchery, the water temperature (27℃-29.8℃) was acclimatized for 30-45 minutes in original transportation Styrofoam box. After acclimation, the breeders were transferred into the aerated glass aquarium for spawning and hatching. After spawning and hatching, the larvae were harvested and rinsed with clean brackish water and transferred into the aerated plastic basin for counting. For tank preparation, the reservoirs and culture tanks were washed and dried for storage, sedimentation and mixing. For water preparation, brackish water (12ppt) was disinfected with 65% chlorine at 10ppm concentration. After chlorination, brackish water was pumped through a filter bag of 3-5 micro mesh into the larval rearing tanks and treated with Furazan Gold at 1-2 ppm concentration. And it was with provided with aeration for 24-48 hours and treated with EDTA at 1-2 ppm concentration.

During the experiments, two different types of feeds, Artemia nauplii and egg custard, were used in feeding of larvae. INVE brand of Artemia cysts were used for Artemia nauplii . The ingredients of egg custard were number of chicken egg, 10gm of milk power, 15 gm of wheat flour and one capsule of cod liver oil. Artemia nauplii was twice a day as initial feeding. The feeding amount of *Artemia nauplii* was 1-3 individuals per ml. After 10 days of stocking, *Artemia nauplii* were increased to 3-5 individuals per 1ml. In the post larval stage 1, the feeding amount of *Artemia nauplii* was decreased to 1-2 individuals per 1ml. Egg custard as supplemental food was fed according to the growth stages of larvae 3 times a day.

For the study of effect on different volume of brackish water in growth and survival rate of giant freshwater prawn larvae under Laboratory condition, the duplicated experiments had been conducted with the same stocking density.

For experiment 1, the stocking rate was 100pcs per 1 liter in tank I and tank II with 500liters water. For experiment 2, the stocking rate was 50pcs per 1 liter in tank III and tank IV with 1000 liters water.

Before the stocking of larvae, the tanks will filled with 50% of culture capacity and provided with continuous aeration. After stocking of larvae, the required amount of water was added daily until the desired capacity of 500 liters and 1000 liters respectively. After five to six days of culture experiment, daily were exchanged with 5-6 % of water. Depending on the condition of water, 7-8% of water was exchanged. During the study period, temperature, pH.DO, salinity and ammonia were recorded 27.0℃-29.8℃, 7.8-8.3, 8.1ppm-8.3ppm, 12-13ppt and 0.01-0.03ppm respectively.

The survival and chronological growth stages and health of the larvae were checked daily by microscopic examination and random samples examination at morning and evening.

After 42 days of culture experiment, the larvae of giant freshwater prawn at larval stages were harvested and counted by volume and estimation method. Then, calculated the survival rates of larvae from each culture tanks.

3. RESULTS

In experiment 1, the larvae stages 1-2 were stocked in the two tanks with 500liters .The stocking rate was 100pcs /L. After 42days, the post larvae were harvested and estimated number as 10500pcs and 11000pcs in the culture tank -I and tank -II respectively.

In experiment 2, the larvae stages 1-2 were stocked in the two tanks with 1000liters .The stocking rate was 50pcs /L. After 42 days, the post larvae were harvested and estimated number as 13000pcs and 12500pcs in the culture tank- III and tank -IV respectively.

The result show that the survival rates were 21% for tank-I, 22% for tank-II, 26% for tank-III and 25% for tank-IV. The lower survival was observed in higher stocking rate. During the operation, temperature, pH, DO, salinity and ammonia were recorded as the range between 27.0°C-29.8°C, 7.8-8.3, 8.1ppm-8.3ppm, 12-13ppt and 0.01-0.03ppm respectively. The water quality parameter resulted in culture tanks during the experiment are expressed in Table-1. No significant variations in the values of each parameter was found among treatments. Developmental stages are also described in Table-2.

4. DISCUSSION

Stocking density has the direct effect on growth and at the same time survival and production (Siddiqui and Al-Hinty, 1993). Stocking densities of *M. rosenbergii* and exogenous factors such as water temperature, seed availability, stress free environment, water quality and feed quality, quantity can affect the result of growth and survival (Baskerville-Bridges and Kling, 2000). Earlier works to optimize stocking density in polders (Kurup et al. 2002), river pens (Son et al. 2005), cages (Cuvin-Aralar et al. 2007) and in polyculture ponds (Hossain and Islam 2006; Marques et al. 2010) suggest that the most important factor for viable freshwater prawn culture is stocking density. The findings of Alikunhi, (1957); Kawamoto et al., (1957) and Haqueet al., (1984) who stated that, the growth rate is high in low density. Reduction in stocking rate resulted in increased weight of prawns and the proportion of marketable yield (Roberts and Bauer, 1978).

The present study, the result on the effect of different volume of brackish water showed that the survival rate of freshwater prawn (*Macrobrachium rosenberghii*) was 26% and 25% in 1000 liters culture capacities of respective experiments and 21% and 22% in 500 liters of respective experiments. Optimal water quality is crucial to all types of aquaculture production and needs to be maintained during the culture period. The variations in the parameters found to be negligible and thus assumed to have no direct impact on the survival and production. According to this result, the survival rates of post larvae in greater volume was higher than of post larvae in less volume in the same stocking density under laboratory conditions due to the availability of adequate space and adequate feeding to grow prawn.

From the result and information, there may be concluded that the research works were successfully achieved for technology to know how giant freshwater prawn post larval production.

5. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Range and average value (Mean±S.D) of water parameters in tanks during rearing

Water parameter	Experiment(1)				Experiment(2)			
	Tank I		Tank II		Tank III		Tank IV	
	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD
Temp: (℃)	27.2-29.8	28.24±0.646	27-29	28.23±0.557	27.3-28.9	28.07±0.326	27-28.5	28.08±0.287
PH	7.8-8.3	8.04±0.157	7.8-8.3	7.89±1.238	7.8-8.3	8.09±0.148	7.8-8.2	8.11±0.108
Salinity (ppt)	10-13	11.87±0.503	10-13	11.94±0.707	10-13	11.91±0.582	10-13	28.08±0.287
DO	8-8.2	8.07±0.078	8-8.3	8.14±0.121	8-8.3	8.1±0.112	8-8.3	8.15±0.11
ammonium	0.02-0.03	0.02±0.005	0.01-0.03	0.02±0.007	0.01-0.03	0.02±0.008	0.01-0.03	0.02±0.007

Table 2. Developmental stages during the larval development of river prawn *Macrobrachuim Rosenbergii* during the culture.

