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editorial

Greetings to
all our contributors
and readers

The year 2011 marks the 7th year of publication of Scientia an annual science journal from Mercy college, Palakkad. We are happy to bring out this issue of Scientia which features 24 articles from various areas of science. Increased competition for the publication of scientific research has led to an increased emphasis on determining the perceived “quality” or “status” of a specific journal. After all, scientists, like everyone else, want to publish papers in journals where their work is likely to have the highest impact. The editors of this journal strive to promote the writing of scientific papers in clear, straight forward, idiomatic English, so that the findings of the authors may be transmitted to the readers as lucidly and unambiguously as possible. Scientia is interested in publishing a wide range of manuscripts presenting original research and reviews in all areas of science. For original research, the common thread is that the work should reveal novel concepts of broad importance to the scientific community. Categories of papers include reviews, mini reviews, full papers and short communications. Scientia covers frontier areas like Physics, Chemistry, Mathematics, Computer science, Bioinformatics and Life sciences. Let the 7th volume of Scientia nurture the diversity with 5 review papers, 2 mini reviews, 15 full papers and 2 short communications. Scientia congratulates all our contributors and readers for your achievement of 2011 and wishes all of you a Happy New Year.



With warm personal regards.



Jayasree

Dr. S. Jayasree
(Chief Editor)

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Gumbel Distributions And Its Applications

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Abstract

Marshall-Olkin Gumbel distributions are introduced. Some properties of the distributions are considered. Expressions for the n th moments are given. The unknown parameters of the distributions are estimated and some numerical results of the estimations are given. Autoregressive time series models of order 1 as well as k are developed with minification structure, having these stationary marginal distributions. Various characterizations are obtained.

Keywords: Marshall-Olkin distribution Gumbel distributions- Estimation -Minification process

1. Introduction

The development of extreme value distributions proceeded to some extent outside the mainstream of statistical distribution theory, with its early stage dominated by work on curve fitting and the later stage by problems encountered in statistical inference. The extreme value theory is a blend of an enormous variety of applications involving natural phenomena such as rainfall, floods, wind gusts, air pollution, and corrosion, and delicate mathematical results on point processes and regularly varying functions. Extreme value theory seems to have originated mainly from the needs of astronomers in utilizing or rejecting outlying observations.

The importance of the Gumbel distribution in practice is due to its extreme value behaviour. It has been applied either as the parent distribution or as an asymptotic approximation, to describe extreme wind speeds, sea wave heights, floods, rainfall, age at death, minimum temperature, rainfall during droughts, electrical strength of materials, air pollution problems, geological problems, naval engineering etc.

In section 2, the new Marshall-Olkin Gumbel maximum (MO-GUMX) distribution is introduced and its distributional properties are investigated. The graphs corresponding to probability density function and hazard rate function are given. Some characteristic properties are established. In section 3, we construct the first order and k^{th} order autoregressive model having minification structure. The sample path behaviour is also considered. In section 4, we considered the new Marshall-Olkin Gumbel minimum (MO-GUMN) distribution and its properties are studied. In section 5, we consider AR (1) model and AR(k) model with MO-GUMN marginal.

2. Marshall-Olkin Generalized Gumbel Family

Consider a survival function F . Then a new family of survival functions is constructed as¹

$$G(x; \alpha) = \frac{\alpha F(x)}{1 - (1 - \alpha) F(x)} \quad ; -\infty < x < \infty, 0 < \alpha < \infty. \tag{2.1}$$

Clearly when $\alpha = 1$, we get $G = F$.

Whenever F has a density, the survival function G given by (2.1) have easily computed densities. In particular, if F has a density f and hazard rate r_F , then G has the density g given by

$$g(x; \alpha) = \frac{\alpha f(x)}{\left[1 - (1 - \alpha) F(x)\right]^2} \quad ; -\infty < x < \infty, 0 < \alpha < \infty$$

and hazard rate

$$r(x; \alpha) = \frac{r_F(x)}{\left[1 - (1 - \alpha) F(x)\right]} \quad ; -\infty < x < \infty, 0 < \alpha < \infty,$$

Consider the Gumbel maximum distribution with survival function (see Castillo (1988))

$$F(x) = 1 - \exp\left(-\exp\left[-\frac{(x - \lambda)}{\delta}\right]\right); \quad -\infty < x < \infty; \delta > 0$$

where λ and δ are constants known as the location and scale parameters. Substituting this in (2.1) we get a new family of distributions, which we shall refer to as MO-GUMX family, whose survival function is given by

$$G(x) = \frac{\alpha \{1 - \exp[-\exp(-\frac{(x - \lambda)}{\delta})]\}}{1 - (1 - \alpha)[1 - \exp[-\exp(-\frac{(x - \lambda)}{\delta})]]} \quad ; x \in R, \delta, \alpha > 0, \lambda \in R.$$

The probability density function is

$$g(x) = \frac{\alpha \exp(-\frac{(x - \lambda)}{\delta}) \{ \exp[-\exp(-\frac{(x - \lambda)}{\delta})] \}}{\delta \{1 - (1 - \alpha)[1 - \exp[-\exp(-\frac{(x - \lambda)}{\delta})]]\}^2} \quad ; x \in R, \delta, \alpha > 0.$$

The probability density function $g(x)$ has a unique mode at $x = x_0$, where x_0 is the solution of the equation

$$-1 + \alpha - (1 - \alpha)s(x) - \alpha \exp(s(x)) = 0, \text{ and } s(x) = \exp(-(x - \lambda)/\delta).$$

Furthermore, $g(-\infty) = g(\infty) = 0$.

The hazard rate function is

$$r(x) = \frac{\exp(-\frac{(x-\lambda)}{\delta}) \{ \exp[-\exp(-\frac{(x-\lambda)}{\delta})] \}}{\delta \{ 1 - (1-\alpha) [1 - \exp[-\exp(-\frac{(x-\lambda)}{\delta})]] \} \{ 1 - \exp(-\exp(-\frac{(x-\lambda)}{\delta})) \}}$$

$$x \in R, \delta, \alpha > 0, \lambda \in R.$$

If $0 < \alpha < 0.5$, then the hazard rate function $r(x)$ has a maximum at $x = x_1$, where x_1 is the solution of the equation

$$1 - \alpha + (1 - \alpha) s(x) + (2\alpha - 1) \exp(s(x)) - \alpha \exp(2s(x)) - \alpha s(x) \exp(2s(x)) = 0.$$

Furthermore, $r(-\infty) = 0$ and $r(\infty) = 1/\delta$. If $\alpha > 0.5$, then the hazard rate function $r(x)$ is an increasing function with $r(-\infty) = 0$ and $r(\infty) = 1/\delta$.

Figure 1 gives the graph of the probability density function and hazard rate function for different values of $r(x)$ for different values of α, λ and δ .

Let us consider the n^{th} moment of the MO-GUMX distribution. We will consider MO-GUMX(0,1, α) distribution, since if $X \sim$ MO-GUMX(α, λ, δ), then $Y = (X - \lambda) / \delta \sim$ MO-GUMX(0,1, α). Then the n^{th} moment of the random variable Y can be written as

$$E(Y^n) = \alpha \int_{-\infty}^{\infty} \frac{y^n e^{-y} e^{-e^{-y}} dy}{(\alpha + (1-\alpha)e^{-e^{-y}})^2} = \alpha(-1)^n \int_0^{\infty} \frac{(\log u)^n e^{-u} du}{(\alpha - (1-\alpha)e^{-u})^2}.$$

Using the expansions

$$\frac{1}{(\alpha + (1-\alpha)e^{-u})^2} = \begin{cases} \sum_{i=1}^{\infty} i(1-\alpha)^{i-1} \sum_{j=0}^{i-1} (-1)^j (i-1) e^{-ju}, 0 < \alpha < 1 \\ \frac{1}{\alpha^2} \sum_{i=0}^{\infty} i \left(\frac{\alpha-1}{\alpha}\right)^{i-1} e^{-i \ln \alpha}, \alpha > 1 \end{cases} \tag{2.2}$$

and (2.6.21.1) from Prudnikov et al. (1986)

$$\int_0^{\infty} (\log u)^n e^{-u} du = \left(\frac{\partial}{\partial a} \right)^n \left(\frac{\Gamma(a)}{j^a} \right) \Big|_{a=1}$$

we obtain that the n^{th} moment of $Y = \text{MO-GUMX}(0,1,\alpha)$ is

$$E(Y^n) = \begin{cases} \alpha (-1)^n \sum_{i=1}^{\infty} i(1-\alpha)^{i-1} \sum_{j=0}^{i-1} (-1)^j (j-1) C_{i,j} \left(\frac{\partial}{\partial \alpha}\right)^n \left(\frac{\Gamma(\alpha)}{(j-1)^r}\right) & -\alpha = 1, 0 < \alpha < 1 \\ \frac{(1-\alpha)^n}{\alpha} \sum_{i=1}^{\infty} i \left(\frac{\alpha-1}{\alpha}\right)^{i-1} \left(\frac{\partial}{\partial \alpha}\right)^n \left(\frac{\Gamma(\alpha)}{i^r}\right) & -\alpha = 1, \alpha \geq 1. \end{cases}$$

We consider now the maximum likelihood estimation of the unknown parameters λ , and α . We estimate the unknown parameters by using the maximum likelihood function. The log-likelihood function is

$$\text{Log } L = n \log \alpha - \frac{1}{\delta} \sum_{i=1}^n (x_i - \lambda) - \sum_{i=1}^n s(x_i) - n \log \delta - 2 \sum_{i=1}^n \log(\alpha + (1-\alpha)e^{-s(x_i)}).$$

The first partial derivatives are

$$\frac{\partial \log L}{\partial \lambda} = \frac{n}{\delta} - \frac{1}{\delta} \sum_{i=1}^n s(x_i) - \frac{2(1-\alpha)}{\delta} \sum_{i=1}^n \frac{s(x_i)e^{-s(x_i)}}{\alpha + (1-\alpha)e^{-s(x_i)}}$$

$$\frac{\partial \log L}{\partial \delta} = \frac{1}{\delta^2} \sum_{i=1}^n (x_i - \lambda) - \frac{1}{\delta^2} \sum_{i=1}^n (x_i - \lambda)s(x_i) - \frac{n}{\delta} + \frac{2(1-\alpha)}{\delta^2} \sum_{i=1}^n \frac{(x_i - \lambda)s(x_i)e^{-s(x_i)}}{\alpha + (1-\alpha)e^{-s(x_i)}}$$

$$\frac{\partial \log L}{\partial \alpha} = \frac{n}{\alpha} - 2 \sum_{i=1}^n \frac{1 - e^{-s(x_i)}}{\alpha + (1-\alpha)e^{-s(x_i)}}$$

Solving the equations $\frac{\partial \log L}{\partial \lambda} = 0$, $\frac{\partial \log L}{\partial \delta} = 0$ and $\frac{\partial \log L}{\partial \alpha} = 0$, we obtain the maximum likelihood

estimates of the unknown parameters.

Definition 1 Let X_1, X_2, \dots be a sequence of i.i.d. random variables with distribution in the family (1) and suppose N is independent of the X_i 's with a geometric(p) distribution such that

$$P(N = n) = p(1-p)^{n-1}, n = 1, 2, \dots$$

Let $U_N = \min(X_1, X_2, \dots, X_N)$ and $V_N = \max(X_1, X_2, \dots, X_N)$. If $F \in \Phi$ implies that the distribution of $U(V)$ is in Φ , then F is said to be geometric minimum stable (geometric maximum stable). If F is both geometric minimum and geometric maximum stable, then F is said to be geometric extreme stable.

Now we have the following theorem

Theorem 2.1

Marshall-Olkin Gumbel Maximum distribution is geometric extreme stable.

Theorem 2.2

Let $\{X_i, i \geq 1\}$ be a sequence of independent and identically distributed random variables with common survival function $\bar{F}(x)$ and N be a geometric random variable with parameter p and $P(N = n) = pq^{n-1}, n = 1, 2, \dots, 0 < p < 1, q = 1-p$, which is independent of $\{X_i\}$ for all $i \geq 1$. Let $U_N = \min_{1 \leq i \leq N} X_i$. Then $\{U_N\}$ is distributed as MO-GUMX if and only if $\{X_i\}$ is distributed as GUMX.

3. An AR (1) model with MO-GUMX marginal distribution

Theorem 3.1

Consider the AR (1) structure

$$X_n = \begin{cases} \epsilon_n & \text{with probability } p \\ \min(X_{n-1}, \epsilon_n) & \text{with probability } (1-p) \end{cases} \quad (3.1)$$

$0 \leq p \leq 1$, where $\{\epsilon_n\}$ is a sequence of i.i.d. random variables independent of $\{X_0, X_1, X_2, \dots\}$. Then $\{X_n\}$ is a stationary Markovian AR(1) process with MO-GUMX marginals if and only if $\{\epsilon_n\}$ is distributed as GUMX distribution.

Theorem 3.2

Consider an autoregressive minification process X_n of order k with structure (3.3). Then $\{X_n\}$ has stationary marginal distribution as MO-GUMX if and only if $\{\epsilon_n\}$ is distributed as GUMX. The sample path of the process for different values of p, λ and δ are given in Figure 2.

$$X_n = \begin{cases} \epsilon_n & \text{w.p. } p_0 \\ \min(X_{n-1}, \epsilon_n) & \text{w.p. } p_1 \\ \min(X_{n-2}, \epsilon_n) & \text{w.p. } p_2 \\ \dots \\ \min(X_{n-k}, \epsilon_n) & \text{w.p. } p_k \end{cases} \quad (3.3)$$

where $0 \leq p_i < 1, (p_0 + p_1 + \dots + p_k) = 1 - p_0$.

4.Marshall-Olkin Gumbel distribution for minimum

Consider Gumbel minimum distribution with survival function ²

$$F(x) = \exp[-\exp(-\frac{(\lambda-x)}{\delta})]; \quad -\infty < x < \infty; \delta > 0, \text{ where } \lambda \text{ and } \delta \text{ are constants known as}$$

the location and scale parameters. Substituting this in (2.1), we get a new family of distributions, which we shall refer to as MO-GUMN family, whose survival function is given by

$$G(x) = \frac{\alpha \{\exp[-\exp(-\frac{(\lambda-x)}{\delta})]\}}{1 - (1-\alpha) \{\exp[-\exp(-\frac{(\lambda-x)}{\delta})]\}}; \quad -\infty < x < \infty, \delta, \alpha > 0.$$

The probability density function is

$$g(x) = \frac{\alpha \exp(-\frac{(\lambda-x)}{\delta}) \{\exp[-\exp(-\frac{(\lambda-x)}{\delta})]\}}{\delta \{1 - (1-\alpha) \{\exp[-\exp(-\frac{(\lambda-x)}{\delta})]\}\}^2}; \quad -\infty < x < \infty, \delta, \alpha > 0.$$

The probability density function g(x) has a unique mode at x = x₀, where x₀ is the solution of the equation

$$1-s_1(x) - (1-\alpha) e^{-s_1(x)} - (1-\alpha)s_1(x)e^{-s_1(x)} = 0$$

And s₁(x) = exp(-(λ - x)/δ). Furthermore, we have that g(-∞) = g(∞) = 0.

