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# *MONODICTYS FLUCTUATA* FROM ROOT SURFACE OF *APLUDA MUTICA*

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The present note deals with the description of a fungus *Monodictys fluctuata* isolated from root surface of *Apluda mutica*.

During investigations on root surface fungi of certain plants, an interesting fungal species *Monodictys fluctuata* was isolated from root surface of *Apluda mutica* L. growing in University Campus, Gwalior. Cultural characteristics of this fungus resemble *Acrospeira fluctuata* which is highly parasitic on leaves of *Monstera delicosa* (Tandon and Bilgrami[5]) and *Sorghum vulgare* (Reddy[4]) and is now treated as a synonym of *M. fluctuata* (Ellis[3]). A scrutiny of the literature (Bilgrami *et al* [1,2]) reveals that this parasitic fungus is hitherto unrecorded from India.

*Monodictys fluctuata* (Tandon and Bilgrami) M.B. Ellis

Colonies effuse, dusty white, mycelium branched septate, 2-4 $\mu$m wide. Conidiophores micronematous, mononematous, irregularly branched, dark, smooth and 6-7 $\mu$m wide. Conidiogenous cell monoblastic, integrated, terminal, bearing a single terminal conidium. Conidia simple, acrogenous, globose to ovoid to broadly ellipsoid, brownish-black, finely echinulate, 4-8 celled with cross and longitudinal septa and measuring 18-35$\mu$m in diam.

The culture is deposited at CMI, Kew, England as IMI 206941.

Authors are thankful to Dr. B.C. Sutton, CMI, England for helping in fungal identification and to Prof. S.B. Saksena for useful suggestions.

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# INFLUENCE OF POST HARVEST FUNGAL INFECTIONS ON THE PHENOLIC CONTENTS OF APPLE, PEAR AND PEACH FRUITS

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Effect of *Gliocladium roseum*, *Sclerotium rolfsii* and *Aspergillus niger* infections on the phenolics of apple, pear and peach fruits respectively was studied. It was noticed that in all the three fruits significantly greater accumulation of already existing phenolic compounds occurred during the course of pathogenesis.

Phenolic compounds have an important role in the biosynthesis of ethylene in most of the fruits and have been shown to play key roles in the resistance of plants to disease causing micro-organisms (Farkas and Kiraly[1]). Recently few investigators[2,3] have observed synthesis and accumulation of phenols and their products in the host in response to the invading pathogen as a major defence mechanism. In the present investigations, an endeavour has been made to study the influence of *Gliocladium roseum* Bain., *Sclerotium rolfsii* Sacc., and *Aspergillus niger* van Tieghem on the phenolic contents of apple, pear and peach fruits respectively. These fungi have been reported earlier[4,5] to cause serious post-harvest spoilage.

Ethanolic extracts of healthy and diseased fruit tissues were prepared after different days of incubation. The phenols of the ethanolic extract were separated by one dimensional ascending paper chromatography using n-butanol : glacial acetic acid : water (4 : 1 : 3, v/v). 2% Ferric chloride solution was used as a spraying reagent. The air dried sprayed chromatograms were developed at 100°C for 10 minutes until brown spots of various phenols developed.

Quantitatively total phenols were assayed by the Folin-Ciocalteau method using catechol as a standard. 0.5 ml of ethanolic extract was added to 25 ml of distilled water. 1 ml from the above solution was diluted with 10 ml of distilled water. To this solution, 0.5 ml of Folin-Ciocalteau reagent was added and the tubes were shaken thoroughly. After 3 minutes, 1.0 ml of saturated sodium carbonate solution was added and the total volume was made to 15 ml with distilled water. These tubes were placed in dark for 1 hour and the blue colour developed was read at 725 nm using Spectronic 20. A blank containing all the reagents minus Folin reagent was used to adjust the adsorbance to zero. The concentration of phenolics was expressed as mg catechol equivalents per gram fresh weight.

It is evident from Table 1 that healthy peach fruits contained four phenolic compounds. During pathogenesis, the amount of two phenols Rf 0.80 and 0.73 remained substantially unchanged but in healthy tissues, they were not observed after 4th and 2nd day of incubation respectively. A phenolic compound (Rf 0.64) was observed in peach tissues only at the time of incubation, whereas, another phenol (Rf 0.39) was noticed both in healthy and diseased tissues. However, its concentration increased two-fold after infection as the disease advanced. Healthy and *G. roseum* infected apple tissues contained only one phenolic compound (Rf 0.39), the

TABLE 1

Chromatographic analysis of phenolic compounds

| Fruit | Pathogen | Incubation period in days | PHENOLS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rf 0.80 H* | I** | Rf 0.73 H | I | Rf 0.64 H | I | Rf 0.39 H | I |
| | | 0 | 3+ | 3+ | 2+ | 2+ | 2+ | 2+ | 4+ | 4+ |
| | | 2 | 2+ | 2+ | 2+ | 2+ | — | — | 4+ | 5+ |
| | | 4 | 2+ | 2+ | — | 2+ | — | — | 4+ | 5— |
| Peach | *A. niger* | 6 | — | 2+ | — | 2+ | — | — | 3+ | 6+ |
| | | 8 | — | 2+ | — | 2+ | — | — | 3+ | 6+ |
| | | 10 | — | 2+ | — | 2+ | — | — | 3+ | 6+ |
| | | 0 | — | — | — | — | — | — | 4+ | 4+ |
| | | 4 | — | — | — | — | — | — | 3+ | 4+ |
| | | 8 | — | — | — | — | — | — | 3+ | 4+ |
| Apple | *G. roseum* | 12 | — | — | — | — | — | — | 3+ | 5+ |
| | | 16 | — | — | — | — | — | — | 3+ | 5+ |
| | | 20 | — | — | — | — | — | — | 3+ | 5+ |
| | | 0 | — | — | — | — | — | — | 4+ | 4+ |
| | | 4 | — | — | — | — | — | — | 3+ | 5+ |
| | | 6 | — | — | — | — | — | — | 3+ | 5+ |
| | | 8 | — | — | — | — | — | — | 3+ | 5+ |
| Pear | *S. rolfsii* | 10 | — | — | — | — | — | — | 3+ | 6+ |
| | | 12 | — | — | — | — | — | — | 3+ | 6+ |

*Healthy **Infected

Quantitative determination of phenols is based upon the size and relative intensity of the spot.

2+, 3+, 4+, 5+ and 6+indicate relative amounts.

—indicates absence of the phenols.

concentration of which decreased in healthy and increased in infected tissues. Similarly, in pear fruits also, the only phenolic compound detected (Rf 0.39) decreased in healthy tissues and showed a notable two-fold increase in the *S. rolfsii* infected tissues.

The changes in the levels of total phenols of healthy and diseased fruits were also estimated and have been presented in Table 2. In healthy peaches, the amount of total phenols showed a gradual reduction due to incubation. After infection with *A. niger*, there was an initial reduction up to 2nd day of incubation, and thereafter, diseased samples showed gradual increase. The total phenolic content of healthy apple and pear tissues also suffered a gradual decline with the increasing incubation period while infected tissues exhibited an increase during the course of pathogenesis.