Experiment - 1				Experiment-2			
Tank-I		Tank-II		Tank-III		Tank-IV	
Cycle Days	Stages	Cycle Days	Stages	Cycle Days	Stages	Cycle Days	Stages
1	I	1	I	1	I	1	I
2	II	2	II	2-3	II	2-3	II
3	III	3-4	III	4-5	III	4	III
4-5	IV	5-6	IV	6-8	IV	5-8	IV
6-8	V	7-9	V	9-11	V	9-12	V
9-13	VI	10-15	VI	12-15	VI	13-17	VI
14-18	VII	16-21	VII	16-18	VII	18-20	VII
19-21	VIII	22-25	VIII	19-21	VIII	21-23	VIII
22-23	IX	26-27	IX	22-24	IX	24-25	IX
24-30	X	28-30	X	25-27	X	26-27	X
31	XI	31	XI	28	XI	28-29	XI
32	PL-1	32	PL-1	29	PL-1	30	PL-1

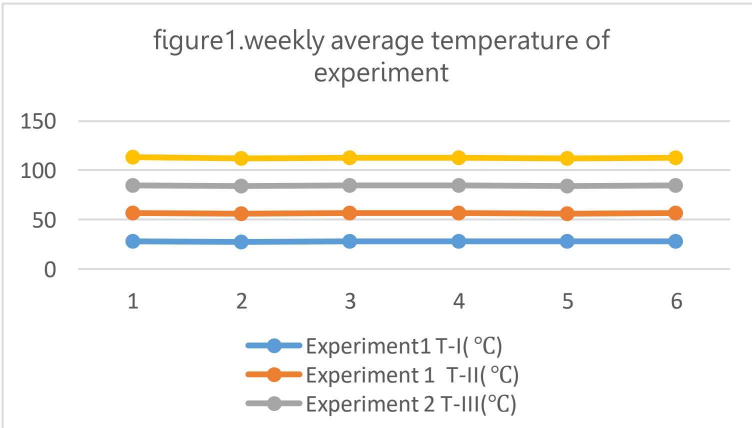
figure1.weekly average temperature of experiment
150
100
50
0
1 2 3 4 5 6
Experiment1 T-I(°C)
Experiment 1 T-II(°C)
Experiment 2 T-III(°C)

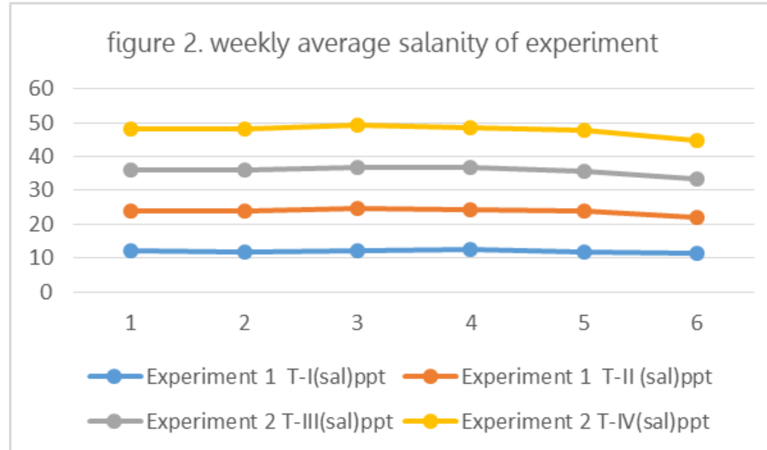
figure 2. weekly average salanity of experiment
60
50
40
30
20
10
0
1 2 3 4 5 6
Experiment 1 T-I(sal)ppt
Experiment 1 T-II (sal)ppt
Experiment 2 T-III(sal)ppt
Experiment 2 T-IV(sal)ppt

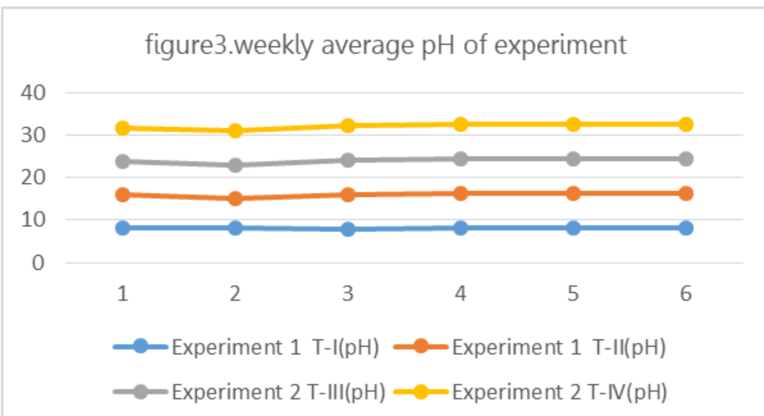
figure3.weekly average pH of experiment
40
30
20
10
0
1 2 3 4 5 6
Experiment 1 T-I(pH)
Experiment 1 T-II(pH)
Experiment 2 T-III(pH)
Experiment 2 T-IV(pH)

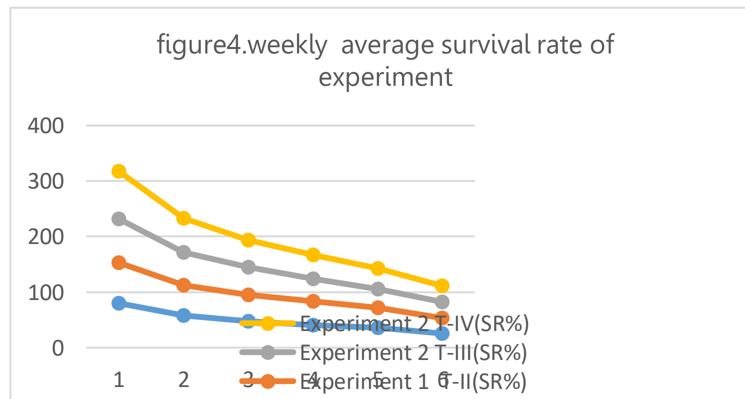
figure4.weekly average survival rate of experiment
400
300
200
100
0
1 2 3 4 5 6
Experiment 2 T-IV(SR%)
Experiment 2 T-III(SR%)
Experiment 1 T-II(SR%)

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METALS MIXTURE (ZINC+LEAD) INDUCED CHANGES IN MOVEMENT PATTERNS OF *CIRRHINA MRIGALA*

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Fish, metal mixture, behavior, toxicity

ABSTRACT

The widespread use of metals for industrial and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices. Metal contamination of surface waters has been well documented worldwide and constitutes a major issue that gives rise to concerns at local, regional, national and global scales. Metals have great impact on the behavioral parameters of fish. Therefore, in present research work was designed to evaluate the behavioral changes in fish, *Cirrhina mrigala* exposed to 96-hr LC_{50} concentration of zinc(Zn)+lead(Pb) of mixture for 4 days. Results showed that the exposure of Zn+Pb mixture increased the jumping, equilibrium status, opercula movement, fin movement, convulsion, hyperactivity and surfacing frequency of fish as compared to control group. It was also concluded that metals exposure altered the behavior of fish.