The hazard rate function is given by

$$r(x) = \frac{\exp(-\frac{(\lambda-x)}{\delta})}{\delta \{1 - (1-\alpha) \exp[-\exp(-\frac{(\lambda-x)}{\delta})]\}}.$$

Figure 3 gives the graph of the probability density function and hazard rate function of the Marshall-Olkin Gumble distribution for minimum.

Let Y ^d = MO-GUMN(0,1,α). Then the nth moment of the random variable Y can be written as

$$E(Y^n) = \alpha \int_{-\infty}^{\infty} \frac{y^n e^y e^{-e^y} dy}{(1 - (1-\alpha)e^{-e^y})^2} = \alpha \int_0^1 \frac{(\log u)^n e^{-u} du}{(1 - (1-\alpha)e^{-u})^2}.$$

Using the expansions

$$\frac{1}{(1-(1-\alpha)e^{-x})^\alpha} = \begin{cases} \sum_{i=1}^{\infty} i(1-\alpha)^{i-1} e^{-ix}, 0 < \alpha < 1 \\ \frac{1}{\alpha^2} \sum_{i=1}^{\infty} i \left(\frac{\alpha-1}{\alpha}\right)^{i-1} \sum_{j=0}^{i-1} (-1)^j (i-1) C_j e^{-ix}, \alpha \geq 1 \end{cases} \quad (4.1)$$

and (2.6.21.1) from ³

We obtain that the n^{th} moment of $Y = \text{MO-GUMN}(0,1,\alpha)$ is

$E(Y^n) =$

$$\begin{cases} \alpha \sum_{i=1}^{\infty} i(1-\alpha)^{i-1} \left(\frac{\partial}{\partial \alpha}\right)^n \left(\frac{\Gamma(a)}{(i)^a}\right), a=1, 0 < \alpha < 1 \\ \frac{1}{\alpha} \sum_{i=1}^{\infty} i \left(\frac{\alpha-1}{\alpha}\right)^{i-1} \sum_{j=0}^{i-1} (-1)^j (i-1) C_j \left(\frac{\partial}{\partial \alpha}\right)^n \left(\frac{\partial}{\partial \alpha}\right)^n \left(\frac{\Gamma(a)}{i^a}\right), a=1, \alpha \geq 1 \end{cases}$$

We consider now the maximum likelihood estimation of the unknown parameters α, λ and δ . We estimate the unknown parameters by using the maximum likelihood function. The log-likelihood function is

$$\text{Log } L = n \log \alpha - \frac{1}{\delta} \sum_{i=1}^n (\lambda - x_i) - \sum_{i=1}^n s_i(x_i) - n \log \delta - 2 \sum_{i=1}^n \log(1 - (1-\alpha)e^{-s_i(x_i)}).$$

and the normal equations are

$$\frac{\partial \log L}{\partial \lambda} = -\frac{n}{\delta} - \frac{1}{\delta} \sum_{i=1}^n s_i(x_i) + \frac{2(1-\alpha)}{\delta} \sum_{i=1}^n \frac{s_i(x_i) e^{-s_i(x_i)}}{1 - (1-\alpha)e^{-s_i(x_i)}} = 0$$

$$\frac{\partial \log L}{\partial \delta} = \frac{1}{\delta^2} \sum_{i=1}^n (\lambda - x_i) - \frac{1}{\delta^2} \sum_{i=1}^n (\lambda - x_i) s_i(x_i) - \frac{n}{\delta} - \frac{2(1-\alpha)}{\delta^2} \sum_{i=1}^n \frac{(\lambda - x_i) s_i(x_i) e^{-s_i(x_i)}}{1 - (1-\alpha)e^{-s_i(x_i)}} = 0$$

$$\frac{\partial \log L}{\partial \alpha} = \frac{n}{\alpha} - 2 \sum_{i=1}^n \frac{e^{-s_i(x_i)}}{1 - (1-\alpha)e^{-s_i(x_i)}} = 0$$

Solving the equations $\frac{\partial \log L}{\partial \lambda} = 0, \frac{\partial \log L}{\partial \delta} = 0$ and $\frac{\partial \log L}{\partial \alpha} = 0$, we obtain the maximum likelihood estimates of the unknown parameters.

Theorem 4.1

Marshall-Olkin Gumbel Minimum distribution is geometric extreme stable.

Theorem 4.2

Let $\{X_i, i \geq 1\}$ be a sequence of independent and identically distributed random variables with common survival function $\bar{F}(x)$ and N be a geometric random variable with parameter p and $P(N = n) = p q^{n-1}; n = 1, 2, \dots, 0 < p < 1, q = 1 - p$, which is independent of $\{X_i\}$ for all $i \geq 1$. Let $U_N = \min_{1 \leq i \leq N} X_i$.

Then $\{U_N\}$ is distributed as MO-GUMN if and only if $\{X_i\}$ is distributed as GUMN.

5. An AR (1) model with MO-GUMN marginal distribution**Theorem 5.1**

Consider an AR (1) structure given by (3.1), where $\{\varepsilon_n\}$ is a sequence of independent and identically distributed random variables independent of $\{X_{n-1}, X_{n-2}, \dots\}$, then $\{X_n\}$ is stationary Markovian AR(1) process with MO-GUMN marginals if and only if $\{\varepsilon_n\}$ is distributed as GUMN distribution.

Theorem 5.2

Consider an autoregressive minification process X_n of order k with structure (3.3). Then $\{X_n\}$ has stationary marginal distribution as MO-GUMN if and only if $\{\varepsilon_n\}$ is distributed as GUMN.

6. Data analysis

In this section we consider some real datasets and compare our distributions with the Generalized Extreme Value (GEV) distribution, Exponentiated Exponential (EE) distribution^{3,2,6}, Exponentiated Gumbel(EG) distribution⁶, Exponentiated Frechet(EF) distribution⁶ and Exponentiated Weibull(EW) distribution^{6,7,8,9,10}.

Data Set 1: First we start with data used in⁹.

The data represent the number of million revolutions before failure 23 ball bearings: 17.88, 28.92, 33.41, 52.42, 12.45, 6.48, 8.51, 84.51, 96.54, 12.55, 56,

67.8, 68.64, 68.64, 68.88, 84.12, 93.12, 98.64, 105.12, 105.84, 127.92, 128.04, 173.4. We estimate the unknown parameters of each distribution by using the function nlm from statistical software R. The maximum likelihood estimators of the unknown parameters of each distributions with log-likelihood, chi-squared and Kolmogorov-Smirnov statistics are presented in Table 1. In Table 2, we present the observed and expected frequencies. We can see that GEV, EE, EG, EF, EW, MO-GUMX, MO-FRMX, MO-WEMX and MO-WEMN very well fit this data set.

Data set 2: Here we consider the annual maximum of daily precipitation amounts at Fort Collins, CO, USA, 1900-1999. We compare the distributions and the maximum likelihood estimators of the unknown parameters in Table 3. We can see that GEV,EE,EG,EF,EW and MOGMX very well fit this data set.

Table 1. Estimated values, log-likelihood, chisquare and Kolmogorov-Smirnov statistics for data set 1.

Distribution	Parameters	LL	X^2	P value	K-S	P value
GEV	$\mu = 54:7058, \sigma = 27:0711, \xi = 0:0647$	-113.1026	0.5446	0.9690	0.1020	0.9704
EE	$\mu = 4:5598, \lambda = 31:8469, \alpha = 4:2039$	-112.9675	0.6667	0.9554	0.1025	0.9690
EG	$\mu = 44:3327, \delta = 19:8066, \alpha = 0:5864$	-113.0214	0.5269	0.9708	0.0972	0.9816
EF	$\mu = 3:0269, \sigma = 3558:6693, \lambda = 0:4063, \alpha = 123:0949$	-112.9581	0.7753	0.9417	0.1083	0.9501
EW	$\mu = 8:9647, \lambda = 1:1450, \delta = 41:4873, \alpha = 2:4418$	-112.9274	0.8458	0.9322	0.1036	0.9659
MO-GUMX	$\lambda = 66:0185, \delta = 31:5734, \alpha = 0:5442$	-113.1061	0.5940	0.9637	0.1004	0.9745
MO-GUMN	$\lambda = 192:1738, \delta = 20:5667, \alpha = 0:0024$	-115.3448	4.0475	0.3996	0.1425	0.7387
MO-FRMX	$\lambda = 479:7238, \delta = 540:8498, \beta = 18:1845, \alpha = 0:6874$	-113.1019	0.4808	0.9753	0.0966	0.9827
MO-FRMN	$\lambda = 409:0825, \delta = 197:0443, \beta = 15:6323, \alpha = 0:0002$	-116.2849	6.0874	0.1927	0.1571	0.6214
MO-W1-MX	$\lambda = 2984:6329, \delta = 2914:8867, \beta = 88:018, \alpha = 0:4527$	-113.1146	0.5641	0.9670	0.1001	0.9752
MO-W1-MN	$\lambda = 10:8413, \delta = 98:2219, \beta = 2:202, \alpha = 0:3065$	-112.7986	0.8499	0.9316	0.1060	0.9582

Table 2. Observed and expected frequencies

Intervals	0-40	40-80	80-120	120-160	160-200
Observed	3	12	5	2	1
GEV	4.0432	11.3180	5.3189	1.6147	0.7052
EE	4.3082	10.9087	5.3139	1.7441	0.7251
EG	4.1551	11.2174	5.1951	1.6839	0.7485
EF	4.2758	10.8528	5.4798	1.7459	0.6457
EW	4.4835	10.6705	5.3666	1.7896	0.6898
MO-GUMX	3.9827	11.4519	5.3249	1.6041	0.6364
MO-GUMN	4.6735	10.0726	6.5696	1.4496	0.2347
MO-FRMX	4.0165	11.4469	5.1933	1.6036	0.7397
MO-FRMN	4.9511	9.3648	6.9822	1.5262	0.1757
MO-WEMX	3.9914	11.4467	5.2945	1.6122	0.6552
MO-WEMN	4.3451	10.7652	5.4057	1.8763	0.6077

Table 3. Estimated values, log-likelihood, chisquared and Kolmogorov-Smirnov statistics for data set 2.

Distribution	Parameters	LL	X^2	P value	K-S	P value
GEV	$\mu = 1:3467, \alpha = 0:5328$ $\xi = 0:1736$	-104.9645	0.7376	0.9466	0.0452	0.9869
EE	$\mu = 0:5276, \lambda = 0:7172$ $\alpha = 2:5985$	-104.1575	0.6884	0.9528	0.0412	0.9958
EG	$\mu = 0:9952, \sigma = 0:2680$ $\alpha = 0:3297$	-104.2126	0.3657	0.9852	0.0348	0.9997
EF	$\mu = 0:4056, \delta = 16:9550$ $\lambda = 0:4962, \alpha = 30:5674$	-104.2775	0.4240	0.9805	0.0365	0.9993
EW	$\mu = 0:5102, \lambda = 0:9328$ $\delta = 0:6236, \alpha = 3:1816$	-104.1431	0.5282	0.9707	0.0396	0.9976
MO-GUMX	$\lambda = 1:4889, \delta = 0:6178$ $\alpha = 0:7271$	-106.6311	3.1698	0.5298	0.0495	0.9673
MO-GUMN	$\lambda = 5:4334, \delta = 0:4393$ $\alpha = 0:0002$	-119.1444	15.8863	0.0032	0.0867	0.4405
MO-FRMX	$\lambda = -0:5170, \delta = 1:5595$ $\beta = 4:3590, \alpha = 2:9404$	-104.9288	0.6027	0.9628	0.0425	0.9937
MO-FRMN	$\lambda = 72:4626, \delta = 67:2462$ $\beta = 166:3343, \alpha = 0:0002$	-119.7381	21.7353	0.0002	0.0847	0.4701
MO-WEMX	$\lambda = 47:5891, \delta = 45:5526$ $\beta = 54:0609, \alpha = 0:2074$	-106.3827	0.9595	0.9159	0.0462	0.9834
MO-WEMN	$\lambda = 0:5351, \delta = 2:4490$ $\beta = 2:1641, \alpha = 0:1618$	-103.8220	0.5151	0.9720	0.0374	0.9990

Table 4. Observed and expected frequencies

Intervals	0-1	1-2	2-3	3-4	4-5
Observed	16	55	20	6	3
GEV	13.6055	58.3578	20.0213	5.2899	2.7255
EE	15.0480	54.9325	21.9571	6.0239	2.0385
EG	14.3310	56.7306	20.4498	6.0078	2.4808
EF	14.5396	56.2729	21.1071	5.7265	2.3539
EW	14.9174	55.3297	21.6208	5.9714	2.1607
MO-GUMX	14.5412	56.9511	22.3332	4.9310	1.2435
MO-GUMN	17.1540	49.7042	28.3075	4.3243	0.5100
MO-FRMX	13.9981	58.0214	20.0507	5.1439	2.7859
MO-FRMN	16.8234	51.0074	27.9628	3.8233	0.3831
MO-WEMX	14.5943	57.7709	20.5075	5.1598	1.9675
MO-WEMN	14.6696	55.9756	20.9220	6.2711	2.1617

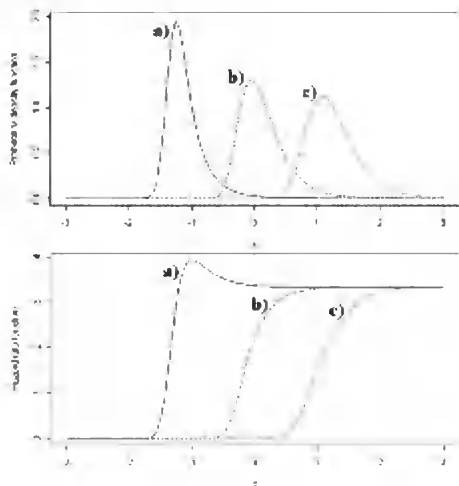


Fig. 1 Probability density function and Hazard rate function

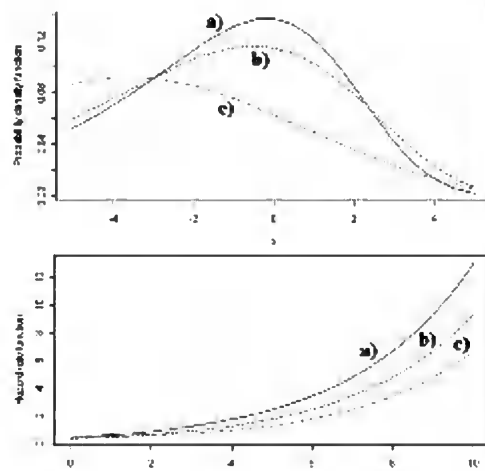


Fig. 3 Probability density function and Hazard rate function

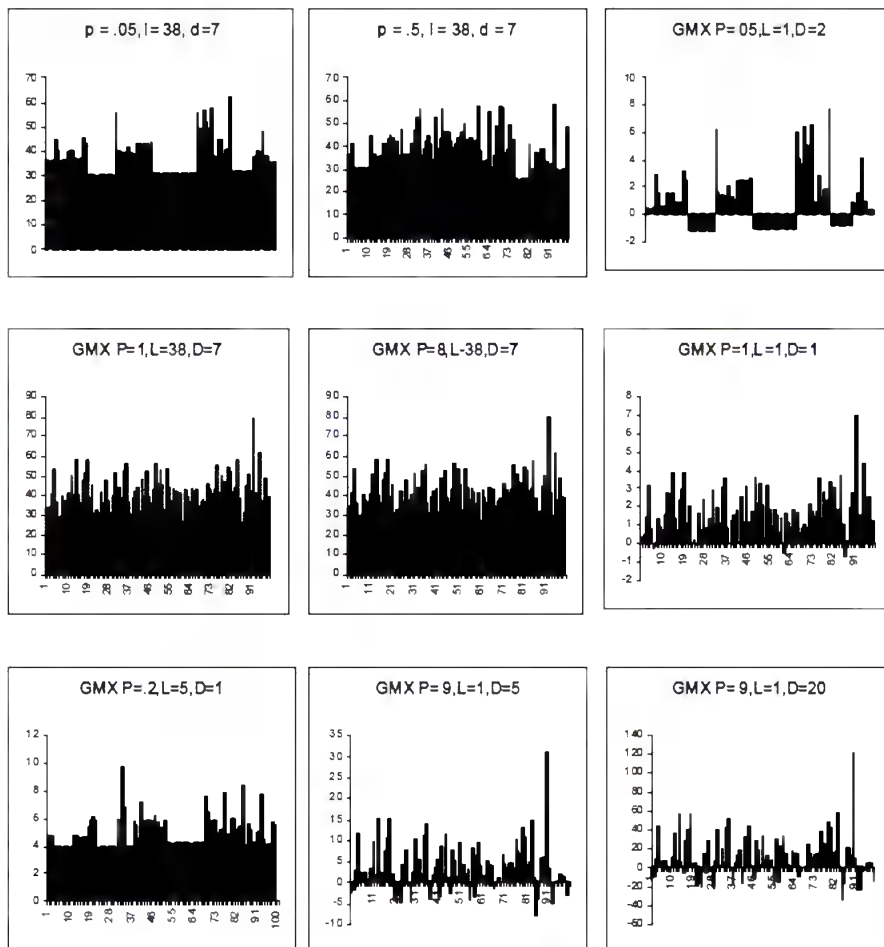


Fig 2. Sample paths of MOGUMAXAR (1) process for different values of p, λ and δ

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Electron transfer mechanism in DSSC