TABLE 2

Changes in total phenols (mg/g of the fruit pulp)

| Fruit | Pathogen | Days of incubation | Phenolic content (mg/g) | |
|---|---|---|---|---|
| | | | Healthy | Infected |
| Peach | *Aspergillus niger* | 0 | 4.66 | 4.66 |
| | | 2 | 3.92 | 3.92 |
| | | 4 | 3.70 | 4.52 |
| | | 6 | 3.81 | 4.66 |
| | | 8 | 2.98 | 5.03 |
| | | 10 | 2.67 | 5.65 |
| Apple | *Gliocladium roseum* | 0 | 3.32 | 3.32 |
| | | 4 | 2.20 | 2.67 |
| | | 8 | 1.73 | 3.70 |
| | | 12 | 1.73 | 4.40 |
| | | 16 | 1.51 | 4.66 |
| | | 20 | 1.05 | 4.66 |
| Pear | *Sclerotium rolfsii* | 0 | 4.96 | 4.96 |
| | | 4 | 4.40 | 4.66 |
| | | 6 | 4.27 | 4.40 |
| | | 8 | 3.92 | 4.96 |
| | | 10 | 2.98 | 5.46 |
| | | 12 | 2.20 | 5.71 |

It could be seen from the results that significantly greater accumulation of already existing phenolic compounds occurred. This rapid increase in phenolics after infection implies that they have a significant role in the pathogenesis. Byrde *et al*[6], concluded that on injury to the fruit, the phenolic compounds are oxidized by the host enzymes and these oxidized phenols inactivate the extracellular pectolytic enzymes of the fungus which are necessary for its pathogenicity. It is also possible that other enzymes which are important in pathogenicity may be similarly inactivated. Cole[7] stated that oxidized phenols probably also limit the extent of pectolytic breakdown in fruits where an infection has become established. This defence mechanism applies in the present host parasite relationship also, particularly as they are wound parasites. The acceleration in the amount of phenolic compounds in response to infection has also been attributed to their synthesis from the intermediates of carbohydrate breakdown.[8]

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# EFFECT OF AZOTOBACTER INOCULATION ON THE GROWTH OF RICE SEEDLINGS

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Rice seedlings were grown in Dapog nursery and plastic trays. The seedlings were inoculated with *Azotobacter* inoculum on 5th day after sowing at the rate of one kilo per hectare. Significant increase in root growth and shoot growth was observed due to inoculation when compared to uninoculated ones. The inoculation of *Azotobacter* bio-fertilizer has shown positive effect on seedling growth.

The inoculation of *Azotobacter* to rice crop is known to fix atmospheric nitrogen. The occurrence and distribution of *Azotobacter* in the rhizosphere of several crop plants and the beneficial effects on yield have been well established[2,3]. The beneficial effect of *Azotobacter* inoculation on rice crop has been reported by Jagannathan *et al*[1]. An attempt has been made to find out the effect of inoculation on rice seedlings.

Rice seedlings (var: TKM-9) were grown in Dapog nursery and plastic trays. The seedlings were grown on pure river sand and were inoculated with peat based *Azotobacter* culture on 5th day after sowing at the rate of 1 kg culture of *Azotobacter* and 10 kg of superphosphate per hectare. The calculated quantities of *Azotobacter* and superphosphate were applied to the Dapog and Tray nursery. Superphosphate was applied to the nur-

TABLE 1

Effect of *Azotobacter* inoculation on growth of rice seedlings on 25th day

| Sl. No. | Treatments | Root growth (mm) | Shoot growth (mm) | Vigour of seedlings (9/100 fresh seedlings) |
|---|---|---|---|---|
| | I *Tray Nursery* | | | |
| 1. | Inoculated | 79.5 | 98.8 | 10.8 |
| 2. | Uninoculated | 49.4 | 89.0 | 9.4 |
| | II *Dapog Nursery* | | | |
| 1. | Inoculated | 88.4 | 96.9 | 8.9 |
| 2. | Uninoculated | 58.5 | 71.8 | 8.4 |

sery in order to induce growth and multiplication of *Azotobacter*. The observations on root growth, shoot growth and vigour of rice seedlings were recorded. The results have clearly shown that the inoculation of *Azotobacter* to rice seedlings have significantly increased the shoot and root growth when compared to the uninoculated ones (Table 1). This trend was observed in both Tray nursery and Dapog nursery. The present results have clearly indicated the positive effect of *Azotobacter* inoculation on rice crop.

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# *NANOSCYPHA TETRASPORA* (SEAVER EX SEAVER) DENISON NEW TO INDIA

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In this short note *Nanoscypha tetraspora*, (Pezizales arocoscyphaceae) is recorded here for the first time from India.

*Nanoscypha tetraspora*, (Seaver ex Seaver) Denison,

Mycologia **64**: 69, 1972.

Basionymum : *Cookeina tetraspora* Seaver, in Stevenson, J. Agric. Univ. P.R. **2**: 160, 1980.

Ascocarps up to 5.5 mm across, sessile; orange-coloured ; hymenium concave, smooth ; margin raised, lighter-coloured, fimbriate. Asci clavate-cylindrical, 4-spored. Ascospores ellipsoid (23--) 24 – 26 (--27) × 10-11.5 $\mu$m, hyaline, finely striated. Paraphyses filiform. Collection examined : PAN 11616, on dead wood, Darjeeling, alt. 2,050m, West Bengal, October 13,1977. Leg. M.P. Sharma.

*N. tetraspora* is being recorded from India for the first time. It is quite a distinct species of the genus and is easily identified by its orange-coloured disc with fimbriate margin, 4-spored asci, and large striated ascospores. The Indian collection examined in quite typical of the species.

The author thanks Drs. W.D. Graddon Ross-on-Wye, England and B. M. Spooner, Royal Botanic Gardens, Kew, England for their help in confirming the identification.

The collection has been deposited in PAN (Herbarium, Botany Department, Panjab University, Chandigarh) and K (Royal Botanic Gardens, Kew, England). The generic concept followed is according to Denison[1].

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# CHANGES IN THE ASCORBIC ACID CONTENTS IN ORANGE FRUITS (*CITRUS RETICULATA* LINN) INFECTED WITH *CURVULARIA LUNATA* (WAKK.) BOEDJIN

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Free ascorbic acid in healthy and diseased orange fruits (*Citrus reticulata* Linn) infected with *Curvularia lunata* (Wakk.) Boed. was determined quantitatively. The fungal invasion caused a sharp decline in the ascorbic acid content of the infected host indicating pronounced effect on the host metabolism as a result of host parasite interaction.

*Curvularia lunata* was isolated from orange fruit. Fruits were inoculated by Granger and Horn's method[4] and incubated at $28\pm1°C$ in a sterilized moist chamber for 12 days.