1. INTRODUCTION

Heavy metals are present naturally in the environment; however, as a consequence of industrial, agricultural, and anthropogenic activities levels of them are increasing rapidly. Heavy metals at high concentrations can cause hazardous effects to many aquatic organisms by changing genetic, metabolic, physiological, biochemical, and behavioral parameters (Atli and Canli, 2007; Ramesh et al., 2009). Fish are most susceptible to the adverse impacts of polltants and provide as an excellent implement for understanding the health condition of aquatic ecosystem (Lushchak, 2008 2011). Fish can uptake heavy metals from the adjacent environment and add them in its different organs and tissues resultant into biomagnifications in the food chain make the fish an important marker of metallic ions pollution (Abdullah *et al.*, 2007).

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Essential heavy metals like zinc, chromium, copper, nickel, molybdenum, iron and cobalt take part an important role in numerous biological processes. However, the necessary metals, when set up at higher concentration can cause toxic effects on the organisms (Sivaperumal *et al.*, 2007). Zinc (Zn) is one of the most important essential trace elements involved in animal growth and the most widely used metal cofactor of many enzymes involved in protein, nucleic acid, carbohydrate, and lipid metabolism (Carpene et al. 2003; Sun et al. 2005). Zinc in certain concentration is desirable for fish growth but its over accumulation is hazardous to exposed fish (Murugan et al. 2008). Zinc is one of the most common contaminants in aquatic systems and is associated with urban runoff, soil erosion, industrial discharges, pharmaceuticals, pesticides and a variety of other activities and sources (Schmitt 2004; Bowen et al. 2006).

The danger of Zn is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically and is only transformed from one oxidation state or organic complex to another (Everall et al. 1989). Lead is extremely toxic metal as it is reported to be accountable for death or sub-lethal changes in reproduction, enlargement and act of the fish (Ramsdorf *et al*., 2009). Lead is one of the most injurious contaminants of aquatic environment .The chief sources of lead contamination are removal and smelting of lead ores, industrial effluents, fertilizers, pesticides and community sewage wastes (Needleman, 2006). Lead may penetrate into the fish body throughout different routes i.e., skin, gills and respiratory region (Olaifa et al., 2004) .Once immersed, lead becomes dispersed in the liver, kidney, gills, heart, gonads with the blood of fish (Astdar, 2005). The studies on fish behaviors provide a lots of knowledge and information because, any behavior alteration can be related to physiological biomarker in aquatic species (Amiard-Triquet, 2009). For example, the monitoring of behavioral response becomes an imminent option to environmental change, disease, stress and the presence of toxic compound in water, which most of this condition initiates the variation of fish behavior (Almazan-Rueda *et al.*, 2004; Gerhardt, 2007).

Fish behavior represents the fish physiological response towards the environmental factor. Moreover, the coordination of fish behavior related to the ecology can be easily observed even if it can quantified (Scott and Sloman, 2004) and at the same times effecting the consequences of metal toxicity upon the concentration and species, including size (Vosyliene *et al.*, 2003; Hussain *et al*., 2011). For instance, the existence of metal ion in the environment mediation increased the mucus like secretion from gill, excessive excretion, anorexia and also the fin movement (Ezeonyejiaku *et al*., 2011). Therefore, present works was designed to see the behavioral changes in *Cirrhina mrigala* due metals mixture exposure.

2. MATERIALS AND METHODS

The experiment was conducted with *Cirrhina mrigala* at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. Acute exposure of zinc (Zn) and lead (Pb) mixture was given to *C. mrigala* for 4-days. Prior to experiment, fish fingerlings were kept in laboratory conditions for two weeks for acclimatization. After acclimation period, fish were transferred to 70-liter glass aquarium for oxidative stress studies. Ten fish were kept in each aquarium. The water temperature (30ºC), pH (7.0) and total hardness (200 mg L^{-1}) were kept constant throughout the study period. Continuous air was supplied to the test and control mediums with an air pump through capillary system. Chemically extra pure compounds of zinc and lead were used to prepare stock solutions of desired dilution. The LC_{50} (96 hr) value of Zn+Pb mixture for *C. mrigala* was computed as 71.55±0.36 (Naz and Javed, 2012). The fish was observed after every 2 hours for 4-days.

Behavioral Study

During Zn+Pb mixture exposure, the behavioral parameters of fish viz. jumping, equilibrium status, opercula movement, fin movement, convulsion, hyperactivity and surfacing frequency were studied.

Data Presentation

Data is presented in the form of Normal (0), Mild (+), Medium(++), High(+++).