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Abstract

Dye Sensitized Solar Cells (DSSCs) have been an arena of intense research since its inception in 1991, as they are a promising photovoltaic technology owing to their low cost and simple manufacturing processes. However, this device has a long way to go before it proves itself in the market. Hence intense research is being done based on the electron kinetics involved in the photovoltaic conversion process of the cell. This paper is a review of the electron transfer mechanism that occurs in DSSC. The maximum current and voltage that can be drawn from this type of cell is limited by the relative energy levels of the photosensitive dye, semiconductor and electrolyte.

Key words: DSSC, HOMO, LUMO, electron transfer mechanism

Introduction

One of the major problems faced by developing countries like India is the increasing cost and demand for fossil fuels. The pollution caused by the combustion of these fuels is also alarming. The most dependable alternative solution for this problem is solar energy. But the present status of photovoltaic is not attractive due to the low commercial index of 'cost effectiveness' of solar cells. It is in this context that Dye Sensitized Solar Cells (DSSC) demands attention of scientists. Eventhough the efficiency of these cells are not upto the solid state cells based on Si, GaAr, InP, CIGS, etc. their production cost is very less. The maximum reported efficiency of these cells are only around 12%, but the commercial viability index of 'cost-effectiveness' continues to attract research in this direction¹⁻⁶. Growth of the Australian company Dyesol Inc., the first in the world to manufacture DSSC modules commercially, is ample evidence of the success of this new photovoltaic contender ever since the conception of the idea of DSSC 20 years ago.

These cells can suit a whole realm of application ranging from the light weight low-power market to large-scale applications. Their excellent performance in diffused light gives them a competitive edge over silicon in providing electric power for both indoor and outdoor stand alone electronic equipment. Application of the DSSC in building-integrated photovoltaic has already begun and will become a rich field for future commercial development. The walls of the Toyota Dream House, Japan where DSSC panels are incorporated to deliver power to the inhabitants is an excellent example.

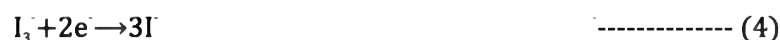
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Basic structure and mechanism

Presently, solid state semiconductor based solar cells dominate photovoltaic science and technology and silicon is the leading material. In these conventional cells, when the energy of the incident photon is greater than the bandgap of a photosensitive semiconductor, excitation of electrons occurs. A p-n junction interface serves to separate these charge carriers, i.e. electrons and holes, thereby creating a potential difference that leads to flow of current through the external circuit. On the other hand, in a Dye Sensitized Solar Cell the major functions of light absorption and charge carrier separation is achieved with the help of a sub bandgap sensitisation of the semiconductor with a photo sensitive dye. An intrinsically stable, wide bandgap semiconductor, such as titanium dioxide with its band gap of 3.1 eV, which normally exhibits a photovoltaic response only under ultra violet irradiation, can then photo respond to visible light of wavelength 400-750 nm or 1.6 – 3.0 eV photons. The photo electrochemical process in this type of cells involves the excitation of the dye from its charge neutral ground state(S) to an excited state(S^{*}) by the absorption of energy of a photon, followed by relaxation through electron loss to the semiconductor substrate (TiO₂).



The dye is left as a surface- adsorbed cation which is then neutralized by reaction with a redox species in the contacting electrolyte. The standard redox system is the iodide tri-iodide couple (I⁻/I₃⁻).



This constitutes a closed cycle for the conversion of incident light into an electric current. This regenerative photo electrochemical device is functionally equivalent to a conventional solid-state photovoltaic cell. The fact that only the dye molecules which are in immediate contact with the semiconductor is capable of transferring the photo excited charge, has lead to the use of nanostructure semiconductor (TiO₂). This will increase the active interface area for light absorption and charge transfer many folds than the projected geometrical area of the surface, thus resulting in enhanced efficiency.

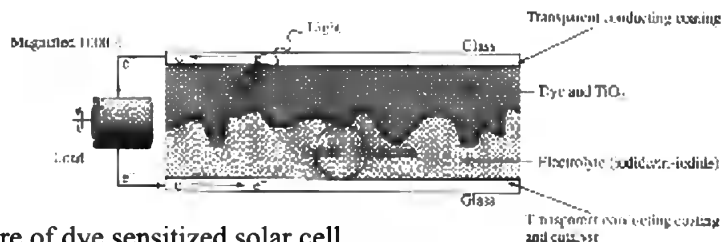


Fig. 1. A schematic structure of dye sensitized solar cell.

A practical DSSC consists of three sandwiched layers: a photo anode, an iodide / tri-iodide redox electrolyte layer and a platinised counter electrode as shown in Fig.(1). The photo anode is a thin porous layer of annealed nanocrystalline semiconductor electrode such as TiO_2 supported on transparent conducting glass. Dye molecules like ruthenium bipyridine derivatives, which are sensitive to the visible light region of the solar spectrum are attached onto the semiconductor electrode.

In a DSSC there are four energy levels that influence the performance of the cell. They are the excited state (LUMO – Lowest Unoccupied Molecular Orbit) and the ground state (HOMO - Highest Occupied Molecular Orbit) of the photosensitiser, the Fermi level of TiO_2 electrode and the redox potential of I^-/I_3^- in the electrolyte. The photo current obtained from a DSSC is determined by the energy difference between the HOMO and the LUMO of the photosensitiser. Smaller the HOMO-LUMO energy gap larger will be the photocurrent. The energy level of the LUMO must be sufficiently negative with respect to the Fermi level (approximately the conduction band level) of TiO_2 for effective injection of electrons from photosensitiser (dye) to TiO_2 . This is marked as ΔE_1 in Fig. (2). The HOMO level of the dye must be sufficiently more positive than the redox potential of the I^-/I_3^- redox mediator to accept electrons effectively (ΔE_2). The energy gaps ΔE_1 and ΔE_2 must be larger than approximately equivalent to 200 mV for optimal electron transfer⁷. The voltage developed in DSSC is determined by the energy gap between the Fermi level of TiO_2 electrode and redox potential of the I^-/I_3^- , which are estimated to be -0.5V versus normal hydrogen electrode (NHE) and 0.4V versus NHE respectively as show in Fig (2). On this basis, the maximum voltage for a DSSC is expected to be approximately 0.9V.

In contrast to a conventional p-n junction solar cell, the mechanism of a DSSC does not involve charge

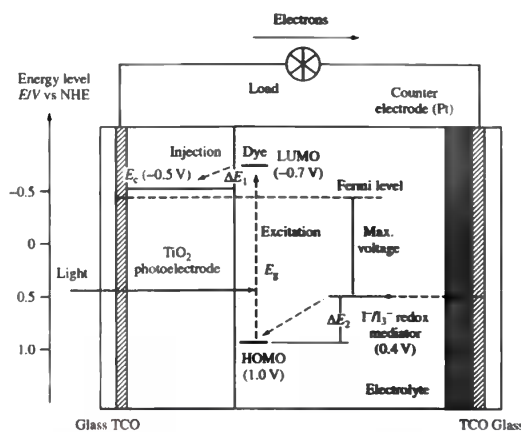


Fig. 2. Schematic energy diagram of DSSC

recombination process between electrons and holes, as electrons are only injected from the photosensitiser into the semiconductor and a hole is not formed in the valence band of the semiconductor. Further, the charge transfer takes place in the TiO_2 film, which is separated from the photon absorption site (i.e. the photosensitiser) leading to effective charge separation. This photon-to-current conversion mechanism in DSSC is similar to the mechanism for photosynthesis in nature, in which chlorophyll functions as the photosensitiser.

Charge-transfer Kinetics

The electron transfer rate from the photosensitiser into the semiconductor depends mainly on configuration of the adsorbed photosensitiser material on the semiconductor surface and also on the energy gap between the LUMO level of the photosensitiser and the conduction band level of the semiconductor. The rate constant for electron injection depends on the electronic coupling between the photosensitiser and the semiconductor and also on the density-of-states of the conduction band. Electronic coupling is attributed to overlap between the wave function of the excited states of the photosensitiser and the conduction band of TiO_2 . Usually, the photosensitiser is strongly adsorbed on the semiconductor surface with carboxyl groups as the anchor, thus resulting in a strong electronic coupling between the Π^* orbital of the excited state of the photosensitiser and the conduction band of TiO_2 , which consists of the unoccupied 3d orbital of Ti^{4+} . Moreover, the conduction band of the semiconductor has a continuous and relatively large density of states. Thus, electron injection from the photosensitiser to the semiconductor occurs at a higher rate than does the relaxation from the excited state to the ground state by the process of energy emission.

To accomplish effective charge separation, the charge recombination process between the injected electrons and oxidized dyes must be much slower than electron injection and electron transfer from I^- ion into oxidized dyes. The approximate time required for various processes are shown in Fig.(3). For

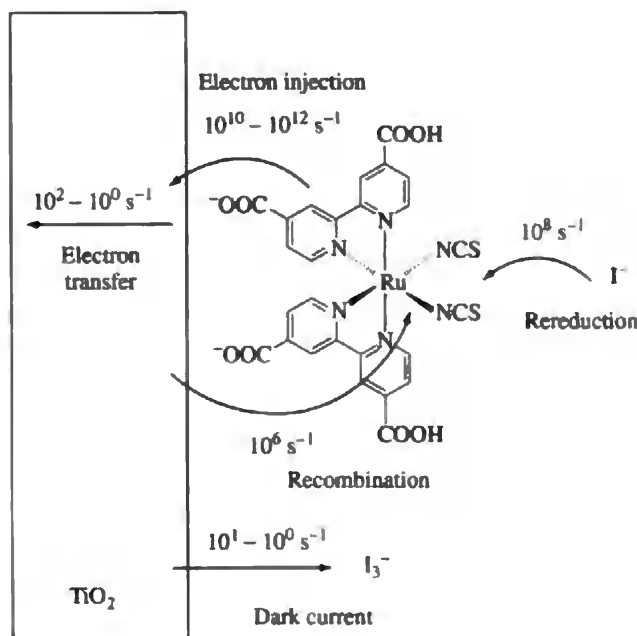


Fig. 3. Schematic diagram of electron-transfer processes

example, it has been observed that the time required for electron injection from N3 dye (a highly efficient Ruthenium complex dye) into TiO_2 is in the order of femto seconds^{8,9}. Charge recombination between injected electrons on TiO_2 and cations of N3 dye require microseconds to milliseconds, in contrast with ultra fast electron injection¹⁰⁻¹³. Thus the much slower charge recombination process compared to electron injection process leads to effective charge separation resulting in high cell performance.

Regeneration rate of the oxidized photosensitiser (dye) by the process of electron transfer from I^- also contributes to the accomplishment of effective charge separation¹⁴. The electron-transfer rate from the I^- ion into cations of the N3 dye is estimated to be 100 ns¹⁵. This reaction rate is much faster than that for charge recombination between injected electrons and dye cations.

Dark current suppresses the efficiency of DSSC. This results from the recombination of injected electrons in TiO_2 with the tri-iodide ions (I_3^-) as per eqn. (4), at the TiO_2 / electrolyte interface where the photosensitisers are not adsorbed. If this reaction occurs predominantly with a large reaction rate, then the cell output will be reduced to zero. In fact the estimated time for this recombination is in the order of 0.1 s to several seconds¹⁶. Dark current can be suppressed to some extent by using suitable co-adsorbates on the TiO_2 surface, resulting in the improvement of photovoltage¹⁷⁻¹⁸.

Basically the electron conductivity in TiO_2 is very small, resulting in slow response of the photocurrent. In DSSC under illumination it is found that electron conductivity increases significantly due to electron injection from photosensitiser¹⁹⁻²⁰. It is expected that, when the injected electrons fill the trap sites and surface defect levels of TiO_2 film, the diffusion coefficient of the electron increases drastically, resulting in increased electron conductivity and good photocurrent response.

DSSC may turn out to be a viable solution for future solar energy conversion issues on the basis of cost, efficiency, stability, availability and environmental compatibility. Recent researches in these cells are focused on increasing the efficiency and lowering the manufacturing cost by using Ruthenium free dyes. A thorough knowhow of the electron kinetics is required for improving the cell performance. Electron transfer mechanism involved in the various steps of photoconversion is discussed in this paper. For maximizing the photo current the HOMO-LUMO energygap of the photosensitive dye should be as small as possible. This would result in maximum utilisation of the visible solar spectrum. Efficient electron transfer from the dye to TiO_2 depends on the co-ordination of the dye molecule over the TiO_2 surface and also on the difference in the oxidation potentials of dye and TiO_2 . For the effective regeneration of the neutral dye, the HOMO level of the dye should be more electropositive than the redox potential of I^-/I_3^- mediator. Choosing suitable co-adsorbates along with the dye will result in the improvement of cell performance.

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Metamaterials

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Abstract

Metamaterials (MTM) have attracted great attention for the last couple of years in existing and emerging research areas. It was only in 1999 that such a media was first developed. From then onwards its properties were fascinating scientists for a wide range of research and applications. Here theoretical aspect of metamaterials having negative index of refraction is presented briefly with a cited application of “electromagnetic cloaking” phenomenon.