On every third day of incubation, 5g of diseased portions, adjacent to the inoculated region, were removed and macerated by grinding with acid washed sand and 10 ml of extraction solution (0.005 m, Ethylene-diaminetetraacetic acid disodium salt in 3% trichloroacetic acid) of Freebaim[5]. Then it was filtered through Whatman No. 42 filter paper using suction pump. The residue was washed twice or thrice and then discarded. The total volume of the filtrate was made upto 25 ml with extracting solution and then centrifuged at 4000 rpm for 15 minutes to obtain a clear solution. Similarly, 5gms of the rind from the healthy fruits were extracted to serve as control.

The indicator dye used for ascorbic acid estimation was 2-6 dichlorophenol indophenol.[6]

It was found that rind in orange contains good amount of ascorbic acid (Table 1). After 12 days of incubation the amount of ascorbic acid was depleted by 19.19% in healthy pulp, whereas in diseased tissue, it decreased upto 54.72%, indicating thereby, a rapid fall in ascorbic acid content during pathogenesis.

Loss of ascorbic acid in the infected fruit may be due to the production of certain degenerating enzymes like polyphenol oxidase, ascorbic acid oxidase, peroxidase and cytochrome oxidase either by the fungus alone or by the host parasite interactions.[7,8,9,10,11]

Increased respiration rate during pathogenesis also results in decrease of ascorbic acid contents. This in turn may induce increased oxidation of ascorbic acid as observed in many fungi.[12,13,14]

TABLE 1

Changes in the ascorbic acid contents in the rind of healthy and diseased orange fruits inoculated with *Curvularia lunata*

| Incubation Period in days | Healthy | | Diseased | |
|---|---|---|---|---|
| | Amount (mg/100 gm) | % loss | Amount (mg/100 gm) | % loss |
| 0 | 50.85 | | | |
| 3 | 47.00 | 7.58 | 45.95 | 9.31 |
| 6 | 43.00 | 15.48 | 32.50 | 34.86 |
| 9 | 42.00 | 17.43 | 25.05 | 49.02 |
| 12 | 40.75 | 19.19 | 22.05 | 54.72 |

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# EXCESS VOLUMES OF BINARY MIXTURES CONTAINING CHLOROBENZENE AND NITRILES

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Excess volumes for binary mixtures of chlorobenzene with acetonitrile and propionitrile have been determined dilatometrically over a range of temperature. The results have been interpreted in terms of charge-transfer complexation between unlike molecules. The effect of temperature on excess volume has been studied.

Mixtures of chlorobenzene with nitriles are of interest from the view point of the existence of specific interaction between unlike molecules. This is attributable to chlorine atom in chlorobenzene, which can act as an electron acceptor towards the nitrile group. As the nitrogen atom in nitriles has a lone pair of electrons, it may be expected to behave as an electron donor. Extensive studies concerning the interaction between components of such systems over an appreciable temperature have not been made. Hence, the present study has been undertaken to report new experimental data for excess volume of the mixtures of chlorobenzene with acetonitrile and propionitrile. Further, the results have been collected over a temperature range, 303.15–333.15 K, to study the influence of temperature on the factors contributing to excess volume.

Chlorobenzene was purified by washing repeatedly with portions of sulphuric acid until the acid was no longer coloured. It was washed with water and dilute potassium carbonate solution, dried with calcium chloride and fractionally distilled[1]. Analytical grade acetonitrile and propionitrile were used without further purification. Purities of the samples were checked by comparing the measured densities with those reported in the literature[2] and the agreement was within experimental error ($\pm 5 \times 10^{-5}$ g cm$^{-3}$).

Excess volumes were measured using a single composition per loading type dilatometer described earlier[3]. The dilatometer consisted of a U-tube with mercury at the bottom to separate the two components. One arm of the U-tube was fitted with a ground glass stopper and the other arm with a capillary that can be replaced by another with thicker (or thinner) bore. Composition was determined directly by weighing. Mixing was carried out by tilting the dilatometer, thereby breaking the mercury seal. Four dilatometers with different capacities were used to cover the entire composition range. The dilatometers were kept in a thermostat maintained at $\pm 0.01$K. The values of $V_E$ were accurate to $\pm 0.003$ cm$^3$ mol$^{-1}$. The data were collected at 303.15, 313.15, 323.15 and 333.15 K.

Excess volume data for the mixtures of chlorobenzene with acetonitrile and propionitrile are presented in Table 1 and 2 respectively. The dependence of $V_E$ on mole fraction may be represented by an empirical equation of the form :

TABLE 1

Experimental molar excess volumes for chlorobenzene + acetonitrile

| 303.15 K | | 313.15 K | | 323.15 K | | 333.15 K | |
|---|---|---|---|---|---|---|---|
| $x$ | $V^E$ | $x$ | $V^E$ | $x$ | $V^E$ | $x$ | $V^E$ |
| 0.0868 | −0.046 | 0.0872 | −0.053 | 0.1161 | −0.076 | 0.0592 | −0.052 |
| 0.1751 | −0.077 | 0.1262 | −0.072 | 0.1773 | −0.101 | 0.1499 | −0.098 |
| 0.2692 | −0.101 | 0.1830 | −0.094 | 0.2686 | −0.126 | 0.2489 | −0.130 |
| 0.3733 | −0.116 | 0.3059 | −0.122 | 0.3210 | −0.136 | 0.3080 | −0.140 |
| 0.4571 | −0.122 | 0.4149 | −0.133 | 0.4277 | −0.148 | 0.4181 | −0.154 |
| 0.5695 | −0.118 | 0.5206 | −0.132 | 0.5591 | −0.145 | 0.5359 | −0.153 |
| 0.6873 | −0.103 | 0.6477 | −0.121 | 0.6814 | −0.128 | 0.6231 | −0.145 |
| 0.7605 | −0.087 | 0.7328 | −0.104 | 0.7570 | −0.112 | 0.7008 | −0.131 |
| 0.8536 | −0.061 | 0.8144 | −0.082 | 0.8602 | −0.076 | 0.8168 | −0.097 |
| 0.9269 | −0.034 | 0.9261 | −0.040 | 0.9263 | −0.045 | 0.9041 | −0.061 |