3. RESULTS AND DISCUSSION

In present study behavioral changes of *C. mrigala* exposed to Zn+Pb mixture were observed (Table 1). During first three days shows the jumping, increased opercula and fin movement, surfacing frequency, convulsion and hyperactivity. The equilibrium status was abnormal. Fish *C. mrigala* exposed to Fe+Ni+Pb+Zn mixture showed increased hyperactivity, swimming rate, mucous secretion and fin movement with increasing the concentration of mixture (Naz et al., 2018). According to Eissa et al. (2009) locomotion parameters are sensitive endpoints and useful biomarkers in behavioral studies of freshwater toxicity. Fish exposed to Zn showed sluggish movement, rapid operculum movement and loss of equilibrium (Abdel-Gawad et al., 2011). Visualized the behavioral changes in fish, Nile tilapia (*O. niloticus*) and catfish in response to copper sulphate (50, 60, 70, 80 100 and 120 mg/l). *O. niloticus* showed more severe effects, as compared to catfish. Avoidance behavior was observed by unsteady swimming pattern with jerky moments. Fish were observed to be suspended in vertical position with tail pointing downwards. Finally fishes sank in the bottom and became motionless (Bhat et al., 2012). The abnormal behavior shown by fish may be due to abnormal level of neurotransmitters. These changes occur much earlier than mortality (Little and Finger,1990). Jumping to and fro signify the avoidance reaction of the fishes to the toxicants. Fish avoid the area containing chemical so mostly fishes remain in the corners of the tank. The increase in surfacing and gulping of sir from surface water after toxicant exposure could be an attempt of the animal escape from the toxicant and to avoid breathing in the contaminated water. Secretion of excessive mucus is probably due to irritation of the skin due to direct contact with the toxicant. Mucus forms a layer between the body and toxicant to minimize irritating effect (Rao, 2006) and also inhibit the diffusion of oxygen during gaseous exchange (Kumar et al., 2015). Preference of upper layer may be due to respiratory stress in the exposed groups (Katja et al., 2005). Lateral swimming and loss of equilibrium is probably due to the impairment of the nervous system (Sinha and Kumar, 1992). Ultimately, fish sank into the tank bottom with a least operculum activity showing failure to fight with stress and ultimately the fish died. Inappropriate behavioral responses to environmental and physiological stimuli due to the toxic effect of aquatic contaminants can have severs implications for survival (Weber and Spieler, 1994).

4. CONCLUSION

The present study concluded that the exposure of metals mixture altered the behavior of fish.

5. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interests regarding the publication of this article.

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Table1 Movement patterns of *Cirrhina mrigala* under Zn+Pb exposure

Parameter	**Control**	**Day-1**	**Day-2**	**Day-3**	**Day-4**
Surfacing frequency	-	+	+++	+++	+
Jumping	-	+	+++	++	+
Fin movement	-	+	+++	++	+
Hyperactivity	-	+	+++	++	+
Equilibrium status	-	+	++	++	+
Opercula movement	-	+	+++	++	+
Convulsion	-	+	++	++	+

Normal (0), Mild (+), Medium (++), High (+++)

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CLOVE (*SZYGIUM AROMATICUM*) AND ITS DERIVATIVES IN RUMINANT FEED

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Clove; methanogenesis; deamination; essential oils; manipulation.

ABSTRACT

Livestock, more prominent of which are ruminants, has a significant contribution to methane emission, a potential greenhouse gas as a good portion of their ingested energy is wasted in the form of methane (2–15%) and major of the ingested nitrogen as ammonia (75–95%). Microbial fermentation in ruminants result in loss of energy in methanogenesis and protein by ammonia nitrogen excretion which causes decreased animal optimal production and also act as environmental pollutants. Previously antibiotics were used to decrease these losses in the rumen, but this approach was restricted due to presence of antibiotic residues in animal products. Some plants or their bioactive extracts/metabolites such as organo-sulphur compounds, saponins, essential oils, flavonoids, tannins and many other metabolites at higher concentration exhibited the potential to limit the methanogenesis by altering the rumen microflora. To overcome this problem, plant extracts including clove bud oil (Syzygiumaromaticum) was introduced as an alternative for manipulating rumen fermentation. Clove bud oil possesses the capability to interact with bacterial cells and inhibits the growth multiplication of methanogenic and deaminating bacteria. This results in reduction in ammonia nitrogen, methane and acetate concentration, while higher propionate and butyrate concentrations were noted. Eugenol is one of the bioactive constituents of clove which has the ability to manipulate rumen fermentation by increasing propionate production, decreasing acetate and methane production, and altering pattern of proteolysis, peptidolysis and amino acid deamination in the rumen. Current review will focus on the use of clove for manipulation of rumen fermentation for inhibition of methanogenesis and energy loss in ammonia – nitrogen waste.

1. INTRODUCTION

Dietary protein is broken down to ammonia to increase nitrogenous waste material instead of becoming part of animal body.

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Almost 75 to 85% of nitrogen taken by lactating animals is excreted out in the urine and feces (Goel and Makkar, 2012) and it badly affect the environment by increasing discharge of nitrous oxide in the air (Patra and Saxena, 2010). To overcome this, ruminal microbial ecosystem is manipulated by adding herbal extracts and spices as feed additives which can eradicate or decrease rumen ciliate protozoa, decrease protein degradation and methane production.

Although, previously antibiotics were recommended as feed additives to manipulate rumen fermentation by decreasing methane and ammonia nitrogen production (McGuffey *et al.*, 2001). But this practice was eliminated due to higher antibiotic residues in animal products(Russell and Houlihan, 2003). So, FDA has recommended the use of natural antimicrobials like plant extracts which are normally considered safe for human consumption by modifying microbial fermentation in the rumen. Plant extracts possess antimicrobial activity due to presence of secondary plant metabolites including phenyl propanoids (e.g.,eugenol, cinnamaldehyde, saponins, anethol, tannins) possessed by the oil extracted from various plants. Main constituent of clove oil are eugenol and b-caryophylene which is a phenolic non-nutrient constituent which have effect on all in vitro rumen fermentation products like volatile fatty acids, N-ammonia and ruminal microorganisms (Busquet *et al.*, 2006a). Essential oils are naturally occurring volatile components responsible for giving plants and spices their characteristic essence and color (Patra and Yu, 2014).

Herbs and spices are most commonly used in feed of ruminants now a day (Chaves *et al.*, 2012). Various anaerobic bacteria, protozoa, fungi and methanogens are present in rumen of animals. Rumen fermentation process is manipulated by using herbs to enhance the efficacy of nutrients digestion and metabolism, reduction of energy loss from unwanted process of formation of methanogens and general increase in the production of animals (Chaves *et al.*, 2011). Previously, some antibiotics and other feed additives were recommended for this purpose, but now trend has been shifted towards the use of herbs and plant extracts to increase the production of animals (Ipharraguerre and Clark, 2003). Herbs and spices mostly exhibit effects on the basis of required dosage, because small dosages show no effect while large amount of dosage can produce toxicity in animals (Goodrich *et al.*, 2018).

2. MECHANISM OF ACTION OF CLOVE AS MANIPULATOR OF RUMEN FERMENTATION

Clove bud (*Syzygiumaromaticum*) contains phenylpropanoids which show antibacterial activity against different bacterial species by interacting with their cell membrane. The activity of clove ows to its unique chemical composition (Fig 4). This interaction produces many changes in the conformation the membrane structure, responsible for its fluidity and expansion (M.Tajodini, P. Moghbeli, H.R. Saeedi, 2014). By disruption of membrane stability, ionic leakage across the cellular membrane occurs and it reduces the ionic gradient across the membrane. Mostly, bacteria equipoise these effects by using ionic pumps and it does not cause cell death but large amounts of energy are distracted to this function and bacterial growth slows down. As a result, this alteration in growth rates causes change in the amount of rumen bacterial populations leading to changes in the profile of rumen fermentation (Benchaar *et al.*, 2008).