Key words: Metamaterials, split ring resonators, negative refraction, permeability, permittivity.

Introduction

Metamaterials ("meta" means "beyond" in Greek), also called Left Handed or Double Negative or Backward Wave media, are a new class of artificial materials with ordered composites that exhibits unusual electromagnetic properties (from microwave to optical frequencies) observed as in natural materials. The unusual behaviors of these materials are due to its negative values of permeability (μ), permittivity (ϵ) and index of refraction (n). The existence of such materials was first predicted by Russian Physicist Victor Veselago¹ in 1968.

Until 1999, a material simultaneously possessing negative permittivity and permeability was merely a theoretical concept, without any prospect of an impact on the technological world. Most of the naturally occurring materials come under the first quadrant of the μ - ϵ curve shown in fig.1. The rest of the region remained unexplored till Veselago¹. It was in 1996 for the permittivities and in 1999 for the permeabilities, that the possibility of realizing plasma-like structures in the microwave region (GHz band) was demonstrated experimentally. The growth of the subject after that was drastic, with the realization of a material simultaneously having negative permittivity and permeability in the GHz band.

Metamaterials are constructed by the periodic arrangement of resonators, similar to a crystalline structure. The 'basic' unit and the 'lattice spacing' should be shorter than the wavelength of the electromagnetic wave used. The simplest way is to construct a periodic structure of pairs of elements, of which one element array (copper wires) produces a negative permittivity (ϵ) and the other array (split ring resonators (SRR)) produces a negative permeability (μ).

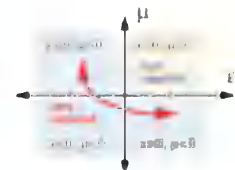


Fig.1. The classification of materials with their permittivity and permeability

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By combining these two arrays, a left-handed medium was constructed by Smith and colleagues at the University of California at San Diego (UCSD) in 2000 for the first time^{3,4}. This review discusses the unique properties of metamaterials in detail. The method of fabrication of arrays of thin conducting wires and SRR's to produce negative permittivity and permeability are also discussed. The possible applications of these materials along with its major research areas are presented in the last section.

Characteristic properties of metamaterials

Metamaterials have many similarities with photonic crystals and frequency selective surfaces. However, these are usually considered different from metamaterials, as they are of similar size to the wavelength at which they function. They cannot be approximated as a homogeneous material also.

Negative refractive index

Negative refraction can be obtained by using a metamaterial which has been designed to achieve a negative value for both electric permittivity (ϵ) and magnetic permeability (μ)^(3,4). Such materials are sometimes called "double negative" materials. The refractive index n is determined using the relation

$$n = \mp \sqrt{\mu\epsilon}.^5$$

Reversing and accelerating the speed of light

Metamaterials refract light at a negative angle, so it emerges on the left side of the incident beam. Inside the metamaterial, both the velocity of the individual wavelengths (phase velocity) and the velocity of the wave packets (group velocity) are both negative, to obtain a negatively refracted light. This condition makes the light move 'backwards' and the peak of the light pulse leaves the metamaterial on the right-hand side before it enters on the left. Consequently, energy is transferred through the material faster than the vacuum speed of light, which is not physical and violates the laws of physics, such as relativity and causality. Thus we can say that the wave packets travel with velocities much higher than the velocities of light, due to the dispersion of the negative index of refraction.^{5,6}

Negative Phase Velocity (NPV) is a property of light propagation through a metamaterial medium. A class of metamaterials which exhibit this novel behavior in the presence of an Electro Magnetic (EM) wave is termed "Double Negative materials" (DNG). For plane waves propagating in a Veselago NPV medium, the electric field, magnetic field and wave vector follow a left-hand rule, rather than the usual right-hand rule as given in fig .2. This gives rise to the name "*left-handed (meta) materials (LHM)*".^{3,4,5}

Left handed media

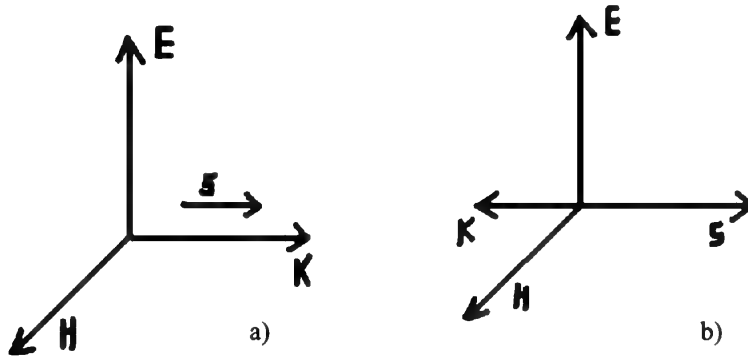


Fig. 2. Illustration of the system of vectors E (electric field vector), H (magnetic field vector), k (propagation vector), and S (poynting vector) for a plane transverse electromagnetic (TEM) wave in an ordinary (a) and a left-handed (b) medium respectively.

Snell's Law

Unlike in ordinary refraction, the angles of incidence and refraction must have opposite signs in LHM which results in the reversal of Snell's Law ($N_1 \sin \theta_1 = -N_2 \sin \theta_2$) as shown below (Fig.3) where N_1 and N_2 are the refractive index of the first and second media respectively and θ_1 and θ_2 their respective angle of incidence and angle of refractions.

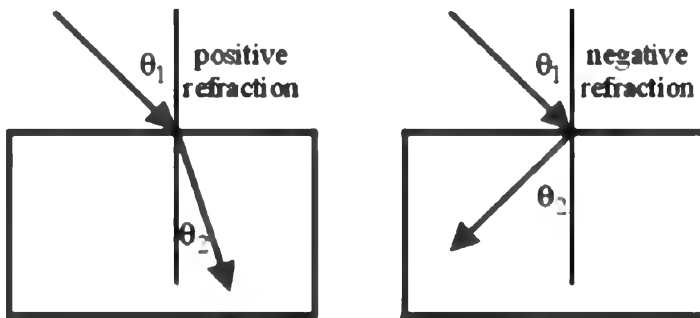


Fig.3. Negative refraction

From Fig. 3,

$$\frac{\sin \theta_1}{\sin \theta_2} = \frac{-|k_2|}{|k_1|} = \frac{n_2}{n_1} < 0$$

which is the well-known Snell's law. So here we can see that concave lenses become convergent and convex lenses become divergent^{3,4,5}.

Metamaterials also exhibit inverse Doppler Shift. Doppler shift is the phenomenon by which a light source moving toward an observer appears to increase its frequency. If an object moves in a LHM, this Doppler frequency shift is reversed. It can be explained using Drude model equation for frequency shift, which shows that the frequency will undergo a blue-shift in normal materials, but red-shift in LHM.^{3,7}

Cerenkov radiation points the other way in metamaterials. Speedy electrons or other charged particles can briefly out-run light in matter, producing a shock wave in the form of a cone of light known as Cerenkov radiation. In normal substances, the radiation is emitted in a forward cone. Left-handed metamaterials, however, have unusual effects on light that should reverse the cone's direction. In 1968 by injecting faster-than-light particles into the metamaterial created an optical analogue of particles moving at twice the

speed of light. This produces a much stronger burst of reverse Cerenkov light which suggests a new possible application of left-handed metamaterials as detectors of high-speed particles in accelerators and other experiments.^{3,8}

Other properties include Goos-Hanchen effect which results from the wave vector direction dependence of the reflection coefficient.^{3,4}

Theoretical models

In order to study the property /behavior of a medium we have to start from its atom level. Drude model⁹ is a good starting point in which the atoms and molecules of conducting materials are represented by a set of harmonically bound electron oscillators, resonant at some frequency ω_0 as shown in Fig.4.

At frequencies far below ω_0 , an applied electric field displaces the electrons from the positive cores and induces a polarization in the same direction as the applied field. At frequencies near resonance, the induced polarization becomes very large, and a considerable amount of energy is stored in the resonator (in this case, the medium) relative to the driving field. This stored energy can change the sign of the applied electric field, and the material exhibits a negative response. If instead of electrons the material response were due to harmonically bound magnetic moments, then a negative magnetic response would exist. In material with negative parameters the usable bandwidth will be relatively narrow when compared with positive materials, hence materials with ϵ and μ both negative are not readily found.

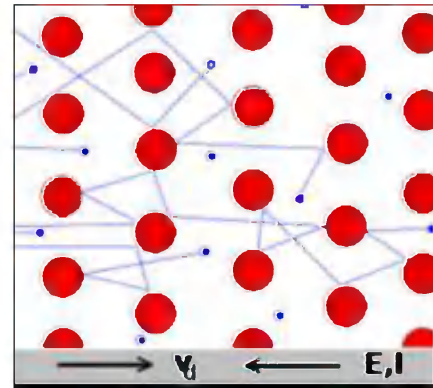


Fig. 4. Drude Model electrons (shown here in blue) constantly bounce between heavier, stationary crystal ions (shown in red).

J. B. Pendry was the first to evolve a practical way to make a double negative metamaterial which show negative ϵ and μ for same frequency.⁽²⁾ The experimental verification of negative refraction is reported for composite materials of SRRs and wires, supporting the existence of the negative refractive index ($n_{eff} < 0$). These structures are called left-handed metamaterials (LHM), attributing to the left-handed (LH) coordinate system formed by the EM wave components in the medium.

Periodically arranged thin metallic wire structures are shown to exhibit the plasma frequency ~~in the~~ microwave regime. Later, Pendry *et al*⁷ proposed split-ring resonator (SRR) structures which strongly

respond to an incident magnetic field resulting in negative permeability near the magnetic resonance frequency ω_{pc} . The studies on SRRs and metamaterials are mainly performed at the gigahertz (GHz) frequency region because of their ease of fabrication. Now frequency range for such materials increases up to terahertz (THz) range.

The SRR structure proposed by Pendry *et al.*⁽²⁾ is commonly used in LHM studies. This structure consists of two concentric rings separated by a gap, both of which have splits at the opposite sides (Fig. 5). LHMs

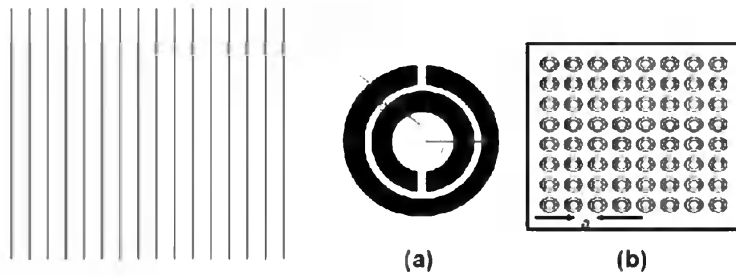


Fig. 5. Thin metallic wire structure and split-ring resonators- (a) Single unit cell (b) array of resonator

are then fabricated as periodic structures of the alternating layers of SRRs and wires. Current implementations of metamaterials rely on “infinite” rods and split-ring resonators (SRRs)⁽¹⁰⁾ to achieve a negative permittivity and a negative permeability, respectively (Fig.6).

The ring and wire units play the role of atomic dipoles: the wire acts as a ferroelectric atom (for negative ϵ), while the ring acts as an inductor L and the open section as a capacitor C. The ring as a whole therefore acts as a LC circuit. When the electromagnetic field passes through the ring, an induced current is created and the generated field is perpendicular to the magnetic field of the light which produces a magnetic resonance that results in a negative permeability.

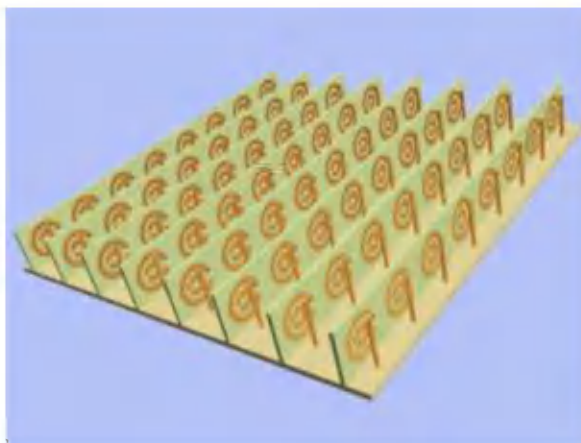


Fig. 6. Schematic diagram of two dimensional arrays of thin wires and SRRs.

The macroscopic view of metamaterials consists in replacing the succession of rings and rods by a homogeneous effective medium characterized by bulk constitutive parameters. This approach is possible since the rings and the separations between the rods are very small compared to the operating wavelength. From the measurements of the reflection (r) and transmission (t) coefficients,¹¹ the retrieved permittivity and permeability were shown to obey a Drude model and Lorentz model,⁵ respectively, described by equations (1) and (2),

$$\epsilon_r = 1 - \frac{f_{ep}^2}{f^2 + i\gamma_e f / 2\pi} \dots\dots\dots(1) \text{ where } f_{ep} \text{ is the electric plasma frequency}$$

and γ_e the electric damping factor

$$\mu(\omega) = 1 - \frac{F\omega^2}{\omega^3 - \omega_{LC}^3 - i\Gamma\omega} \dots\dots\dots(2) \text{ where } \omega_{LC} \text{ is the resonance}$$

frequency and F a geometrical factor, Γ describes resistive losses in the split ring resonator. ¹²

For $\omega \gg \omega_{LC}$ the dipole response is completely out of phase with the driving field and the metamaterial is diamagnetic ($\mu < 1$). For the frequency region just above ω_{LC} , the permeability is negative. ($\mu < 0$)

The above discussions shows that the shape of the rings, their effective radii, the width of their metallization and many other factors influence metamaterial properties. They also govern their resonant and plasma frequencies, which are directly related to the bandwidth where negative values occur. The rings can take various shapes, which have shown to obey the frequency-dispersive Lorentz model ^{1,13}.

Applications

The potential take-up of these structures in applications like communication and sensing systems is primarily due to the control of the amplitudes, frequencies and wave-numbers of propagating and non-propagating electromagnetic modes enabled by metamaterials to an extent that was not previously possible. The control of electromagnetic modes in the application fields relevant to Metamorphose includes, super lenses (can focus 10 times more sharply than a conventional lens) ^{2,4}, and military (Current military tactical networks and communications systems).

Metamaterials have been proposed as a mechanism for building a cloaking device. A three-dimensional invisibility cloak would hide an object completely. The microwave cloak is also slightly reflective and casts a partial shadow. Plasmon's (Plasmon's occur at the interface of a material with a positive dielectric constant and a negative dielectric constant.), could be used to cancel out visible light or radiation coming from an object. This 'plasmonic cover' would work by suppressing light scattering by resonating with illuminated light, which could render objects "nearly invisible to an observer." But such an object made invisible in red light would still be visible in multi wave length daylight. ¹⁴

The above description of advantageous properties of metamaterials gives a small insight into the large potential industrial applications. An exhaustive review of applications is beyond the scope of this short overview. Nevertheless, envisaged applications at microwave and millimeter waves are clearly centered on communication systems, sensing and imaging systems for biomedical applications, environmental monitoring, food quality control, security/chemical detection scanning systems and terrestrial/space observation. Intense further analysis and research will however be necessary to ascertain these concepts to reality.