$x$ indicates mole fraction of chlorobenzene, $V^E$ in $cm^3\ mol^{-1}$

TABLE 2

Experimental molar excess volumes for chlorobenzene + propionitrile

| 303.15 K | | 313.15 K | | 323.15 K | | 333.15 K | |
|---|---|---|---|---|---|---|---|
| $x$ | $V^E$ | $x$ | $V^E$ | $x$ | $V^E$ | $x$ | $V^E$ |
| 0.0867 | −0.081 | 0.0726 | −0.077 | 0.0806 | −0.099 | 0.0772 | −0.109 |
| 0.1640 | −0.140 | 0.1534 | −0.149 | 0.1404 | −0.158 | 0.1698 | −0.207 |
| 0.2309 | −0.188 | 0.2179 | −0.198 | 0.2341 | −0.239 | 0.2220 | −0.250 |
| 0.3172 | −0.238 | 0.3041 | −0.251 | 0.3178 | −0.293 | 0.3029 | −0.306 |
| 0.4220 | −0.275 | 0.4223 | −0.294 | 0.4003 | −0.326 | 0.3705 | −0.336 |
| 0.5034 | −0.280 | 0.5110 | −0.300 | 0.5150 | −0.332 | 0.4956 | −0.354 |
| 0.6281 | −0.251 | 0.6279 | −0.272 | 0.6271 | −0.301 | 0.6268 | −0.322 |
| 0.7426 | −0.192 | 0.7133 | −0.230 | 0.7322 | −0.241 | 0.7514 | −0.248 |
| 0.8350 | −0.134 | 0.8260 | −0.154 | 0.8291 | −0.169 | 0.8340 | −0.182 |
| 0.9259 | −0.061 | 0.9329 | −0.067 | 0.9059 | −0.098 | 0.9173 | −0.102 |

$x$ indicates mole fraction of chlorobenzene, $V^E$ in $cm^3\ mol^{-1}$

TABLE 3

Values of the parameters for equation (1) and the values of standard deviation, $\sigma(V^E)$

| T/K | $a_0$ | $a_1$ | $a_2$ | $\sigma(V^E)$ / $cm^3\ mol^{-1}$ |
|---|---|---|---|---|
| | | $xC_6H_5Cl+(1-x)CH_3CN$ | | |
| 303.15 | −0.482 | 0.043 | −0.078 | 0.001 |
| 313.15 | −0.533 | 0.060 | −0.136 | 0.001 |
| 323.15 | −0.588 | 0.057 | −0.168 | 0.001 |
| 333.15 | −0.602 | 0.082 | −0.272 | 0.003 |
| | | $xC_6H_5Cl+(1-x)C_2H_5CN$ | | |
| 303.15 | −1.108 | 0.061 | 0.230 | 0.003 |
| 313.15 | −1.187 | 0.057 | 0.137 | 0.003 |
| 323.15 | −1.326 | 0.110 | 0.142 | 0.003 |
| 333.15 | −1.400 | 0.085 | −0.023 | 0.003 |

$$V^E = x(1-x)\,[a_0 + a_1\,(2x-1) + a_2\,(2x-1)^2] \quad (1)$$

where $a_0$, $a_1$ and $a_2$ are the adjustable parameters. $x$ represents mole fraction of chlorobenzene. The values of the parameters, calculated by the method of least squares are given in Table 3 along with the values of standard deviation, $\sigma(V^E)$.

The data included in Table 1 and 2 show that the volumes of $V^E$ are negative for the two systems over the whole range of composition and the entire range of temperature covered. The values of $V^E$ may be explained in terms of two opposing effects ; (1) expansion in volume due to loss of diplor association between like molecules and dispersion forces, (2) contractiou due to charge-transfer, dipole-dipolc and dipole-induced dipole interactions. The actual value of $V^E$ would depend upon the relative strengths of the two opposing effects. The experimental results suggest that the latter effect is dominant over the former in both the systems at all temperatures.

The negative values of $V^E$ for the mixtures of chlorobenzene with acetonitrile and propionitrile at all the temperatures follow the order acetonitrile< propionitrile. This may be attributable to the greater donor property of propionitrile.

A comparision of the data at 303.15, 313.15, 323.15 and 333.15 K, reveals that the values of $V^E$ become more negative with increasing temperature in both the systems. This may be explained by assuming decrease in molar volume of the complexes with increase in temperature. This contention receives support from the work of Fenby *et al.*[4], Kowalski *et al.*[5], Krishnaiah and Naidu[6].

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# FORMATION OF BENZIMIDAZOLE-2-ALDOXIME COMPLEXES WITH Cu(II), Co(II), Ni(II) AND Fe(II) IN 50% v/v AQUEOUS-ETHANOL MEDIUM

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Proton-ligand formation constants of benzimidazole-2- aldoxime and the formation constants of its complexes with Cu(II), Co(II), Ni(II) and Fe(II) have been determined potentiometrically in 50% V/V aqueous-ethanol medium at a constant ionic strength of 0.1M ($KNO_3$) and at 30°C using the Irving-Rossotti titration technique. The order of formation constants Cu(II)>Fe(II)>Co(II)>Ni(II) violates the Irving-Williams order. The reasons for the unusual stabilities of Fe(II) and Co(II) has been discussed in the light of strong field nature of the ligand.

Benzimidazoles play an important role in biology and analytical chemistry. In continuation of our earlier work on complexing tendencies of 2-substituted benzimidazoles[1], we have undertaken benzimidazole-2-aldoxime (BAOXH) as complexing agent. BAOXH is known to form solid complexes with copper.[2] Its 1-benzyl derivative has also been used for extractive photometric determination of Cu and Co in steels[3]. No work has been reported on the formation of BAOXH complexes in solution. Hence, a potentiometric study has been undertaken to determine the formation constants of BAOXH complexes with Cu(II), Co(II), Ni(II) and Fe(II) in 50% V/V aqueous-ethanol medium at a constant ionic strength of 0.1 M $KNO_3$ and at 30°C using the Irving-Rossotti titration technique[4].

The general experimental procedure was as given in our earlier work[1]. All chemicals used were of Analar grade. The metal nitrate solutions prepared, using conductance water, were standardised by conventional methods.[5] The ligand BAOXH was prepared as per procedure given in the literature[6].

The experimental procedure involved the potentiometric titrations of the following 50% V/V aqueous-ethanol mixtures against carbonate free standard KOH solution[7].

(i) $HNO_3$ (0.005 M) + $KNO_3$ (0.1M)

(ii) $HNO_3$ (0.005 M) + $KNO_3$ (0.1M) + Ligand (0.002 M)

(iii) $HNO_3$ (0.005 M) + $KNO_3$ (0.1M) + Ligand (0.002 M) + metal nitrate (0.0004 M)

All the titrations were performed in an inert atmosphere of oxygen and $CO_2$ free nitrogen. The pH measurements were made with an Elico Digital

pH meter model LI 120 equipped with a CN 67 combination electrode. The system was thermostated at 30° ±0.1 throughout the experiment. The pH metes readings (B) for 50% V/V aqueous-ethanol medium were corrected using the method suggested by Van Uitert and Haas[8].

From the titration curves, the values of $\bar{n}_H$, $\bar{n}$ and pL were evaluated using Irving and Rossotti method[4].

Proton-ligand formation curve was obtained by plotting pH vs $\bar{n}_H$ (the average number of protons bound to ligand). From this curve, the proton-ligand formation constants log $K_1^H$ and log $K_2^H$ have been evaluated by Bjerrum's half-integral method and also by point-wise calculation method. The value of log $K_1^H$ corresponds to the association of proton to the "Pyridyl nitrogen" of the imidazole ring while log $K_2^H$ to the oxime (-OH) group.