Eugenol (4-allyl-2-methoxyphenol; C10H12O2) is a phenolic compound present in clove bud (*S.aromaticum*) and it is responsible for broad spectrum antimicrobial activity against both gram-positive and gram-negative bacteria due to the presence of a hydroxyl group (M.Tajodini, P. Moghbeli, H.R. Saeedi, 2014).It is reported in a continuous culture study that low doses of clove bud oil (2.2 mg/L) decreases production of acetate and branched-chain volatile fatty acidsand increases production of propionate (Busquet *et al.*, 2006a). Clove bud oil has also effects on nitrogenmetabolism by increasing peptide nitrogen and by decreasing ammonia nitrogen concentrations, indicative of reduced peptidolytic activity in the rumen. (Busquet *et al.*, 2006a)reported in an *in vitro* batch culture study that clove bud oil helps in manipulating rumen fermentation, decreasing total volatile fatty acids and ammonia nitrogen concentrations and exhibit a linear increase in the molar proportion of propionate and a quadratic effect on the molar proportions of acetate and butyrate. As a result, clove bud oil helps in improving overall rumen fermentation.

Clove bud oil contains 85% eugenol which is responsible for increase in pH of rumen and reduced total volatile fatty acids and ammonia nitrogen concentrations (M.Tajodini, P. Moghbeli, H.R. Saeedi, 2014).Eugenol causes increase in propionate concentration and clove bud oildecreases acetate concentration (fig. 1). Clove bud oil and eugenol increasedbranched chain volatile fatty acids at 300 mg/L compared with control, but this value decreased in eugenol at dose rate of 3,000 mg/L. So, it is reported that other components of clove bud oil may interact with eugenol and showed additional impacts. Results of*in vitro* continuous culture study revealed that lower inclusion rate of clove bud oil (2.2 mg/L)decreased the molar concentration of acetate and branched chain volatile fatty acids whilean increase in the molar concentration of propionate was noted, however similar dose of eugenol did not show any effects(Busquet *et al.*, 2005a).

3. CLOVE NUTRACEUTICS

Phenolics in herbal oils showed nutritional significance as common antimicrobials and antioxidant and may directly respond with, and extinguish, free radicals to prevent lipid peroxidation, along these lines improving health and preventing certain infections(Botsoglou *et al.*, 2004; Brenes and Roura, 2010; Tariq *et al.*, 2015). 2-grams clove powder possesses 6 calories, 1 gram of carbohydrates and fiber each and small quantity of vitamin K and manganese. Scientifically reported nutraceutical spectrum of clove and clove oil includes cancer prevention, malignancy inhibition, tumor growth suppression, liver health improvement, decrease in oxidative pressure on cell lines, antibacterial action, lowering of blood glucose, enhance bone health and repair, enhance gastric ulcer healing (fig.2) (Shabestari *et al.*, 2011; Morsy *et al.*, 2012a; Roy *et al.*, 2015). Clove is being abundantly produced in some countries and most of its part is of exported (Fig 3)

4. EFFECT OF CLOVE ON METHANE PRODUCTION

Livestock, more prominent of which are ruminants, has a significant contribution to methane emission, a potential greenhouse gas as a good portion of their ingested energy is wasted in the form of methane (2–15%) and major of the ingested nitrogen as ammonia(75–95%)(Bodas *et al.*, 2012; Cobellis *et al.*, 2016a). Ruminant production system is a major point of emission of all the greenhouse gases mainly CH_4(18%) and CO_2(9%). Amongst the greenhouse gases, methane possessessignificant global warming properties (about 23 times) more than carbon dioxide(Ugbogu *et al.*, 2019). Methane production in ruminants mainly depends on forage to be fed and time of harvest of forage (ZHONG *et al.*, 2016). The emission of greenhouse gases from livestock systems is emerged a great global concern asthese emitted gases substantially contribute in global warming.

Some plants or their bioactive extracts/metabolites such as organo-sulphur compounds, saponins, essential oils, flavonoids,tannins and many other metabolites at higher concentration exhibited the potential to limit the methanogenesis by altering the rumen microflora(Patra and Saxena, 2010). The possible mechanisms and effects of these constituents on rumen microbiota are not fully understood yet. The well documented antimicrobial activity of these plant extracts/metabolites has provoked the interest of researchers whether these compounds are worth to use as selective inhibitorsof rumen methanogens(Benchaar *et al.*, 2008). Studies reported plant essential oils as very promising entities for selective reductionin the abundance of methanogens and deamination of protein. Many studies haveinvestigated essential oils as feed additives to reduce enteric methane production. Essential oils derived from oregano, thyme, cinnamon, horse radish, garlic, frangula and rhubarb showed decrease in methane production *in vitro* in a dose dependent manner (Benchaar and Greathead, 2011). However, some reports also stated theessential oils as feed additives can result in decreased feed degradability in rumen and lower amounts of volatile fatty acids due to theirnon-specific and broad antimicrobial activities in the rumen (Cobellis *et al.*, 2016b). The challenge to identify such plant constituents that canpossess selective inhibition of ruminal methanogens at practically applicable feeding rates, with long duration effects and without any negative alteration in the feed digestion and animal productivity (Benchaar and Greathead, 2011). Recent studies showed the adverse effects of high doses of these feed additives on intake and rumen degradation indicating that a single high dose of essential oil may not practically and effectively lower down the methane productionin the ruminants until and unless used in smaller doses and with combination of other anti-methanogenic products(Patra and Yu, 2012). Combinations of these oils in lower doses may be a practical strategy to minimizethe greenhouse gases emission and nitrogen excretion from ruminant without any adverse effect (Cobellis *et al.*, 2016a).

Essential oils showedinhibitory effects against methanogenic microfloraof rumen along with reduced methanogenic protozoal abundance. Although plant secondary metabolites may adversely deteriorate the utilization of nutrients, there are studies depicting that methane production can bemitigated without any negative effect on rumen functioning and feed utilization (Patra and Saxena, 2010). Ruminal microbiota possesses the capability to adopt feed additives in a duration of 7 days. However, some plant extracts/ oils can modify the rumen fermentation patterns by the manipulation of ruminal microbiota(Busquet *et al.*, 2005a). As determined by 16S rRNA sequencing, the α biodiversity of ruminal bacteria was noted similar but relative abundance and methane production level changes depending upon inclusion rate of plant oils as feed additive (Zhou *et al.*, 2020).