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Quantum Computing: Future Technology

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Abstract

Quantum computing is the one of our most significant fundamental research project now. These are systems that use the behavior of subatomic particles to conduct calculations now performed with transistors on a chip. An ordinary computer is a collection of bits that can either be a 0 or a 1. But quantum bits can hold those states, 0 and 1, simultaneously. Instead of doing a calculation one after the other, the processing power in a quantum computer can increase exponentially. Two quantum bits, or qubits, can hold four distinct states, which can be processed simultaneously, three qubits can hold eight and 10 qubits can hold 1,024 states. In time, researchers expect machines with thousands of qubits.

Key words: quantum computer, quantum memory, qubits, photons, Shor's algorithm, quantum dots.

Introduction

Civilization has advanced as people discovered new ways of exploiting various physical resources such as materials, forces and energies. The history of computer technology has involved a sequence of changes of physical realization - from gears to relays to valves to transistors to integrated circuits and so on. Today's advanced lithographic techniques can squeeze fraction of micron wide logic gates and wires onto the surface of silicon chips. Soon they will yield even smaller parts and inevitably reach a point where logic gates are so small that they are made out of only a handful of atoms.

Quantum computing was first theorized less than 30 years ago, by a physicist at the Argonne National Laboratory. Most digital computers are based on the Turing Theory. Paul Benioff is credited with first applying quantum theory to computers in 1981 (Quantum Turing Machine).¹ As Moore's Law states, the number of transistors on a microprocessor continues to double every 18 months, the year 2020 or 2030 will find the circuits on a microprocessor measured on an atomic scale. And the logical next step will be to create quantum computers, which will harness the power of atoms and molecules to perform memory and processing tasks. Quantum computers have the potential to perform certain calculations significantly faster than any silicon-based computer.

Defining the Quantum Computer

The Turing machine, developed by Alan Turing in the 1930s, is a theoretical device that consists of tape of unlimited length that is divided into little squares². Each square can either hold a symbol (1 or 0) or be left

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blank. A read-write device reads these symbols and blanks, which gives the machine its instructions to perform a certain program.

In a quantum Turing machine, the difference is that the tape exists in a quantum state, as does the read-write head. This means that the symbols on the tape can be either 0 or 1 or a superposition of 0 and 1. While a normal Turing machine can only perform one calculation at a time, a quantum Turing machine can perform many calculations at once. In figure the numbers θ and φ define a point on the unit three-dimensional sphere, called *the Bloch sphere*, and it provides a useful means to visualize the state of a single qubit. A quantum computer operates by manipulating those qubits with a fixed sequence of quantum logic gates. The sequence of gates to be applied is called a .

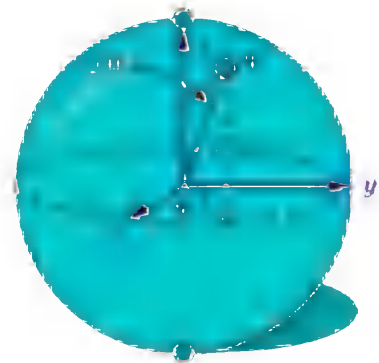


Fig. 1. The Bloch sphere is a representation of a qubit, the fundamental building block of quantum computers

A quantum computer is a device for computation that makes of direct use quantum mechanical phenomena, such as superposition and entanglement (when two particles have the same properties and behave identically while being separate), to perform operations on . The superposition of qubits is what gives quantum computers their inherent parallelism (ability to perform multiple computations simultaneously or in parallel). According to physicist David Deutsch, this parallelism allows a quantum computer to work on a million computations at once, while desktop PC works on one. A 30-qubit quantum computer would equal the processing power of a conventional computer that could run at 10 teraflops (trillions of floating-point operations per second).

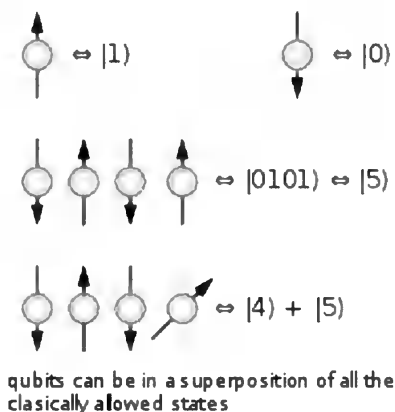


Fig. 2. Bits vs. Qubits

Qubits represent atoms, ions, photons or electrons and their respective control devices that are working together to act as computer memory and a processor³. Because a quantum computer can contain these multiple states simultaneously, it has the potential to be millions of times more powerful than today's most powerful supercomputers.

Photon: Under the photon theory of light, a *photon* is a discrete bundle (or *quantum*) of electromagnetic (or light) energy. Photons are always in motion and, in a vacuum, have a constant speed of light to all observers.⁴

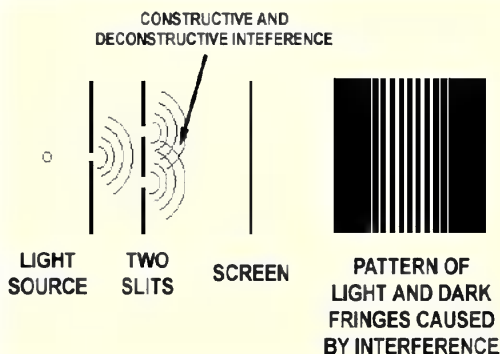


Fig. 3. Young's two slit experiment: photons

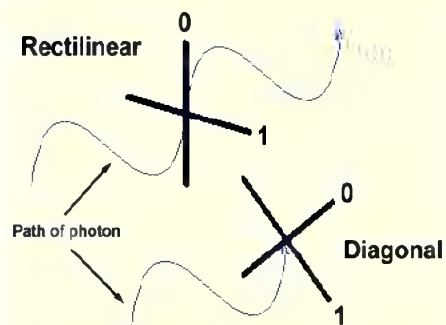


Fig. 4. path of photon

Photon polarization is the quantum mechanical description of the classical sinusoidal plane electromagnetic wave. Individual photons are completely polarized. Their polarization state can be linear or circular, or it can be elliptical, which is anywhere in between of linear and circular polarization. The polarisation of photons can be used to encode data. In order to receive the data, the polarisation of the filter must match that of the photons.

Qubit Control: Computer scientists control the microscopic particles that act as qubits in quantum computers by using control devices like, Ion traps, Optical traps, Quantum dots, Semiconductor impurities, Superconducting circuits.

Enhancements in Recent years

- 1998: Los Alamos and MIT researchers managed to spread a single qubit across three nuclear spins in each molecule of a liquid solution of alanine (an amino acid used to analyze quantum state decay) or trichloroethylene (a chlorinated hydrocarbon used for quantum error correction) molecules.⁵
- 2000: In March, scientists at Los Alamos National Laboratory announced the development of a 7-qubit quantum computer within a single drop of liquid. The quantum computer uses nuclear magnetic resonance (NMR) to manipulate particles in the atomic nuclei of molecules of trans-crotonic acid, a simple fluid consisting of molecules made up of six hydrogen and four carbon atoms⁶. Researchers at IBM-Almaden Research Center developed quantum computer in August. The 5-qubit quantum computer was designed to allow the nuclei of five fluorine atoms to interact with each other as qubits, be programmed by radio frequency pulses and be detected by NMR instruments similar to those used in hospitals.

- 2001: Scientists from IBM and Stanford University successfully demonstrated Shor's Algorithm on a quantum computer.
- 2005: The Institute of Quantum Optics and Quantum Information at the University of Innsbruck announced that scientists had created the first qubyte, or series of 8 qubits, using ion traps.
- 2006: Scientists in Waterloo and Massachusetts devised methods for quantum control on a 12-qubit system. Quantum control becomes more complex as systems employ more qubits.
- 2007: Canadian startup company D-Wave demonstrated a 16-qubit quantum computer, selling the computer, called the "D-Wave One," for \$10 million per computer. The company will also perform maintenance on the computer and other professional services⁷.
- 2011: The Australian Centre of Excellence for Quantum Computation and Communication Technology, established in 2011, is an international research effort to develop the science and technology of a global quantum information network, encompassing ultra-fast quantum computation. On May 25th, 2011, Columbia. D-Wave announced that it had sold its first full system to Lockheed Martin. The company's research was also published last month in the scientific journal *Nature*. The company, which has been in business for 12 years, is working on a 128-qubit processor that is in its 23rd generation.⁸

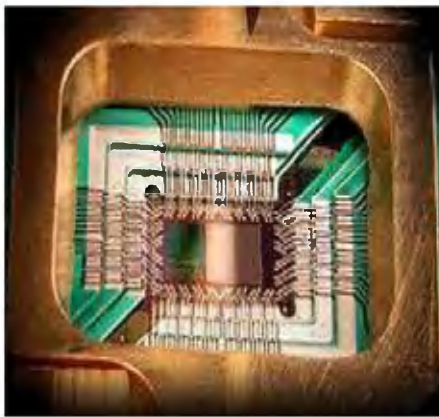


Fig. 5. Photograph of a chip constructed by D-Wave Systems Inc., designed to operate as a 128-qubit superconducting adiabatic quantum optimization processor, mounted in a sample holder.



Fig. 6. D-Wave Systems, Inc.
D-Wave's 16-qubit quantum computer

Quantum memory

Large scale implementations of quantum information processing technology will require a quantum memory. The process of measurement collapses a quantum state. Therefore, to build a quantum memory, you need to devise a system that store optical quantum states by mapping the light into atomic ensembles. We can reverse this mapping and recall stored light on demand. This is known as the Gradient Echo Memory (GEM) and can work with ensembles of both two and three level atoms.

The quantum logic operations in optical quantum computers will require optical quantum memory to buffer intermediate results and create deterministic logic gates. Storage of quantum states of light requires a coherent optical memory. The Gradient Echo Memory (GEM) is a coherent light storage technique based on photon echoes. The idea was first demonstrated at the Australian National University in a 2-level solid state ensemble.

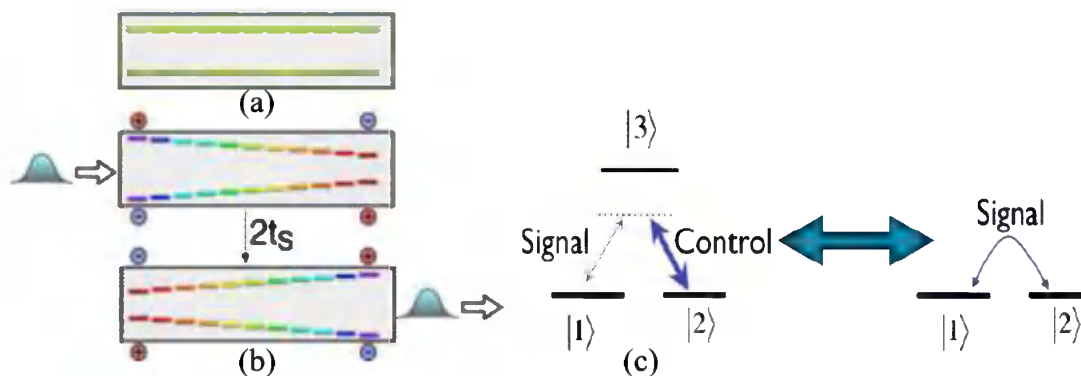


Fig.7. An ensemble of two level atoms

The storage mechanism works as shown (Fig.7). An ensemble of two level atoms (a) is frequency shifted (b) to create a gradient of atomic frequencies along the length of the ensemble. The frequency width of the atomic sample is adjusted to capture the entire bandwidth of the optical pulse. After flipping the frequency gradient at time ts , a photon echo is generated at time $2ts$ (c). The atomic ensemble can be composed of either two-level atoms or three level atoms. In the case of three level atoms, we use an additional control beam to couple atomic ground states (Fig.7c). In this way we enable a range of other atomic systems for GEM, in particular the alkali atoms, which have long lived atomic ground states.

Shor's algorithm

This is an algorithm invented by Peter Shor in 1995 that can be used to quickly factorise large numbers. Consider the problem of finding the prime factors of the number 15^9 . Since the algorithm consists of three key steps, this explanation will be presented in 3 stages.

Stage 1:- Place a memory register into a coherent superposition of all its possible states. The letter 'Q' will be used to denote a qubit that is in the coherent state.

When a qubit is in the coherent state, it can be thought of as existing in two different universes. In one universe it exists as a '1' and in the other it exists as a '0' (Fig.8). Extending this idea to the 3 bit register we can imagine that the register exists in 8 different universes, one for each of the classical states it could represent (i.e. 000, 001, 010, 011, 100, 101, 110, 111). In order to hold the number 15, a four bit register is required (capable of representing the numbers 0 to 15 simultaneously in the coherent state). A calculation performed on the register can be thought of as a whole group of calculations performed in parallel, one in each universe. In effect, a calculation performed on the register is a calculation performed on every possible value that register can represent.

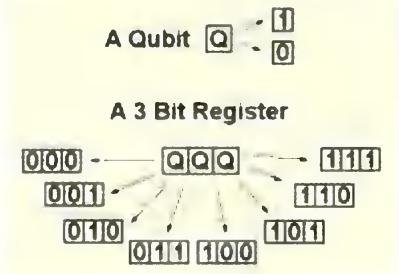


Fig. 8. A three-qubit register can represent 8 classical states simultaneously.

Stage 2: The second stage of the algorithm performs a calculation using the register:

- A random number X is chosen, where $1 < X < N-1$
- X is raised to the power contained in the register (register A) and then divided by N
- The remainder from this operation is placed in a second 4 bit register (register B).

After this operation has been performed, register B contains the superposition of each universes results. This is best illustrated with an example, if we choose X to be 2, then the contents of register B, for every possible value in register A

$$\text{Register B } \boxed{Q\,Q\,Q\,Q} = X^{\text{Register A } \boxed{Q\,Q\,Q\,Q}} \text{ MOD } N$$

Fig. 9. Operation performed in stage 2.

are as follows. Notice that the contents of register B follows a repeating sequence (1, 2, 4, 8, 1,2,4,8...), the frequency at which this repeats can be named f . In this case the repeating sequence (1, 2, 4, 8) has four values so $f=4$. Stage 3: In final stage the frequency of repetition, f , can be found using a quantum computer. This is done by performing a complex operation on register B and then looking at its contents which causes the results from every universe to interfere with each other. The resulting value for f is then used in the following equation to calculate a (possible) factor.

$$\text{Factor P} = X^{\frac{f}{2}} - 1$$

Table 1: Contents of Register B, when $N = 15$ and $X = 2$.

Register A	Register B
0	1
1	2
2	4
3	8
4	1
5	2
6	4
7	8
8	1
9	2
10	4
11	8
12	1
13	2
14	4
15	8

Developments

There are a number of quantum computing *models*, distinguished by the basic elements in which the computation is decomposed. The four main models of practical importance are:

- the *quantum gate array* (computation decomposed into sequence of few-qubit quantum gates),
- the *one-way quantum computer* (computation decomposed into sequence of one-qubit measurements applied to a highly entangled initial state (cluster state)),
- the *adiabatic quantum computer* (computation decomposed into a slow continuous transformation of an initial Hamiltonian into a final Hamiltonian, whose ground states contains the solution),
- and the topological quantum computer (computation decomposed into the braiding of anyons in a 2D lattice)

A quantum computer would be able to perform calculations on a far greater order of magnitude than traditional computers¹⁰.