The metal-ligand titration curves were well below the metal ion hydrolysis curves, indicating the completion of complexation before the onset of hydrolysis of metal ions. The low concentrations of the metal ions used are likely to discourage the formation of polynuclear complexes. The maximum $\bar{n}$ values for all the metals were found between 1.55 to 1.65 indicating the formation of both 1 : 1 and 1 : 2 complexes. The metal-ligand formation curves were drawn by plotting $\bar{n}$ vs the free ligand exponent, pL. From these curves, the metal-ligand formation constants were determined by Bjerrum's half-interg-ral method. The refinement of the values was also done by other computational methods[9] such as point-wise calculation and least-squares methods. Their average values along with the values of log $K_1^H$ and log $K_2^H$ are summarised in Table-I. The order of stability constants obtained in this investigation Cu(II)>Fe(II)>Co(II)>Ni(II) does not conform to the Irving-Williams[10] order. Such a deviation was also observed in our earlier studies on other 2-substituted benzimidazoles[1].

TABLE I

Proton-Ligand and metal-ligand formation constants of BAOXH in 50% V/V Aqueous-ethanol medium

Ionic strength = 0.1 M and t = 30°C

| Cation | $H^+$ | Cu(II) | Fe(II) | Co(II) | Ni(II) |
|---|---|---|---|---|---|
| log $K_1$ | 10.15 | 9.49 | 8.84 | 8.47 | 4.35 |
| log $K_2$ | 3.42 | 8.34 | 5.87 | 3.28 | 2.82 |
| log $\beta_2$ | 13.57 | 17.83 | 14.71 | 11.75 | 7.17 |

One of the reasons for the higher stabilities of Fe(II) and Co(II) complexes could be the possible oxidation of the systems in alkaline medium. However, this was ruled out as the maximum $\bar{n}$ values (which will indicate the completion of complexation) lie within the acidic region. It was also found that these systems do not get oxidised even upto pH 10 so readily under $N_2$ atmosphere, and the stability constants can be determined under these conditions[11].

Further a correlation of this data with that of pyridine-2-aldoxime (PAH)[12,13] (a structurally analogous compound) reveals that the results are not anamolous, but are due to the strong field nature of the ligand[13]. Like PAH, benzimidazole was proved to be a strong field ligand by Goodgame *et al*[14]. Both the ligands have free $\pi$-orbitals capable of accepting electrons from the metal atom (back coordination). This back coordination strengthens the metal donor bond, which accounts for the higher stability of the system. From the present investigations it would be concluded that BAOXH acts as

a bidentate ligand coordinating through pyridyl nitrogen of imidazole ring[15,16] and oxime nitrogen[2,13].

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# SOME SUBSTITUTED BENZENEAL KANAMINES AS ANTIAMEBIC AGENTS

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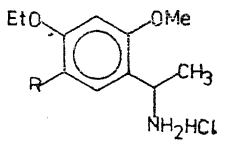
Some 4-ethoxy-2-methoxy-5-alkyl-*a*-methylbenzene methanamines and 4-ethoxy-2-methoxy-5-alkylbenzeneethanamines have been synthesised from the corresponding acetophenones and benzaldehydes. These have been screened for in vitro antiamebic activity against E. histolytica which is reported.

Substituted benzenealkanamines have been described to possess antiamebic activity[1-4]. Retention of the dimethoxy moiety on the phenyl group was regarded as being essential for the maintenance of activity[5,6]. We have observed that the presence of an ethoxy group ortho to the alkyl chain increases the activity of these compounds. We report the synthesis and in vitro antiamebic activity of 4-ethoxy-2-methoxy-5-alkyl-*a*-methylbenzenemethanamines and 4-ethoxy-2-methoxy-5-alkylbenzeneethanamines (Table I).

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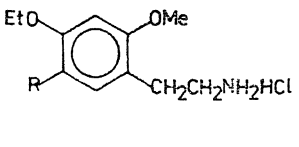


II

R = Et, n-Pr, n-Bu

All m.ps are uncorrected. 'H NMR was recorded with TMS as internal standard and chemical shifts are reported in δ (ppm) units.

*4-ethoxy-2-methoxy-5-alkyl-α-methylbenzenemethanamines (I)* :

The 2, 4-dihydroxy-5-alkylacetophenones were prepared by the Nencki reaction of 4-alkylresorcinols. The nonchelated hydroxyl group was easily ethylated by the $K_2CO_3$/acetone method in 3 hrs, while the chelated hydroxyl group was methylated in 36 hrs. The ketones were crystallizcd from ethyl acetate-pet. ether and characterised by elemental analysis and spectral data (UV, IR and 'H NMR). The 4-ethoxy-2-methoxy-5-alkyl acetophenones were converted into the oximes and reduced by zinc dust/$NH_4OH$ to the corresponding benzenemethanamines, isolated as hydrochlorides by passing dry HCl through their etheral solutions. Their IR spectra in KBr showed the following prominent bands. $\nu$ $cm^{-1}$: 3020-2860 (N-H stretch), 1605, 1475 (C-C ring stretch), 1580 and 1505 (N-H band), 1290 ( C-O-C assymetric stretch), 1045 (C—O—C symmetric stretch). 'H NMR (TFA) gave the signals at : δ 1.0—1.66 (m, $-CH_3$ and saturated $CH_2$—groups), 2.56-2.60 (2H, t, Ar—$CH_2$—), 4.0 (3H, s, $-OCH_3$—), 4.16-4.20 (2H, o, $-OCH_2$—), 6.73 -6.75 (1H, s, Ar—H), 7.10-7.16 (1H, s, Ar—H).

*4-ethoxy-2-methoxy-5-alkylbenzeneethanamines*(II):

4-ethoxy-2-methoxy-5-alkylbenzaldehydes ware

TABLE 1

4-Ethoxy-2-methoxy-5-alkylbenzenealkanamine hydrochlorides* and their antiamebic activity

| Compound | R | m.p. | Mol. formula | Antiamebic Activity $\mu$g/ml (min. inhib. conc.) |
|---|---|---|---|---|
| Ia | Et | 221° | $C_{13}H_{22}O_2NCl$ | N.A.** |
| Ib | n-pr | 205-6° | $C_{14}H_{24}O_2NCl$ | 1000 |
| Ic | n-Bu | 185-7° | $C_{15}H_{26}O_2NCl$ | 500 |
| IIa | Et | 227-8° | $C_{13}H_{22}O_2NCl$ | 250 |
| IIb | n-pr | 158° | $C_{14}H_{24}O_2NCl$ | 125 |
| IIc | n-Bu | 156-7° | $C_{15}H_{26}O_2NCl$ | 62.5 |