Effects of essential oils on methanogens mainly dependent on the oil source and its dose. Clove oil can reduce methanogenesis without any negative impact on rumen fermentation at the inclusion rates of 125, 250, and 500 mg/L (Günal *et al.*, 2017). Clove oil supplementation has the ability to reduce methane production by 34.4%, higher than eucalyptus oil (17.6%) and peppermint oil (25.7%), when used at 1.0 gram per liter without affecting total VFAs production (Patra and Yu, 2012). Clove oil usage at 600 ppm dose results in reduction ($p<0.05$) of total gas production without any alteration intotal VFA,feed degradability and acetate to propionate ratio. Methanogenesis was noted to be significantly lowered ($p<0.05$) when clove oil and peppermint oil was used at 300 and 600 ppm, respectively(Roy *et al.*, 2015). Clove bud oil and clove leaf oil can also reduce the methanogenesis by 12.9% and 8.0% respectively as compared to lemongrass and turmeric oil (7.3% andno effect, respectively) (Pawar *et al.*, 2014). (Patra *et al.*, 2006)evaluated that ethanolic and methanolic extracts of clove can inhibit methanogens*in vitro*, but feed digestibility was also reduced.Inhibition of methane production occurred at higher doses (i.e., >300mg/L of culture fluid) and mostly associated with a decrease in total VFA concentrations and feed degradation(Benchaar and Greathead, 2011). According to the results of in vitro study, a significant decrease on methane production was observed by administration 0.5mL clove methanol extract at 16, 24, 48, 72 and 96 h post incubation ($p<0.05$). Additionally, a significant descending procedure on methane production was observed using 1mL clove methanol extract during the study except 4 and 24 h after injection ($p<0.05$)(Shabestari *et al.*, 2011). *Invitro* investigation of (Patra *et al.*, 2010) showed that methanogenesis was not affected by clove water extract. However, methanolic and ethanolic extracts of clove inhibited CH_4 production by 35 and 83% respectively. The spices do not alter the ruminal pH and total VFA concentration. However, the presence of clove can reduce acetic acid concentrationsignificantly (Chaudhry and Khan, 2012).(Patra and Yu, 2012)showed that use of essential oil mixture(1.0 g/L) from clove, oregano, eucalyptus,peppermint and garlic can reduce methane production without altering dry matter and NDF degradability.

5. EFFECT OF CLOVE ON PROTEIN METABOLISM

Ruminant nutritionistsand microbiologists are continuously exploring the alternative waysfor the manipulation of rumen fermentation for improvement of feed efficiency and animal performance. Recent studies revealed that plants secondary metabolite and their derivatives possess the capabilities to improve dietary energy and nitrogen in animals. These metabolic improvements are more likely to be carried out by lowering the abundance of hyper-ammonia producing ruminal microflora which results in reduction in amino acids deamination and ammonia nitrogen production (Busquet *et al.*, 2006b; Patra *et al.*, 2006; Calsamiglia *et al.*, 2007). However, these improvements are noted only at higher concentrations of these additives but such higher inclusions adversely affect microbial fermentation and decrease total VFAs concentration(Benchaar *et al.*, 2007). Essential oils in combination are reported to have promisingresults as feed additives to improve feed efficiency, nutrients utilization and pathogenic control livestock (Benchaar *et al.*, 2008). Unlikely, essential oils supplementation are also reported to have limited effects on apparent ruminal microbial fermentation,nutrient digestibility, metabolites, blood cells and protozoa and bacteria count (Khateri *et al.*, 2017).Same observations of plant oils additives on nutrient utilizationand milk performance inlactating cows has also been reported (Benchaar *et al.*, 2007).

Essential oilssupplementation significantly reduced ($p<0.05$) the rate of amino acids deamination by affecting ammonia-hyperproducing bacteria and anaerobic fungi(McIntosh *et al.*, 2003). Ruminal ammonia production was notedlowest($p<0.01$) for clove when compared to cumin, coriander and turmeric supplementation in ruminal feed. The addition of spices does not alter total VFAsand the pH of ruminal fluid. However, the supplementation of clove in feed can significantly reduce($p<0.05$) acetic acid concentration(Chaudhry and Khan, 2012).

Right combination mixture of essential oils may practically prove to boost up the animal productivity by reducing methanogenesis and mitigating protein degradation by ruminal microflora. Clove oil usage at 600 ppm dose results in reduction ($p<0.05$) of total gas production without any alteration in total VFA, feed degradability and acetate to propionate ratio(Roy *et al.*, 2015). Addition of essential oils showed inhibitory effects on proteolytic bacteria of rumen which results in reduction of ammonia production (Patra and Yu, 2014).(Lin *et al.*, 2013)reported that the inclusion of essential oils mixture (1 g/day) from clove, cinnamon, lemon andoreganocan inhibit ammonia production in sheep. A study on the investigation of effects of essential oils mixture (from clove, peppermintand oregano) supplementation on proteolytic bacteria of rumen revealed that this mixture effectivelyreduced the number of *S. ruminantium*, *P. ruminicola*and *P. bryantii*(Patra and Yu, 2014). Eugenol Inclusion (chief bioactive component of clove) was noted to enhance protein utilization and energy efficiency in the ruminants (Calsamiglia *et al.*, 2007; Lin *et al.*, 2013; Abo-EL-Sooud, 2018).

Essential oils mixturehas the potential to inhibit the multiplication of methanogenic speciesprominent of which are*Fibrobacter succinogenes* and*Butyrivibriofibrisolvens*.(Busquet *et al.*, 2006b)described that mixture of clove bud oil, anise oil, capsicum oil, oregano oil,cinnamon oil, ginger oil, dill oil, garlic oil, cade oil and tea tree oil and their bioactive components (*i.e.*, eugenol,anethol, carvacrol,cinnamaldehyde, carvone andbenzyl salicylate) significantly decreased the ammonia – nitrogen amounts at higherinclusion rates (*i.e.* 3000 mg/L), marginal effects atmoderate concentrations (*i.e.* 300 mg/L) and non-existent effects at low concentrations (*i.e.* 3 mg/L). This reduction was also accompanied by total VFAs reduction, main source of energy in ruminants, revealing the negative impact on microbial fermentation in the rumen and nutritional consequences in the animals.