Building a quantum computer

Quantum dots: An example of an implementation of the qubit is the 'quantum dot' which is basically a single electron trapped inside a cage of atoms. When the dot is exposed to a pulse of laser light of precisely the right wavelength and duration, the electron is raised to an excited state: a second burst of laser light causes the electron to fall back to its ground state.¹¹ The ground and excited states of the electron can be thought of as the 0 and 1 states of the qubit and the application of the laser light can be regarded as a controlled NOT function as it knocks the qubit from 0 to 1 or from 1 to 0. If the pulse of laser light is only half the duration of that required for the NOT function, the electron is placed in a superposition of both ground and excited states simultaneously, this being the equivalent of the coherent state of the qubit.

More complex logic functions can be modelled using quantum dots arranged in pairs. Therefore quantum dots are a suitable candidate for building a quantum computer¹².

The latest development in quantum computing takes a radical new approach. It drops the assumption that the quantum medium has to be tiny and isolated from its surroundings and instead uses a sea of molecules to store the information. When held in a magnetic field, each nucleus within a molecule spins in a certain direction, which can be used to describe its state; spinning upwards can signify a 1 and spinning down, a 0. Nuclear Magnetic Resonance (NMR) techniques can be used to detect these spin states and bursts of specific radio waves can flip the nuclei from spinning up (1) to spinning down (0) and vice-versa. The quantum computer in this technique is the molecule itself and its qubits are the nuclei within the molecule. This technique does not however use a single molecule to perform the computations; it instead uses a whole 'mug' of liquid molecules. The advantage of this is that even though the molecules of the liquid bump into one another, the spin states of the nuclei within each molecule remain unchanged.

Applications of quantum computers

In order for a quantum computer to show its superiority, it needs algorithms that exploit its power of quantum parallelism, such as Shor's algorithm and Grover's algorithm¹³. By using these algorithms a quantum computer will be able to outperform classical computers. For example, Shor's algorithm allows extremely quick factoring of large numbers. While a classical computer may be estimated to take 10 million billion billion years to factor a 1000 digit number, a quantum computer would take only around 20 minutes to do the same.

Artificial Intelligence: Computers will be capable of simulating conscious rational thought and a quantum computer will be the key to achieving true artificial intelligence. The theory of quantum computation allows us to look at the question of consciousness from a slightly different perspective. The first thing to note is that every physical object, from a rock to the universe as a whole, can be regarded as a quantum computer and that any detectable physical process can be considered a computation. Under these criteria, the brain can be regarded as a computer and consciousness as a computation. The next stage of the argument is based in the Church-Turing principle and states that since every computer is functionally equivalent and that any given computer can simulate any other, therefore, it must be possible to simulate conscious rational thought using a quantum computer. In this situation, the applications of artificial intelligence is also applicable to quantum computers. Quantum computing has wide applications, which range from communication to factorization and searching to teleportation¹⁴.

- Quantum teleportation.
- Quantum parallelism with Deutsch's and Deutsch-Jozsa algorithm.
- Super dense coding.
- Quantum communication.

With classical computers gradually approaching their limit, the quantum computer promises to deliver a new level of computational power. With them comes a whole new theory of computation that incorporates the strange effects of quantum mechanics and considers every physical object to be some kind of quantum computer. A quantum computer thus has the theoretical capability of simulating any finite physical system and may even hold the key to creating an artificially intelligent computer. The quantum computers power to perform calculations across a multitude of parallel universes gives it the ability to quickly perform tasks that classical computers will never be able to practically achieve. This power can only be unleashed with the correct type of algorithm. Some algorithms have already been invented; they are proving to have huge applications in the world of cryptography. This is because they enable the most commonly used cryptography techniques to be broken in a matter of seconds.

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Nanotechnology in medicine

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Abstract

Nanotechnology today is growing very rapidly and has infinite applications in almost everything we do. The basic definition of nanotechnology is the engineering of functional system at the molecular scale. A complete list of potential application of Nanotechnology is too vast and diverse to discuss in detail, but without doubt one of the greatest value of Nanotechnology will be in the development of new and effective medical treatment. This review focus on the application of nanotechnology in medicine. Nanomedicine, which is the process of diagnosing, treating and preventing disease and relieving pain and preserving human health using molecular tools. Nanorobots are introduced into human body to perform cellular repairs at molecular levels. This technology will build fleets of computer controlled molecular tools much smaller than a human cell and built with the accuracy and precision of drug molecules. Such tools will let medicine; intervene in a sophisticated and controlled way at the cellular and molecular level. They could remove obstructions in the circulatory system, kill cancer cells, or take over the function of sub-cellular organelles.

Key words: nanotechnology, nanomedicine, nanorobots, molecular level cell treatment

Introduction

Nano is derived from “*vavOζ*” the Greek word for dwarf, and usually is combined with a noun to form words such as Nanometer, Nanotechnology or Nanorobot. A nanometer is 10^{-9} meter or one billionth of a meter. Since it is not easy to visualize the scale of a nanometer, a comparison with concepts and objects of appreciable dimensions is helpful¹. The genesis of nanomedicine concept was a visionary idea of late Nobel Physicist Richard P Feynman who proposed a nanomedical procedure to cure heart diseases in 1959². Nanomedicine today has branched out in hundreds of different directions, each of them embodying the key insight that the ability to structure materials and devices at the molecular scales, which can bring enormous immediate benefits in the research and practice of medicine³. Programmable nanorobotic devices would allow physicians to perform precise intervention at the cellular and molecular levels. Medical nanorobots have been proposed for gerontological applications, in pharmaceutical research, clinical diagnosis and in dentistry. Other applications include mechanically reversing arteriosclerosis, improving respiratory capacity enabling near instantaneous hemostasis, supplementing the immune system, rewriting or replacing DNA sequences in cells, repairing brain damage and resolving gross cellular insults whether caused by irreversible processes or by cytogenic storage of biological tissues.⁴

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Over last few years truly remarkable progress has been made in such diverse areas as proteomics, gene therapy, vaccine development, stem cells tissue engineering, nanotechnology and drug development through combinatorial chemistry. Although much of this progress has been at the preclinical, translational research stage and some notable advances have already entered the clinic⁵.

Medical applications

Immunoisolation

Supplying encapsulated new cells to the body could also be a valuable way to treat enzyme or hormone deficiency disease. Encapsulated neurons that could be implanted in the brain and then be electrically stimulated to release neurotransmitters, possibly as a part of a future treatment for Alzheimer's or Parkinson's disease. Microcapsules containing easily harvested replacement pig islets cells could be implanted beneath the skin of some diabetic patients⁶. Nano-egg that is all trans retinoic acid might contribute to the regeneration of beta cells in vivo and provide useful information for future therapy of diabetes mellitus⁷. Engineered Hepatitis B virus envelop L particles were allowed to form hollow nanoparticles displaying peptide that is indispensable for liver specific entry by the virus in humans.

Gated Nanosieves

The flow of materials through nanopores can also be externally regulated. Recent efforts have been directed at immobilizing biochemical molecules recognition agents such as enzymes antibodies other proteins and DNA, inside nanotubes to make active biological nanosensors. They also serve the purpose of drugs separation and selected bio catalysis.

Ultra fast DNA sequencing

It is useful in genetic testing and DNA microarray.

Fullerene-based pharmaceuticals

It is soluble derivatives of Fullerene such as C_{20} – a soccer ball-shaped arrangement of 60 carbon atoms per molecules show great promise as pharmaceutical agents. Clinical trials have shown good biocompatible and low toxicity even at relatively high dosages. They will be used as Antiviral (against HIV), Antibacterial (*E. coli*, *Streptococcus*) agents, Photodynamic anti tumor agents, in anticancer therapies, as antioxidants therapies, as antioxidants and anti apoptosis agents.

Nanoshells

It is a platform for nanoscale drug delivery, consisting dielectric metal (gold coated silica) treatment of diabetes, targeting micro-metastasis, detections of immunoglobins in blood, serum and saliva. Nanoshells

may be used to concentrate the heat from infrared light to destroy cancer cells with minimal damage to surrounding healthy cells.

Single Virus detector

Arrays modified with viral antibodies detect viruses suspended in fluids, whether bodily or otherwise. The Lieber group tested nano-wire arrays having receptors specific to influenza A, paramyxovirus and adenovirus and found that detectors could differentiate among the 3 viruses.

Tectodendrimers

They are tree shaped synthetic molecules up to a few nanometers in diameter that are formed with a regular branching structure. A combinatorially large number of smart therapeutic nano-devices can easily be synthesized from library of dendrimeric components performing the following tasks: Diseased cell recognition, Diagnosis of disease state, Drug delivery, Location reporting, Reporting outcome of therapy. Distinct cancer recognition or targeting dendrimers, creating a nanodevices customarized to destroy a specific cancer type and no other while also is sparing the healthy normal cells. In 3 nanodevices synthesized using a 5 generation, ethylenediamine-core polyamidoamine dendrimers with folic acid, fluorescein and methotrexate covalently attached to the surface to provide targeting imaging and intracellular drug delivery improved the cytotoxic response of a cell to methotrexate 100 fold over free drug. Its long term goal is to apply the antennas to living systems and control gene expression via remote electronic switching.

Biologic robots

These synthetic microbes could be designed to produce useful vitamins, hormones, enzymes, or cytokines in which a patient's body was deficient or to selectively absorb and metabolize harmful substances such as poisons and toxins.

Respirocytes

Artificial mechanical red blood cell or respirocyte, a blood borne, spherical 1- μm diameter, 1000 atm pressure vessel with active pumping powered by endogenous serum glucose, able to deliver 236 times more oxygen to the tissue per unit volume than natural red blood cells and to manage carbonic acidity. Primary medical applications of respirocytes would include transfusion blood substitution, partial treatment for anaemia, perinatal/neonatal and lung disorder, enhancement of cardiovascular/neurovascular procedures, tumour therapies and diagnostics, prevention of asphyxia, veterinary and other uses.

Chromosome replacement therapy

In one simple cryosurgical procedures called “chromosome replacement therapy”, a nanorobot controlled by physician would extract existing chromosomes from a particular diseased cell and insert new ones in the place in that same cell. If the patient chooses, inherited defective genes could be replaced with non defective base-pair sequences permanent curing genetic diseases. Nanovectors are tried in non viral gene delivery systems of gene therapies.²

Nanostructure mediated drug delivery

Many times, the success of a drug is dependent on the delivery method. The efficiency of drug delivery to various parts of body is directly affected by particle size. Nanostructured mediated drug delivery, a key technology for realization of nanomedicine, as potential to enhance drug bioavailability, improve the timed release of drug molecules and enable precision drug targeting⁷. Nanoskill drug delivery system can be implemented within pulmonary therapies, as non viral gene therapy vector and in stabilization of drug molecule that could otherwise degrade to rapidly. Additional benefits are reduced drug toxicity and more efficient drug distribution. The advantages of nanostructure mediated drug delivery include the ability to deliver drug molecules directly into cells and capacity to target the tumour within healthy tissues⁸. For example, DNA and RNA that is packaged within nanoscale delivery system can be transported into the cell to fix genetic mutations or alter gene expression profiles. Nanoscale drug delivery architectures are able to penetrate tumours due to the discontinuous or 'leaky' nature of tumour microvasculature, which typically contains pores (100-1000 nm in diameter). The micro-vasculature of healthy tissue varies by tissue types but most tissues involving heart, brain and lung, they have tight inter-cellular junction less than 10 nm therefore, tumour within these tissue types can be selectively targeted by creating drug delivery nanostructures greater than the intercellular gap of the healthy tissue but smaller than the pores found within the tumour vasculature.

Biodegradable polymer nanoparticles are being investigated for the delivery of proteins and genes, vaccines, anticancer drugs, ocular drugs and cytokines.

BioDelivery Sciences (a company) which has progressed to the clinical testing stage with a drug for treating systemic fungal diseases, which is using a nanoparticle called a cochleate.

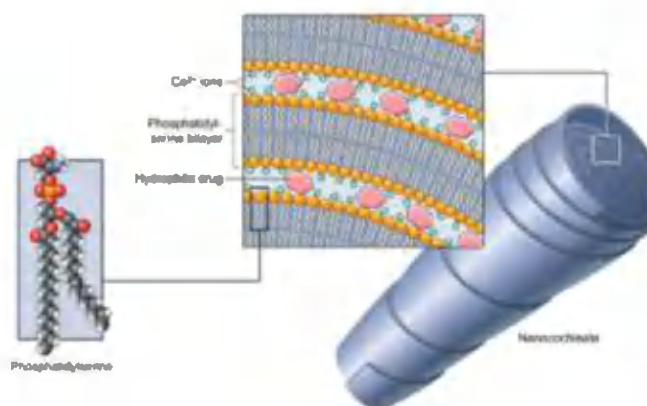


Fig.1. Bioral is a novel drug delivery system

Bioral is a novel drug delivery system, based upon cochleate technology. Bioral encapsulates and protects a drug without chemically bonding to it and may facilitate oral dosing of drugs that typically need to be given by intravenous administration. Alternating layers of lipids spiral around a drug molecule, encapsulating it and potentially protecting it from degradation by acid or digestive enzymes in the stomach. The Bioral technology is being evaluated as a new means of overcoming the poor oral absorption of drugs, such as the antifungal drug Amphotericin B. Pre-clinical studies have demonstrated the potential value of agents delivered using the Bioral technology^{14,15}.

Dendrimers (polyamidoamine polymers) are being investigated for both drug and gene delivery, as carriers for penicillin and for using anticancer therapy⁹. Generation 5 (G5) dendrimers can be conjugated to folic acid for targeting, iron oxide for magnetic resonance imaging. These agents have been tested invitro and invivo on animal model using KB cancer cells showed tenfold increase in chemo-therapeutic efficacy while significantly limiting systematic toxicity. Onyx-0115 is a type 2/5 chimeric virus that has been modified by attenuation of the E1B-55, this complex with other proteins binds to and inactivates the p53 tumor suppressor gene.

A number of tumor specific antibodies, angiogenesis inhibitors and drugs targeting specific proteins and small molecules are undergoing both preclinical and clinical trails. If these tumour specific inhibitors can be conjugated to fit the models of biodegradable nanoparticals, then the difference between cancerous cells and normal cells can be exploited. There is much synergy between imaging and nanotechnology in biomedical applications. Multifunctional nanoparticles serve as both diagnostic and therapeutic tools are engineered. Translation of this technology to head and neck squamous cell carcinoma is presently under way¹⁰. The multifaceted effect of gold nanoparticles (colloidal gold) has been successfully demonstrated in both diagnostic and treatment of cancer. Nanogold has been shown to have 600 times more absorption in cancer cells than normal human cells. This and optical property of nanogold to absorb the scattered light have been utilized by El-Sayed et al to demonstrate the detection and imaging of malignant cells who conjugated the antibody of Epidermal growth factor receptor (Egfr), which is only expressed by malignant cells onto nanogold after the absorption of nanogold-antiEgfr conjugate into cancer cells, the cells were to be shining in dark field microscopy. This antibody nanogold conjugate will be used as vector for delivering drug molecules or ionizing radiation in the form of radioactive nanogold. It has been used to deliver anticancer protein, tumour necrotic factors⁸.