*All compounds gave satisfactory C,H and N analysis results
**Non active at 1000 $\mu$g/ml

prepared by the Vilsmeir reaction of the corresponding 2-ethoxy-4-methoxyalkylbenzenes. The aldehydes were crystallized from light petroleum and also characterised by elemental analysis and spectral data (UV, IR and $^1$H NMR). These were converted into corresponding $\beta$-nitrostyrenes by condensation with nitromethane. The yellow crystalline nitrostyrenes were characterised and reduced to the corresponding benzeneethanamines with LAH, and isolated as hydrochlorides. Their IR spectra in KBr showed the following prominent bands, $\nu cm^{-1}$ : 3020 -2840 (N—H stretch) 1605, 1475 (C—C ring stretch). 1580 and 1505 (N—H bend), 1290 (C—O—C asymmetric stretch), 1045 (C—O—C symmetric stretch). Their NMR (TFA) gave the signals at : $\delta$ 1.0 (t,—$CH_3$), 1.50 (3H, t,—$OCH_2-CH_3$ and m, saturated —$CH_2$—gps) 2.56—2.60 (2H, t. Ar—$CH_2$—), 3.06—3.10 (2H, t, Ar—$CH_2$—$CH_2$—$NH_2$), 3.50 ( 2H, m, Ar—$CH_2$—$CH_2$—$NH_2$, 3.96—4.0 (3H. s,—$OCH_3$), 4.16—4.20 (2H, q,—$OCH_2$—), 6.70—6.73 (1H, s, Ar—H), 7.03—7.06 (1H, s, Ar—H).

The in vitro testing of the compounds against axenically grown E. histolytica was carried out with solutions in sterile distilled water after seitz filteration. The minimum inhibitory concentration was then determined by the cavity shide method[7]. Substituted benzeneethanamines were more active than the corresponding benzenemethanamines and 4-ethoxy-2-methoxy compounds more than the corresponding 2,4-dimethoxy compoundwith the increasing length of the alkyl chain it was observed that the antiamebic activity increased regularly. The length of the alkyl chain thus seems to have a direct

relation to the antiamebic activity of these compounds.

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# DISLOCATION TRAJECTORIES IN SOLU TION GROWN NICKEL SULPHATE SINGLE CRYSTALS

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Well defined square shaped etch pits are produced on etching with methanol (010) basal cleavages of solution grown nickel sulphate single crystals which are confirmed to nucleate at the sites of intersection of dislocations with the cleavage face. On successively etching isolated cleavages, it is concluded that the trajectories of dis locations in the case of nickel su'phate sirgle crystals, to the extent of region of surface removal by etching, are not all straight but curved.

Controlled dissolution of single crystals by suitable solvents predicts very important and useful information regarding the defects and history of growth of the crystals.[1-10] In the present note, an attempt is made to study the trajectory of dislocations in solution grown Nickel sulphate single crystals using methanol as the etchant.

In order to grow the crystals, a saturated solution of Nickel sulphate at 100°C was prepared and cooled in SICO furnace at the rate of 5°C per hour till the room temperature was reached. It was then kept undisturbed for a week. One of the crystals from the above lot was used as the seed hung in the mother liquor and allowed to grow bigger. The maximum size of the crystal that could be got by the above process was 27mm × 20mm × 15 mm. The crystals grown by the present method were ascertained to be single crystals from (i) the Laue pattern obtained by allowing X-ray beam incident normal to the well-defined (010) basal cleavage (ii) one-to-one correspondence on match pair of (010) basal cleavages [Figs. 1 (a) and 1 (b) ] (iii) from the square shaped point and flat bottomed well defined etch pits on (010) basal cleavages formed by etching in methanol for the required time [Fig. 2], meaning ordered dissolution rates[9].

That the isolated and clustered etch pits nucleate at the sites of dislocations, has been confirmed by (i) successive etching of isolated cleavages where the pits grow in size and depth as the duration of etching increases (ii) etching of matched faces and thin flakes, in cases of which, perfect correlation in the etch patterns exists.

Figures 3 and 4 are the photomicrographs of the etch patterns formed on etching a basal cleavage for 2 minutes and 10 minutes in methanol. The following points merit discussion.

1. The pit marked 'A' continues to grow in size and depth, as the etching progresses.
2. The pit marked 'B' grows bigger in size and depth, and becomes flat bottomed in the second stage of etching, and
3. The pit marked 'C', which is flat bottomed in the first stage of etching, disappears during the second stage of etching.

These observations can be explained by postulating the trajectories of dislocations as schematically represented in Fig. 5. As long as the etch pit remains point bottomed only enlarging the size and depth, suggests that the dislocation runs straight in the body of the crystal undeviated, at least to the depth of the crystal investigated by etching (as in the case of pit 'A'). Once the pit enlarging in size and depth changes to a flat bottomed one, suggests that the dislocation is getting deviated (as in the case of pit 'B'). The disappearance of a flat bottomed pit on continued etching (as in the case of pit 'C') gives the clue that the dislocation deviates almost parallel to the cleavage face.

It is concluded that the trajectories of dislocations in the case of Nickel sulphate single crystals, to the extent of region of surface removal by etching (37 to 40 $\mu$), are not all straight but curved as shown in the Fig. 5.

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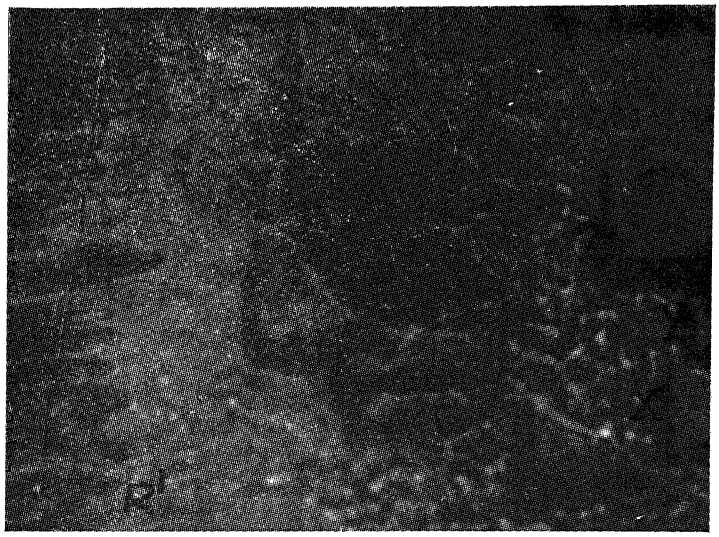

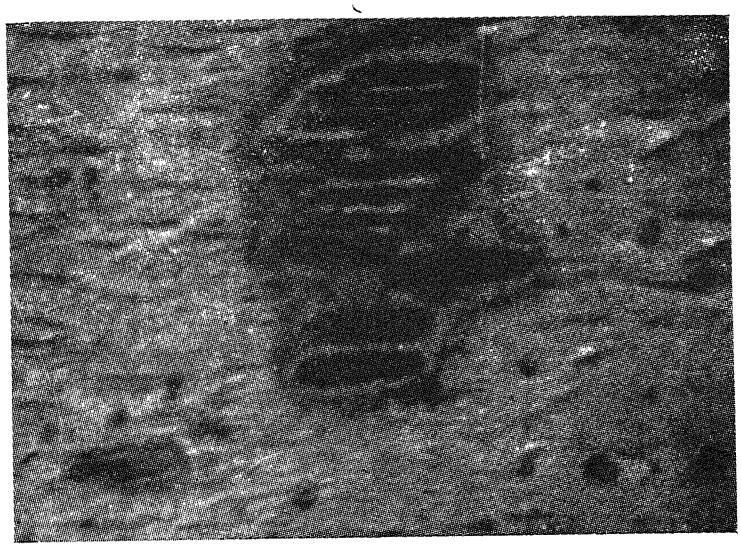

Fig. 1 (a) and (b) X 175 Photomicrographs of a matched cleavage pair.