(Busquet *et al.*, 2005b)demonstrated that clove bud oil addition (at 2.2 mg/L) to a continuous culture fermenter can reduce (80%) large peptides concentration without any alteration on ammonia – nitrogen amount, revealing that clove bud oil can alter microbial proteolytic patterns in the rumen. However, eugenol addition (chief bioactive component of clove oil) at the same concentration showed no effect on nitrogen metabolism, suggesting that anti-peptidolytic properties of clove oil is due to some unidentified oil fractions.Clove oil supplementation has no significant effect on milk yield and milk composition in lactating animals, while milk non-protein nitrogen and milk fat contents decreased and an increase in milk protein was noted compared to control(Morsy *et al.*, 2012b). The mixture of both clove and cinnamon essential oils have a potent use in animal feed, favoring a greater sarcomere length, enhancing meat tenderness, without any negative change in the chemical composition or fatty acid profile of meat(Monteschio *et al.*, 2019).

Overall, considering that body size is a master trait driving fundamental characteristics of organisms, its study along altitudinal gradients under different bioclimates may allow better understanding of the factors driving elevational patterns in the populations structure of Carabidae and ecogeographical rules as well. The proposition that Carabidae generally follow Bergmann rule or any common pattern is clearly challenged by available studies. The results suggest that to improve understanding of the drivers of the observed patterns further investigations on changes in ground beetles communities along altitudinal gradients should consider different species and bioclimatic contexts and use similar sampling designs.

6. WHAT INFLUENCE WILL CLIMATE CHANGE HAVE ON THE PRODUCTION OF CLOVES?

The climatic conditions have largely influenced the cloves production on worldwide scale in past few years. The yield recorded in Madagascar in 2018, due to unexpected moisture conditions, was an imperceptible disaster. The production in Malagasy appeared nearly 10 to 20% of a mean year. A similar observation was made in Brazil, Comoros and Zanzibar, where the yield was reduced from 7000 tonnes in 2017 to 1300 tonnes in 2108. Meanwhile, the production in Indonesia was high because of favorable climatic conditions. This raises a concerning question for the stakeholders that, whether these are primarily important indications of the impact of climate change?

7. WHAT IS THE POTENTIAL IMPACT OF THE COVID-19 PANDEMIC?

This last but prospective section aims to evaluate the aftermath of health crisis related to COVID-19 and its spread on a global scale. Although it affects the horticultural sectors as a whole, depending upon the considered sector, this effect will be uncommon and unusually linked to the span of current pandemic. Regarding clove sector, it is not easily possible to forecast the effect of the financial crisis following the COVID-19 on domestic consumption in main consumer countries.

Furthermore, it is still not known that to what extent this pandemic will affect the economic conditions of two major cloves purchasing countries, namely India and Indonesia. Industrial purchaser, distributors, banks, freight forwarders and transporters, all are deeply concerned with this.

However, the question for the cloves will arise at the end of 2020, at the time of harvesting. We can expect for the improvement of COVID-19 situation by the end of this year along with the liberation euphorbia resulting in revised interest on markets.

Regarding clove oil, the eugenol market seems to remain unaffected by this pandemic as there is no decrease in demand of eugenol based vanillin which is the main outlet and acknowledged as a natural product by the USA.

Additionally, the major applications of eugenol are found in food, livestock, pharmaceutical and cosmetic industries which are relatively less affected by the COVID-19 unlike perfumery, which is adversely impacted by the abandoned tourism in 2020.

Resultantly, the raw materials sector for perfumery will have to face the economic consequences in next few months in the form of a noticeable decline in the prices of the raw materials.
The producer countries can take the advantage of this economic setback by adapting to the market demands, regarding the time of delivery and quality of product as well as assuring the competitive prices of products. These pre-COVID-19 issues seem to become even worse by this pandemic.

8. CONCLUSION

Livestock systems are major contributors of greenhouse gases globally. Plant originated metabolites are choice of time to mitigate this challenge. Clove and its derivatives are considered as an important feed additive for boosting up the milk yield and growth rate in ruminants by reducing methanogens is and deamination of dietary amino acids. Its chief bioactive component is eugenol which is responsible to enhance animal productivity by reducing methane producing microflora and inhibiting protein degradation in rumen. Now a days, clove bud oil is being used as essential oil to add in the feed of ruminants for better milk and meat production.

9. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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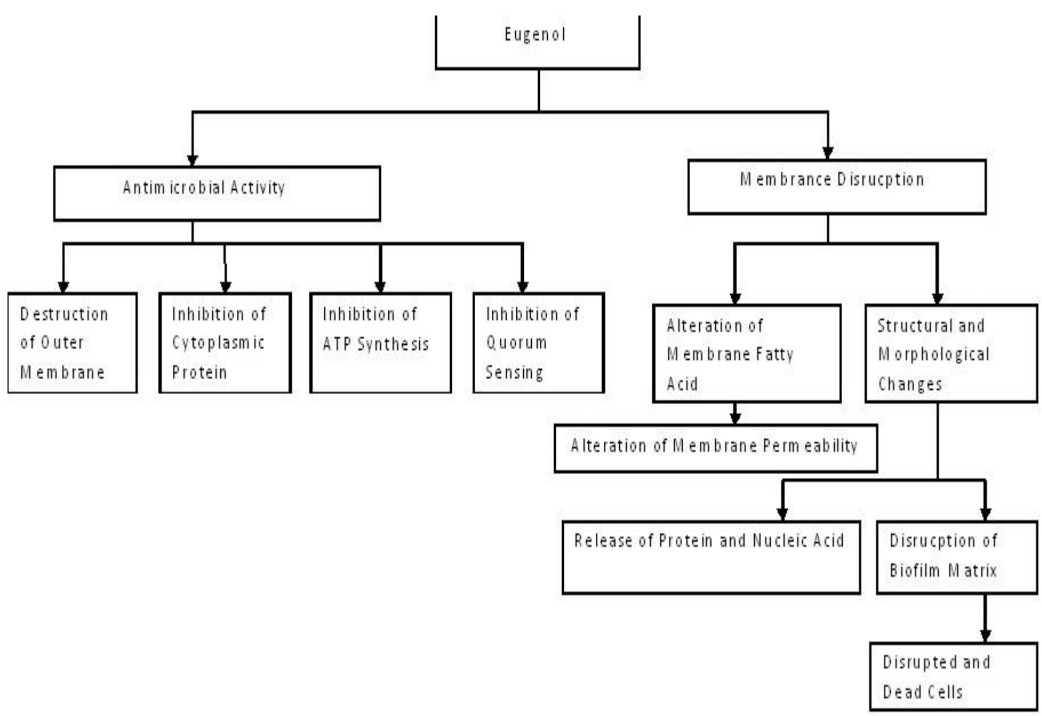