Cancer Nanotechnology

Cancer nanotechnology is currently under intense development for application in cancer imaging, molecular diagnosis and targeted therapy. The basic rationale is the nano-meter sized particles, such as biodegradable micelles, semiconductor quantum dots and iron oxide nanocrystals, have functional or structural properties that are not available from either molecular or macro-scopic agents. When linked with biotargeting ligands, such as monoclonal antibodies, peptides or small molecules, these nanoparticles are used to target malignant tumour with high affinity and specificity. In the “mesoscopic” size range of 5-100 nm in diameter, nanoparticles also have large surface area and functional groups for conjugating to multiple diagnostic (e.g.: optical, radioisotopic or magnetic) and therapeutic (e.g. anticancer) agents. Recent advances have led to multifunctional nano-particles probes for molecular and cellular imaging and integrating nanodevices for early cancer detection and screening. These developments have opened exciting opportunities for personalized oncology in which cancer detection, diagnosis and therapy are tailored to each individual molecular profile progression and clinical outcome. During the past years, progress has been made in the oral cancer genetic markers field, which includes alterations of the p53 tumor suppressor protein, the inactivation of cyclin dependent kinase inhibitors and the over expression of the epidermal growth factor receptor. Genomic and proteomic studies of oral cancer tissues, plasma and saliva of oral cancer patients, have allowed the identification of several promising (likely to succeed) cancer signatures. The increased efforts in translational research will result in earlier diagnosis of oral cancer, better knowledge of prognostic factors and the development of targeted treatment regimens based on patients' clinical and biological characteristics at presentation¹¹.

Adriano Cavalcanti and co-workers are using an innovative approach to develop a nanorobot for diagnosis of cancer before metastases. They plan to incorporate a higher gradient of signal intensity of E-cadherin for chemical parameters identification in guiding nanorobots to identify malignant issues. Malignant issues and tumors such as squamous cell carcinoma are difficult to remove during surgical treatment due to their hardness. Ultrasonic piezo-electric actuators attached to scapel/surgical cutter, resonate at the tip of the tool at ultrasonic frequencies, to assist in rapidly cutting through tough and hardened malignant tissues¹¹.

Oral Transmucosal Fentanyl Citrate (OTFC) a new opioid formulation with nanotechnology based delivery system, is an effective treatment to cancer patients who are already receiving opioids and continue to receive such flares of pain¹².

Radiology and molecular imaging

The recent development of a nanophosphor screen technology by Bhargava RN and associates (Naocrystal technology, Briarcliff Manor, NY) started the exciting new phase of digital nanocrystal X-ray technology and its application to the continually changing field of digital radiology. The new developed and patented nanophosphor screen converts the X-ray photons to light and captures the light by way of reflection and micro channeling to light collecting devices and or electronics detectors¹³.

Otto Zhou and his co-workers developed a new technology of Carbon Nanotube (CNT) based X-ray machines which are smaller in size, low in cost and provides clear images and operates at lower temperatures as compare to metallic filaments in conventional X-ray machines. CNT x-ray machines can take multiple images for fast succession such as high speed camera for applications such as X-ray images of beating heart. CNT becomes a potential promising tool for cellular research and cell level therapeutics and functionalized version of CNT have demonstrated photo thermal ablation of cancer cells during in vitro studies.

Molecular imaging

MTI: Superparamagnetic nanocrystals (dark contrast effects in images) are available in the form of superparamagnetic iron oxide (SPIO) in nanoparticles in 50-500 nm and ultrasmall superparamagnetic iron oxide (USPIO) <50 nm size. Paramagnetic nanoparticles (bright contrast) and several nanoparticles are in developmental stage e.g. X@C60, Gd-conjugated dendrimers.

Optical imaging: Targeted quantum dots (nanocrystals of 2-10 nm made up of calcium selenide capped by zinc sulphide) used for fluorescence imaging, Non invasive visualization (1 cm) of lymph nodes as an aid for surgical therapy and Imaging combined with drug targeting.

Ultrasonic imaging: Microbubbles for enhanced contrast and nanobubbles-development phase.

Scintigraphy: Radionuclides attached to perfluoro carbonnanop articles are helpful in early detection of tumours

Radiotherapy

Biodegradable silicon delivering P (radioactive) nanomaterials for brachytherapy currently under clinical investigation.

Other Medical Applications: Nanorobots could actually be programmed to repair specific diseased cells, functioning in a similar way to antibodies in our natural healing process. Nanofibers can stimulate the

production of cartilage in damaged joints. Femtosecond laser system is applicable in neurosurgery, ophthalmology and dermatology. Photo-thermal ablation using gold coated silica nano shells and magnetic field induced thermotherapy using superparamagnetic iron oxide nanoparticles may be tried for treating malignancies. For the diagnosis of diabetes, cancer, detection of bacteria, viruses and fungal spores, ultrasensitive mass detection technology (detection limit 10⁻¹⁶) such as cantilever array sensors (NEMS/MEMS) will be used. Silicon nanowire based field effect transistors and electrical detection technology will be used for virus detection, cancer markers and cystic fibrosis. Nanotechnology based implantable materials will be used for stent coatings, retinal implants trace makers hearing aids, bioprosthesis and drug delivery system. DNA microarrays will be employed for the detection of DNA sequences and lan on a chip (LOC) will be used for enzyme detection and molecular diagnosis. Treatment of brain tumors, Alzheimer's and Parkinson's diseases may be possible by drug delivery across blood brain barrier using nanotechnology.²

Nanotechnology still faces many significant challenges in realizing its tremendous potential. They are ranging from basic engineering problems like precise positioning and assembly of molecular scale parts, to economical mass production techniques, to biocompatibility and simultaneous coordination of the activities of large number of independent micrometer scale robots. In addition there are larger social issues of public acceptance, ethics, regulation and human safety that must be addressed before molecular nanotechnology can enter the modern medical armamentarium. However there are equally powerful motivations to surmount these various challenges such as the possibility of providing high quality medical care to the 80% of world's population that currently receives no significant medical care.

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***Hybanthus enneaspermus* (L) F. Muell.: A versatile medicinal herb**

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Abstract

Hybanthus enneaspermus (L) F. Muell is an important medicinal herb widely used in the traditional systems of medicine. It is a sub-erect or erect herb, distributed in the tropics and the subtropics. Morphological features of the plant has been studied and described here. Threat status of the plant has been reviewed and various reasons of rarity are quoted. Uses of this herb in Ayurveda, Siddha and folklore systems of medicine have been appraised. Previous scientific studies revealed the diverse biological activities and phytochemical constituents of the herb. In prospect of the multifarious properties of the herb, further scientific studies are necessary to exploit the unknown activities and measures should be adopted to conserve the depleting taxa.

Key words: padmacarini, orithalthamarai, cyclotide, *Hybanthus enneaspermus*

Introduction

The healing property of plants has been used by man from time immemorial. Studies on the scientific validation of traditional uses and the search for novel properties open up great opportunities in the herbal drug industry. Wider acceptance of herbal drugs all over the world promotes the economic development of the nations to a great extent. A number of plant-derived drugs or chemical intermediates used in modern medicine are obtained from plants. About 95% of the medicinal plants consumed by the Indian industry are collected from the wild¹. Over exploitation of herbs from wild source, shrinking of forests/wilderness areas and habitat destruction are the major threats to medicinal plant resources². Presently, herbal drugs are widely adulterated mainly due to the unavailability of genuine raw materials. Documentation of traditional health care knowledge is an important area of herbal drug research which opens new vistas in the discovery of novel drugs. Traditional knowledge based drug development can improve or accelerate the drug discovery and development processes. Currently, the general procedure of drug discovery, *i.e.*, 'laboratories to clinics' become 'clinics to laboratories - a true reverse pharmacology approach³. For instance, tribes like Thottianaickans use *Hybanthus enneaspermus* against diabetes and this property was recently evaluated by Patel *et al*⁴. The present review describes the traditional and modern uses of a multipotent medicinal herb, *H. enneaspermus* along with its morphology, chemical constituents, biological activities, conservation etc.

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***Hybanthus enneaspermus* (L.) F. Muell**

H. enneaspermus is an important medicinal herb of the family Violaceae. The name *Hybanthus* is originated from the Greek words 'hypos' (hump-backed) 'anthos' (flower) and the word *enneaspermus* is from 'ennea' (nine) and 'sperma' (seed) referring to the characteristic features of the species such as spurred anterior petal and capsules which contain about nine seeds.

Synonyms

Ionidium suffruticosum Ging., *Ionidium banksianum* (DC.) Steud., *Viola enneasperma* L., *Viola suffruticosa* L., *Pigea banksiana* DC., *Hybanthus aurantiacus* (Benth.) F. Muell.

Vernacular names

Pink ladies slipper (English), 'ratanpurus' (Hindi), 'padmacarini' (Sanskrit), 'orilathamara' (Malayalam), 'orithaltamarai' (Tamil) and 'purusha rathnam' (Telugu).

Morphology

H. enneaspermus is a sub-erect or erect herb with somewhat woody base and spreading branches. Leaves are simple, alternate, subsessile, linear to lanceolate, 10-20 mm long, 3-7 mm wide and the midrib is prominent on both the leaf surfaces. Flowers are many, solitary in upper axils and zygomorphic. Sepals are free and slightly unequal. Corolla consists of five unequal, pink coloured and free petals. Lower petal is large (6-9 mm), broadly spatulate, clawed and prominently 3 veined. Stamens are five, dimorphic and the anterior filaments are appendaged. Ovary is tricarpeal and unilocular. Capsules are sub-globose, 4-5mm in diameter, loculicidally and elastically 3 valvate. Seeds are 5-12, ovoid, yellowish and longitudinally ribbed.

Distribution

The genus *Hybanthus* consists of about 150 species that are widely distributed in the tropics and the subtropics⁵. In India, *H. enneaspermus* is found in the warmer parts of the Deccan peninsula. Generally, it grows mixed with weeds like *Ammania baccifera* L., *Oldenlandia alata* J. Koenig ex Roxb., *Heliotropium bracteatum* R. Br. and *Lindernia oppositifolia* (Retz.) Mukerjee⁶. Authors collected the plants from Thanjavoor, Tamil Nadu and identified with authentic literature. Voucher specimens (NSKGRC 102A, B, and C) were deposited in the herbarium of Navajyothi Sree Karunakara Guru Research Centre for Ayurveda and Siddha, Uzhavoor, Kottayam, Kerala.

Threats

The plant is under threat in the natural habitat due to over exploitation by natural healers, overgrazing by animals, seasonal habitat, sporadic distribution and poor germination frequency of seeds^{7,8}. Even though the seeds and developing capsules are often found on the ground, loss due to rodents and inundation is considerable⁸.

Medicinal uses

H. enneaspermus is widely used Ayurveda, Siddha and other traditional systems of medicine. In Ayurveda, the botanical identity of the drug 'padmacarini' is controversial. Physicians of Kerala have considered it as *Nervilia aragoana* (locally called as 'orilattamara'). However, Kirtikar and Basu , Nadkarni and Chopra have described 'padmacarini' as *H. enneaspermus*^{9,10,11}. The plant has been reported to cure conditions of 'kapha' and 'pitta', urinary calculi, strangury, painful dysentery, vomiting, burning sensation, wandering of mind, urethral discharge, blood trouble, asthma, epilepsy, cough and to give tone to the breasts⁹. It has aphrodisiac, demulcent, tonic and diuretic actions and is used against urinary infections, diarrhoea, leucorrhoea, dysuria, inflammation and sterility¹².

In Siddha system, 'orithalthamarai' (*H. enneaspermus*) is used in the preparation of 'Orithalthamarai choornam'. The drug when administered with milk twice a day may cure megham diseases or gonorrhoeal diseases in ladies, leucorrhoea, increase sexual power or libido and improve the quality of semen. The plant is widely used by various tribes in their health care practices. Leaves of *H. enneaspermus* are being used as a vegetable by Palliyar tribals of the Western Ghats¹³. Decoction from shade dried and powdered leaves are used against diabetes by Thottianaickans of Semalai reserve forest of Tamil Nadu¹⁴. However, Konda Reddy tribals of Andhara Pradesh administer its leaf paste in milk to increase fertility in women¹⁵. Juice from the leaves is applied in the eye against red eye disease by the rural poor of South Kerala¹⁶. Natarajan et al. reported that the people of Malligainatham village, Tamil Nadu used the plant as a remedy for relaxation of nerves, because it helps the regulation of blood circulation. They use the cooked plants to change the hair colour (white to black)¹⁷. Furthermore, *H. enneaspermus* is also used in the treatment of herpes in coastal Karnataka¹⁸.

Adulteration of the drug

Retnam and De Britto suggested that it is difficult to identify *H. enneaspermus* from its co-existing weeds in the absence of flowers. They reported some of its pharmacognostic characters which can be used for its identification⁶. Further, the rarity and controversy in the botanical identity may also lead to adulteration of the genuine drug.

Biological activities

Pharmacological studies reveal that the plant possesses a diverse array of activities. Most of these studies provide scientific validation of its ethnomedicinal uses. The list of bioactivities cited here gives a clear picture of the pharmacological properties of *H. enneaspermus* (Table 1).

Phytochemical constituents

H. enneaspermus is reported to contain aurantiamide acetate, isoaborinol, β -sitosterol and triterpene^{8, 29}. Presence of alkaloids, flavonoids, cyanogenic-glycosides, tannin phenols and carbohydrate have been noticed in the aqueous leaf extracts^{25,12}. The plant also contains short cyclic peptides known as cyclotides. Distribution of cyclotides is varied in different parts of the plant, 3 cyclotides has been detected from leaves, 4 from flowers and 13 from fruits³⁰. Cyclotides have a well defined three-dimensional structure, unique circular backbone topology and the knotted arrangement of three disulfide bonds makes them exceptionally stable to thermal and enzymatic degradation. Their well defined structures have been associated with a range of biological activities, including uterotonic, inhibition of neurotension binding, hemolytic, anti-HIV, and insecticidal as well as trypsin inhibitory activity. 'Hypa A' and 'B' are the two cyclotides isolated and sequenced from *H. enneaspermus*³⁰.

Steps towards conservation

Plant regeneration was achieved indirectly from seed derived callus. Murashige and Skoog (MS) medium supplemented with 8.8 μ M benzyl adenine and 2.6 μ M naphthalene acetic acid produced 8.9 shoots per culture⁸. However, Sonappanavar and Jayraj reported rapid callogenesis and subsequent formation of shoots from leaf explants⁷. These *in vitro* culture techniques may help to conserve this rare species.

Conservation of depleting phyto-resources along with the promotion of cultivation of herbs is one of the pivotal steps to sustain pharmaceutical industries. Moreover, the development of good agricultural practices and good manufacturing practices are also crucial for the market sustainability of herbal drugs in the international markets. *H. enneaspermus*, a rare medicinal herb of the family Violaceae has been used in traditional and folklore health care practices. Scientific validation of these medicinal properties has been studied using various pharmacological parameters. Moreover, most of the scientific evaluation reported on this herb is an acceptance of its traditional uses. Elaborate pharmacological and photochemical studies may lead to the discovery of many more hidden facts regarding its medicinal uses.

Table 1. List of various pharmacological properties reported in *H. enneaspermus*.