Fig. 2 X 350 Etch pattern revealing well defined square pits.

Fig. 3 X 350 Etch pattern on (010) cleavage of the crystal etched in Methanol for two minutes.

Fig. 4 X 350 The same region of Fig. 1, after etching for 10 minutes.

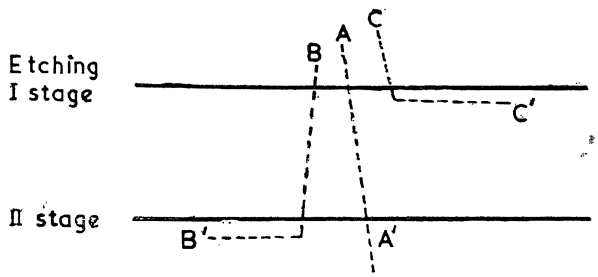


Fig. 5 Schematic diagram representing the trajectories of dislocations.

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# EFFECT OF MALATHION ON SOME FUN-CTIONAL ASPECTS OF NITROGEN UTILITY IN THE TELEOST, *TILAPIA MOSSAMBICA* (PETERS)

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The ammonia and urea content of the tissues in mala thion exposed (ME) fishes decreased considerably, suggesting a fall in their production. The glutamine content of the ME tissues enhanced remarkably. Thus the ammonia seems to be recycled thereby facilitating the nitrogen metabolism for the better utilisation of nitrogen. This higher potentials of nitrogen conservation along with nucleic acids (DNA and RNA) suggest the diversion of nitrogen moity probably towards higher protein synthetic potentials, during sublethal stress.

The biological activity of the organophosphorus insecticides is generally attributed to the inhibition of the enzyme, cholinesterase[1]. However, changes in the physiological events other than acetylcholinesterase inhibition cannot be ignored, since pathological changes in liver and testes of rats treated with diazinon were reported[2]. Elevation in the ratios of serum proteins and lipoproteins in the blood of carps treated with dimethyl-thiophosphate[3] and changes in RNA contents of the tissues treated with α-napthyl thiourea[4] were reported in a number of species. However, literature on physiological changes associated with nitrogen metabolism in the fish exposed to sublethal concentration of malathion is not covered. Hence this report is intended to give the regulative potential of the fish in counteracting the toxicity of malathion, the organophosphorous pesticide being extensively used in a variety of formulations to control a wide range of insect species under different conditions.

The fish, *T. mossambica*, were collected from fresh water ponds in and around Tirupati, and acclimatised to laboratory conditions. They were fed with groundnut cake daily and frog muscle twice a week. Fishes in batches of 8 each were exposed to 70 litres of 2ppm malathion solution (sublethal) in tap water for 48 h. These arrangements tend to give similar ratio of fish biomass to water volume. Other factors which may influence the toxicity of malathion were nullified by maintaining suitable controls in tap water. After exposure to malathion these fishes were chosen for experimental analysis. The tissues like muscle (red), gill and liver were separated quickly and kept in ice-jacketed pyrex petridish. In each tissue nucleic acids (DNA and RNA) were extracted by the method of Smiley and Krotokov[5]. The DNA content was estimated using diphenylamine reagent[6] and RNA content was estimated using orcinol reagent[7]. The ammonia and glutamine contents were estimated by the method of Bergmeyer[8] and the urea content by the method as described by Natelson[9]. The data was subjected to statistical analysis as per Bailey[10].

From Table 1 it is evident that ammonia and

TABLE 1

Changes in ammonia, urea, glutamine and nucleic acid (DNA RNA) content of the muscle, gill and liver tissues of normal and malathion exposed (ME) fishes at 48 h of malathion exposure. The value are expressed in $\mu$ moles/g wet wt. of tissue for ammonia, urea and glutamine, and mg/g wet wt. tissue for DNA and RNA. Each value is the mean ±S. D. of 6 individual observations. NS=not significant; + or − indicate percent increase of decrease over normal respectively.

| | MUSCLE | | | GILL | | | LIVER | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normal | ME | 't' test | Normal | ME | 't' Test | Normal | ME | 't' test |
| Ammonia | 12.89 | 11.41 | | 9.38 | 7.52 | | 10.58 | 7.73 | |
| | ±2.62 | ±2.76 | NS | ±1.40 | ±1.04 | P<0.001 | ±1.08 | ±2.52 | P<0.001 |
| | | −11.48 | | | −19.83 | | | −26.94 | |
| Urea | 5.01 | 4.27 | | 5.71 | 5.05 | | 12.52 | 9.73 | |
| | ±1.68 | ±1.46 | NS | ±1.64 | ±2.04 | NS | ±1.87 | ±0.66 | P<0.001 |
| | | | | | −11.39 | | | −22.28 | |
| Glutamine | 96.60 | 132.96 | | 32.20 | 41.25 | | 130,81 | 182.12 | |
| | ±7.34 | ±8.05 | P<0.001 | ±3.21 | ±4.74 | P<0.05 | ±6.51 | ±11.05 | P<0.001 |
| | | +37.63 | | | +28.10 | | | +39.23 | |
| DNA | 1.037 | 1.448 | | 1.240 | 1.640 | | 3.00 | 3.905 | |
| | ±0.171 | ±0.182 | P<0.001 | ±1.60 | ±0.122 | P<0.001 | ±0.181 | ±0.262 | P<0.001 |
| | | +39.63 | | | +32.26 | | | +30.16 | |
| RNA | 2.610 | 3.378 | | 2.393 | 2.940 | | 6.852 | 10.132 | |
| | ±0.448 | ±0.008 | P<0.05 | ±0.301 | ±0.473 | P<0.05 | ±0.671 | ±0.706 | P<0.001 |
| | | +29.43 | | | +22.86 | | | +47.87 | |

urea contents of muscle, gill and liver tissues tend to decrease considerably. The decrease in ammonia content of muscle and urea content of muscle and gill tissues were found to be statistically insignificant (Table 1). The glutamine and nucleic acid (DNA and RNA) levels were enhanced considerably and were found to be statistically significant (Table 1).

The decreased ammonia content of the tissues in ME fishes envisages the low production of ammonia, probably by a fall in the glutamate dehydrogenase, the enzyme that catalyses the ammonia production. This appears reasonable since glutamate dehydrogenase was found to decrease during malathion stress[11]. Hence, it is likely that the nitrogen metabolism of ME fishes seem to be shifted towards lessening of the ammonia production, thereby restoring the higher levels of glutamate to be used for various biosynthetic and energetic requirements during malathion imposed toxic stress.