Figure 1. Spectral line of Eugenol activities

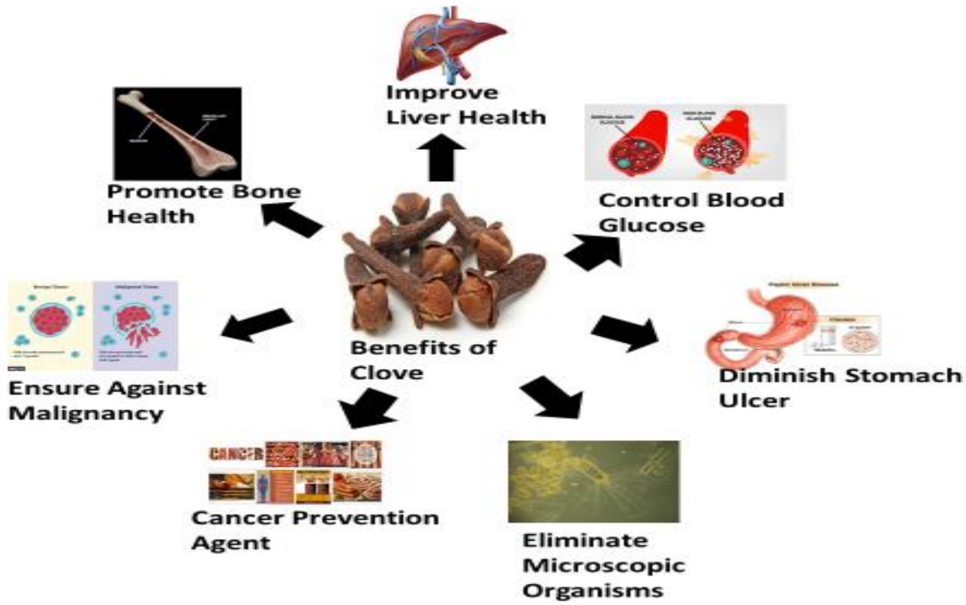

Figure 2. Nutraceutical spectrum of Clove and Clove essential oil

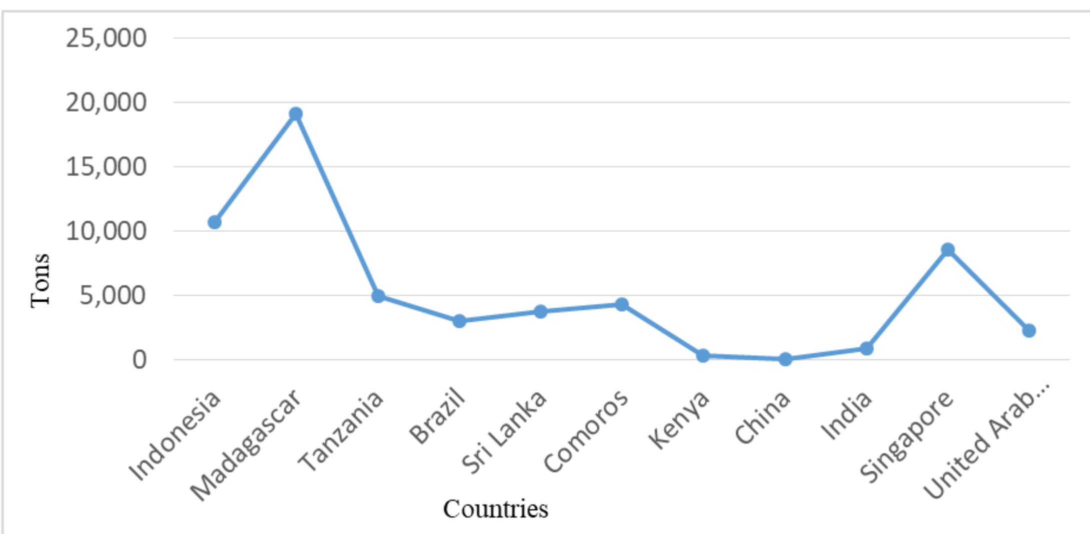

Figure 3. Export potential of Clove observed in different countries (Adapted from Danthu et al., 2020)

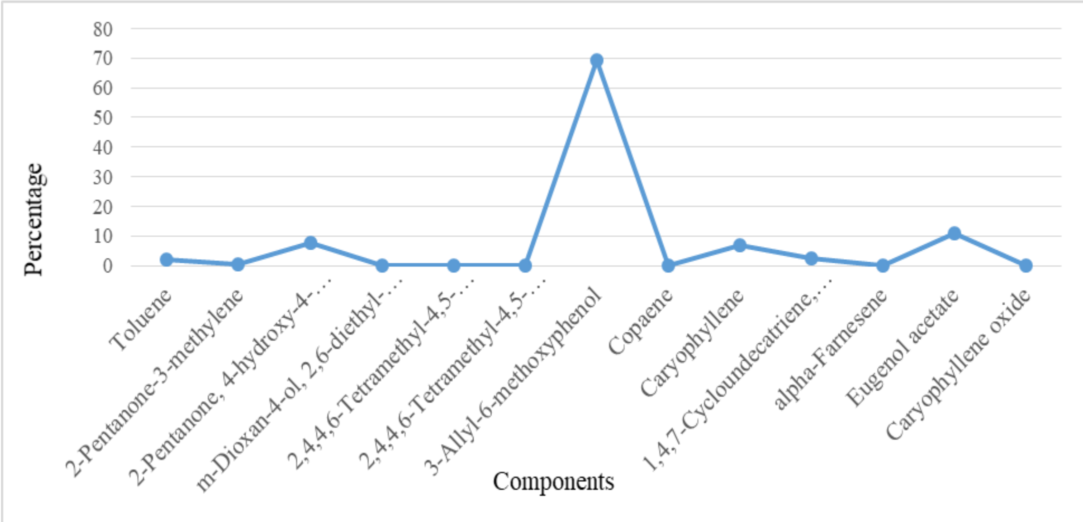

Figure 4. Chemical composition (%) of clove bud oil (Adapted from Uddin et al., 2017)

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