Though teleost fishes were mostly ammonotelic, in the present study, the presence of urea in the tissues of normal and ME fishes envisages the presence and synthesis of urea. It was also reported that a full complement of urea cycle enzymes could be detected in a wide variety of fresh water and marine teleosts, albeit with low levels of activity[12]. However, except for liver the urea content of the muscle and gill tissues may be due to vascular mobilisation from liver, since the presence of ornithine cycle enzymes in these tissues is rather doubtful[13]. In line with the decreased production of ammonia, the urea content of the tissues was also decreased during malathion stress. This decrease in urea content might be due to a reduction in the supply of ammonia to ornithine cycle. However, the glutamine content of the ME tissues increased considerably (Table 1) suggestive of a shift in emphasis towards the mobilisation of ammonia for glutamine production[14]. Thus the ammonia seems to be recycled, thereby fecilitating the nitrogen metabolism for the better utilisation of the nitrogen source. This higher potentials of nitrogen conservation in ME fish tissues may be utilised for higher protein synthetic potentials. This appears true, since the nucleic acids are associated with protein synthesis, the increased RNA content of the ME tissues (Table 1) evidently suggest enhanced protein synthesis during malathion exposure. Patil and Murthy[4] also reported increased nucleic acid content in rats administered with α-napthylthiourea, a rodenticide. However, It is not known how the DNA content of ME tissues was enhanced. It is likely that the toxicity of malathion may induce higher proteolysis leading to cell destruction which might have resulted in higher DNA content in the tissues of ME fishes.

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# A STUDY ON THE LIPID CONTENT OF A SPIDER *HIPPASA GREENALLIAE* (BLACKWELL) (FAMILY : LYCOSIDAE)

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In Hippasa *greeenalliae* (Blackwell ) the amount of total lipid is directly proportional to body weight of both the sexes. But the amount of free fatty acids is inversely proportional to the weight of the body irrespective of sex. The female has a slightly higher amount of total lipid while the male has a higher percentage of free fatty acids.

A wide variation in lipid content of insects has been reported by many workers[1–5]. In most of the female insects the amount of lipid present in the body is more than that present in the males. This may be due to the fact that the lipid is the most efficient substrate for egg development in them. But in many species of Lepidoptera the males have greater amount of lipid in their body than the females. The higher content of lipid in the male moths may be utilized during flight in search of the virgin females[6-8]. In migratory moths there is no sexual dimorphism in lipid content because both the sexes migrate and use equal amount of lipid.[9] While the information on the quantitative and qualitative aspects of lipids in insects is enormous very little is known about them in spiders.

Millot[10] has reported that the spiders have more amount of lipid than glycogen in their body. Blum *et al.*[11] have shown the presence of seven fatty acids in the spider *Nephila clavipes*. The investigation by Stewart and Martin[12] on the spider, *Dugesiella hentzii* reveals that the average fat content in this species is 10.3% of the body weight. Thus the information available on the amount of lipid and free fatty acids in spiders in relation to sex and body weight is meagre. Therefore the present investigation is undertaken to estimate the amount of total lipid and free fatty acids in the spider *Hippasa greenalliae* in relation to sex and body weight.

A total number of 200 specimens were collected from the fields around Coimbatore for the present study and reared individually in plastic containers. Each animal was fed with three immobilized cockroach nymphs per day for three days. For the next two days no food was given to them. On the sixth day 48 females and 48 males were selected for the experiments. The lipid content was estimated separately in males and females following Mishra and Mishra[13]. The percentage of free fatty acids was determined as described by Plummer[14].

The results are given in Tables 1-4. They reveal that the females of *H. greenalliae* on average, contain 11.4 mg total lipid for 301.68 mg body weight, i.e., 3.81%. The males have 8.0 mg total lipid for 212.14 mg body weight, i.e., 3.78%. The

TABLE 1

Data showing the total lipid content in the female *Hippasa greenalliae.*

| Body weight in mg | Lipid content in mg | Percentage of lipid |
|---|---|---|
| 126.86 | 4.9 | 3.89 |
| 229.26 | 8.9 | 3.88 |
| 235.63 | 9.1 | 3.86 |
| 300.87 | 11.6 | 3.85 |
| 310.36 | 11.9 | 3.83 |
| 355.75 | 13.3 | 3.74 |
| 405.36 | 15.1 | 3.72 |
| 450.38 | 16.7 | 3.71 |
| Average | | |
| 301.68 | 11.4 | 3.81 |

Each value is an average of 3 estimations.

TABLE 2

Data showing the total lipid content in the male *Hippasa greenalliae*

| Body weight in mg | Lipid content in mg | Percentage of lipid |
|---|---|---|
| 141.38 | 5.3 | 3.74 |
| 155.86 | 5.8 | 3.77 |
| 161.49 | 6.1 | 3.77 |
| 205.54 | 7.8 | 3.80 |
| 216.72 | 8.5 | 3.80 |
| 247.04 | 9.4 | 3.80 |
| 260.44 | 9.9 | 3.80 |
| 293.63 | 11.4 | 3.81 |
| Average | | |
| 212.14 | 8.0 | 3.78 |

Each value is an average of 3 estimations.

TABLE 3

Data showing the free fatty acid value in the female *Hippasa greenalliae*

| Body weight in mg | Acid value | Percentage of acid value |
|---|---|---|
| 149.72 | 0.157 | 0.10 |
| 174.22 | 0.150 | 0.08 |
| 198.94 | 0.147 | 0.07 |
| 241.06 | 0.122 | 0.05 |
| 329.99 | 0.120 | 0.03 |
| 356.65 | 0.107 | 0.03 |
| 378.79 | 0.097 | 0.02 |
| 392.96 | 0.087 | 0.02 |
| Average | | |
| 277.79 | 0.124 | 0.05 |

Each value is an average of 3 estimations.

TABLE 4

Data showing the free fatty acid value in the male *Hippasa greenalliae*

| Body weight in mg | Acid value | Percentage of acid value |
|---|---|---|
| 169.57 | 0.183 | 0.10 |
| 182.41 | 0.174 | 0.09 |
| 191.44 | 0.169 | 0.08 |
| 208.45 | 0.164 | 0.07 |
| 210.10 | 0.154 | 0.07 |
| 214.74 | 0.151 | 0.07 |
| 221.80 | 0.148 | 0.06 |
| 246.01 | 0.138 | 0.05 |
| Average | | |
| 205.44 | 0.160 | 0.07 |

Each value is an average of 3 estimations.

total amount of lipid is directly proportional to the weight of the body in both the sexes. Though there is no great difference in total lipid content between sexes the female has a little more than the male. This greater amount of lipid in female may be required for the synthesis and storage of yolk in the eggs as in the case of many insects[3].

The average value of free fatty acids in the females is 0.05% while in the males it is 0.07%. The amount of free fatty acids in the males of *H. greenalliae* is slightly higher than that in the females. It is also observed that the acid value is inversely proportional to the weight of the body and the amount of lipid in both the sexes.

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