Activity studied	Method adopted/ Models	Part used	Extract used	References
a) Antidiabetic	Oral glucose tolerance test & normoglycemic effect	Whole plant	Ethanol	4
b) Hypoglycemic activity	Alloxan-induced diabetic female sprague dawley rats	Leaves	Methanol & aqueous	19
Hepatoprotective	CCl ₄ -induced liver damage in rats	Whole plant	Aqueous	20
Cardioprotective	Isoproterenol-induced rats	Whole plant	Ethanol	21
Anxiolytic	Elevated plus maze, Elevated T-maze, Vogel conflict test and isolation induced aggression models	Whole plant	Hydro-alcoholic	22
Antioxidant	<i>In vitro</i> models	i) Whole plant	Ethanol	19
		ii) Leaves	Hydro-alcoholic	23
Antimicrobial				
a) Antibacterial	Disc diffusion assay	i) Leaves	Methanol	24
		ii) Whole plant	Aqueous, ethanol, petroleum ether & chloroform Methanol	25
b) Antifungal	Disc diffusion assay	Whole plant		26
Antiarthritic	Freund's adjuvant induced arthritis model	Whole plant	Alcohol & aqueous	12
Antiplasmodial	<i>Plasmodium falciparum</i> K1 chloroquine resistant and 3D7 chloroquine sensitive strains	Whole plant	Methylene chloride	27
Anticonvulsant	Maximal electric shock & Strychnine induced convulsions model	Whole plant	Aqueous & ethanol	28
Anticancer	Ascites tumour model	Whole plant	Methanol	Unpublished data

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Solar cell

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Abstract

Ever since the discovery of photoelectric effect in 1839, technology has traversed an eventful path to reach the present status of photovoltaic. This paper is a review of the developmental landmarks in the history of solar cells. Classification of solar cells is made on the basis of technological development. Review of materials starting from wafer silicon to quantum dots has also been mentioned.

Key words: Solar cells –history, generations, materials.

Introduction

One of greatest challenges of mankind is to find ways to replace the diminishing fossil fuel supply. The sun is a source of practically unlimited energy providing us with millions of kilowatts of power, most of which is wasted. A solar cell (photovoltaic cell) is a device that converts light energy from sun into electrical energy by photovoltaic effect.

Certain semiconductor when placed in sunlight will absorb photons from the sun's radiation. If these photons are of high enough energy, an electron in the valance band will use the absorbed energy to move up into the conduction band of the semiconductor. The electrons and holes thus created near a p-n junction will be separated by the potential barrier existing at the junction. Then they move freely through the semiconductor as majority carriers to an electrical contact and from there to the wire as current. In short, the device (solar cell) needs to fulfill fundamentally two functions: - Photo generation of charge carries (electrons and holes) in a light adsorbing material and separation of the charge carriers to a conductive contact that will transmit electricity.

History of solar cell

The word photovoltaic is a combination of the Greek word photo for light and the name of the physicist Allesandro Volta.

The solar energy conversion process is based on the photovoltaic effect discovered by a nineteen year old French physicist Edmund Becquerel in 1839¹. In the 1860s, Willoughby Smith discovered that electricity travelled through selenium very well when it was in light. After a few years William Adams and Richard

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Day discovered that the sun's energy creates a flow of electricity in selenium. In 1905, Albert Einstein explained that photons have enough energy to knock loose electrons off some materials. Later Daryl Chapin, a Bell Scientist was excited to find that silicon cells made nearly five times more electricity than selenium cells² In 1980s, PV cells were also being made from other materials apart from silicon such as gallium arsenide, copper sulfide and cadmium sulfide. After ten years PV cells were built into flexible plastic sheets. In the next year efficient photo electrochemical cells were developed and it was the birth of dye sensitized solar cell. In 2006 polysilicon cells came into the scene. Recently scientists at the NREL have designed an inverted metamorphic triple junction solar cell.

Generations of solar cells

Solar cells are classified into four generations based on technological development.

First generation

The first generation of solar cell is currently the most commercially wide spread as well as the oldest. It consists of high quality large area silicon wafer cells in single junction structure. They use thinly sliced wafers of silicon as the active media. Even today 86% of the solar cell market is based on this generation of cells.

Second generation

Second generation photovoltaic include highly efficient multiple junction cells based on thin film. These solar cells are thinner, more flexible and more inexpensive than first generation. Here the types of semiconductor materials vary from amorphous silicon (a-Si) to cadmium telluride (CdTe) and copper indium-gallium selenide (CIGS). These technologies also hold promise of higher conversion efficiencies.

Third generation

This generation solar cells include plastic (polymer) solar cells, photoelectro- chemical solar cells and organic dye sensitized cells (DSSC). Third generation solar cells promise incredibly low production costs. It has now conceived new ideas like solar paint and homemade solar cells. The major problem of this generation cells include fast degradation of the cells and very low efficiencies. The maximum efficiency range of DSSC is 10 – 13%.

Fourth generation

Fourth Generation solar cells are considered the future of solar technology. This category includes cutting edge technologies like quantum dots and nano wires. Silicon nano structures consisting of quantum wire or quantum dot super lattices can result in control of the effective band gap of silicon and this is a promising route towards more efficient solar cells.

Solar cell materials

Solar cells can be made from a wide range of semi conductor materials.

Crystalline Silicon cells

These conventional cells are generally made from layers of silicon a few hundred micrometers in thickness. Silicon for bulk cells is refined and grown into lightly p-type doped crystalline ingots that are then sliced into extremely thin wafers. However, the size that the crystalline wafers can be cut to is still very thick³. To make the wafers into solar cells, n-type dopants are diffused across the surface creating p-n junction.

Mono crystalline Silicon cells

These cells are made from a single large crystal wafer of Si. These cells have high efficiency but are expensive. These crystals are grown using the Czochralski process as cylindrical ingots and hence do not completely cover all the area of a square solar cell.

Poly crystalline Silicon Cells

These cells are less expensive and less efficient than monocrystalline cell. They are made of Si wafers cut from square cast ingots of Si. The method of casting a poly crystalline wafer of Si as opposed to a mono crystalline wafer requires much less precision and expense.

Ribbon Silicon cells

Ribbon Silicon is a type of multicrystalline silicon and it is formed by drawing flat thin films from molten Si. These cells have lower efficiencies than poly – Si but save on production costs due to great reduction in Si waste, as this approach does not require sawing from ingots.

Thin film technologies

Thin film technologies reduce the amount of material required in creating the active material of solar cell. Most thin film solar cells are sandwiched between two panes of glass to make a module. Amorphous Si (a-Si), cadmium telluride (CdTe), copper indium gallium selenide (CIGS), is three thin film technologies. CdTe technology costs about 30% less than CIGS technology and 40% less than a-Si technology.

Amorphous Si cells

Amorphous Silicon can be deposited onto a conductive substrate in a layer of few micrometers thick to create a thin film solar cell. The deposition process of applying a-Si allows it to be less than 1% of the thickness of a crystalline cell^{4,5} Amorphous Si cells are often built using two or three junctions to increase

the amount of the solar spectrum they can utilize. Typically alloys of a-Si and Ge are used to create the additional junctions in the multi-junction cells. These cells are lighter, use much less material and are less energy intensive to produce than bulk Si cells. However, the cell efficiency of amorphous Si is much lower than crystalline Si due to the increased recombination of the electron-hole pairs that result from the lower carrier mobility.⁶

Cadmium-Telluride cells

CdTe is an effective solar cell material. It is a very strong absorber of light and has a band gap almost perfectly tuned to match the solar spectrum. To create a p-n junction for solar cells a layer of cadmium sulfide is added to the CdTe. CdTe solar cell uses only about 10% of the semiconductor material when compared with bulk silicon cells. It is less efficient than bulk Si cells but has lower costs.

Copper-Indium/Gallium – Diselenide Cells

These cells have recently been shown to be upto 19.9% efficient, the highest efficiency of any thin film cell. The mixture of the two materials creates a more complex and effective heterojunction, where the junction is formed by semiconductors of dissimilar band gaps. The band gap of semi conductor in this cell can be varied by altering the ratio of Indium to Gallium.

Gallium – Arsenide multijunction cells

A triple junction gallium – arsenide cell may use Gallium – Indium – Phosphide (GaInP), gallium arsenide, and germanium p-n junctions. Each type of semi conductor will have a characteristic band gap energy which causes it to absorb light most efficiently at a certain colour, or more precisely, to absorb electromagnetic radiation over a portion of the spectrum. GaAs cells have reached a record efficiency of 40.7%⁷.

Organic cells

Organic solar cells are polymer solar cells built from thin films of organic semiconductors such as polymers and small molecule compounds like polyphenylene vinylene, copper phthalocyanin and carbon fullerenes. Its function is slightly different from most other cells: instead of semiconductor p-n junctions, organic cells utilize electron donor and acceptor materials. Typical choices are polymers for electron donors and fullerenes for electron acceptors. When an electron – hole pair is created by the absorption of a photon in the donor, the electron and hole stay together as exciton. The exciton diffuses through the cell until it reaches the acceptor where the electron is transferred to the acceptor material, creating a current through the acceptor. Because the exciton will only travel for a few nanometers before

the electron and hole recombine, it is highly beneficial to have donor and acceptor blended together, forming a bulk heterojunction⁴. The efficiency achieved by these cells is low as 6%.

Dye sensitized solar cells (DSSC)

The material components of DSSC are thin film of electrolyte sandwiched between two electrodes with a lattice of dye-coated nano-TiO₂ particle coating in one of the electrodes. In a DSSC, the incident photons excite electrons in the dye molecules. If sufficient energy is given, the excited electrons will escape from the dye to the conduction band of the TiO₂ particles and will then diffuse to the electrode, thus generating current. The electrons return to the dye through electrolyte.

Nanocrystalline solar cell (nc-si)

Nanocrystalline silicon is a form of porous silicon. It is an allotropic form of silicon with para crystalline structure. nc-Si has small grains of crystalline silicon within the amorphous phase. It has higher electron mobility due to the presence of the silicon crystallites. It also shows increased absorption in the red and IR wavelengths, which make it an important material for use in a-Si solar cell⁵.

This article depicts stage by stage development in the field of photovoltaic. In spite of these developments, we have to go a long way to witness a day where photovoltaic is the prime supplier of energy.

Acknowledgements

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A study on Chemi-informatics interpretation of bioactive substance

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Abstract

Bioinformatics and chemi informatics tools are of immense value to biologist in the extraction of information from vast repository of biological and chemical data. Computational tools are employed here to discover specific biological properties of newly generated chemical compounds. The objective of this work is to these molecules based on the structure and biological role they play and to elucidate the physical and chemical features of those molecules which contribute to their said biological function. This would help to maximize the pharmacodynamic and pharmacokinetic properties of those compounds. A commercial tool name by the TSAR which performs Quantitative Structure Activity Relationship Analysis(QSAR) is used to cluster the molecules on their similarity in steric, lipophilic and electronic parameters. Using regression analysis the correlation between their predicted biological property and their structural parameters are studied. These could be after further refinement can be used as drug against bacterial infection and other specific targets.

Key words: computational tools, QSAR, pharmacodynamics, regression analysis

Introduction

Cheminformatics is transforming the data into information and information into knowledge for the intended purpose of making better decisions faster in the area of drug lead identification and optimization. Chemical information systems are concerned with storing, retrieving, and searching information about chemical compounds, and with storing relationships between bits of chemical data. These *in silico* techniques are used in pharmaceutical companies in the process of drug design. Chemical structures are represented *in silico* using formats such as XML based chemical Markup language or SMILES. These representations are often used for storage in large chemical databases. WLN was the first line notation to feature a canonical form, that is rules for WLN meant there was only one "correct" WLN were able to write molecular structure in a line format , communicate molecular structure to one another and to computer programs. The best known and most widely used similarity metrics compare the two-dimensional topology that is; they only use the molecule's atoms and bonds without considering the shape. 3D Similarity Searches compare the Configuration of a molecule to other molecules. The "electronic surface" of the molecule is the important bit – the part that can interact with other molecules, 3D searches compare the surfaces of two molecules, and how polarized or polarizable each bit of the surface is. 3D Similarity

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Searches are uncommon, for two reasons: It is difficult and it is slow. The difficulty comes from the complexity of molecular interactions. The properties are compared, which are computed or measured or both and declares that molecules with many properties in common are likely to have similar structure. "Clustering" is the process of differentiating a set of things into groups where each group has common features. Molecules can be clustered using a variety of techniques, such as common 2D and/or 3D features.

Materials and Methods

Quantitative Structure Activity Relationship (QSAR)

QSAR is a process whereby the structures of a set of compounds are qualified and then compared to the numerical values of a biological activity or a physical property. Structure information and the measured property or activity is then processed into a mathematical model of relationship. An important advantage of QSAR is that it models the *in vivo* situation since it is based on activity data. Calculation of Quantitative Structure – Activity Relationship (QSAR) and Quantitative Structure property Relationship values used to predict the activity of a compound from its structure.

Pass Prediction

Biological Activity Spectrum (BAS)

Biological Activity Spectrum (BAS) of a compound represents the pharmacological effects, physiological and biochemical mechanisms of action, specific toxicity which can be revealed in compounds interaction with biological system finding the most probable new leads and selecting the most prospective compounds for high throughout screening from the set of available samples.

Mathematical Approach

n is the total amount of compounds in the training set;

n_i is the total amount of compounds, that have the descriptor i ;

n_j is the total amount of compounds, that reveal the activity j ;

n_{ij} is the amount of compounds, that have both the descriptor i and the activity j ;

$P_i = n_{ij} / n_j$ is the estimate of a prior i probability of activity j ;

Algorithm of prediction

For the compound under prediction structural descriptors are generated,

For each activity the following values are calculated;

$$U_i = \alpha_i \text{Arc sin } \{n_i (2p_{ij}-1)\}, U_{oj} = \alpha_j \text{Arc sin } \{r_j (2p_j-1)\}$$

$$s_j = \sin(U_j/m)$$

Result and Discussion

Preparing molecular structure files

Two Dimensional Structures of the selected compounds were converted into 3D structures using the chemical structure drawing tool the chemsketch. The 3D structures were saved into SD files. The structure files of the selected compounds were sent through Internet for predicting the Biological properties are shown in the Table: 1

Table 1. Predicting the Biological properties

Sl. No.	Name of the compound	Biological property
1.	1-2-4-Triazole -3-Alanine	NMDA receptor agonist
2.	2- Amino-3 Benzylamino propanic acid	Hydroxy tryptamine
3.	2- Amino-3 Benzylamino propanic acid.2	Hydroxy tryptamine
4.	2-Amino-3-Phenylbutanic acid	Hydroxy tryptamine
5.	2-AminoAdipic acid-hydrate2	Phosphatase inhibitor
6.	2-Aminoadipic acid-hydrate	NMDA receptor agonist
7.	2-Aminoheptanoic acid	NMDA receptor agonist
8.	2-Amino-N-Caprylic acid	NMDA receptor agonist
9.	2S-3R-2-Amino-3hydroxy-4methylpentonic acid	Phosphatase inhibitor
10.	2S-3R-2-Amino-3-methoxybutonic acid	Membrane integrity

Prediction of Cluster Analysis

The SD files of selected compounds were opened into TSAR. Molecular properties concerning to steric, lipophilic and electronic nature of the molecules were calculated.

Using cluster algorithms, the molecules were grouped based on their similarities in their molecular properties was shown in the Fig. I.

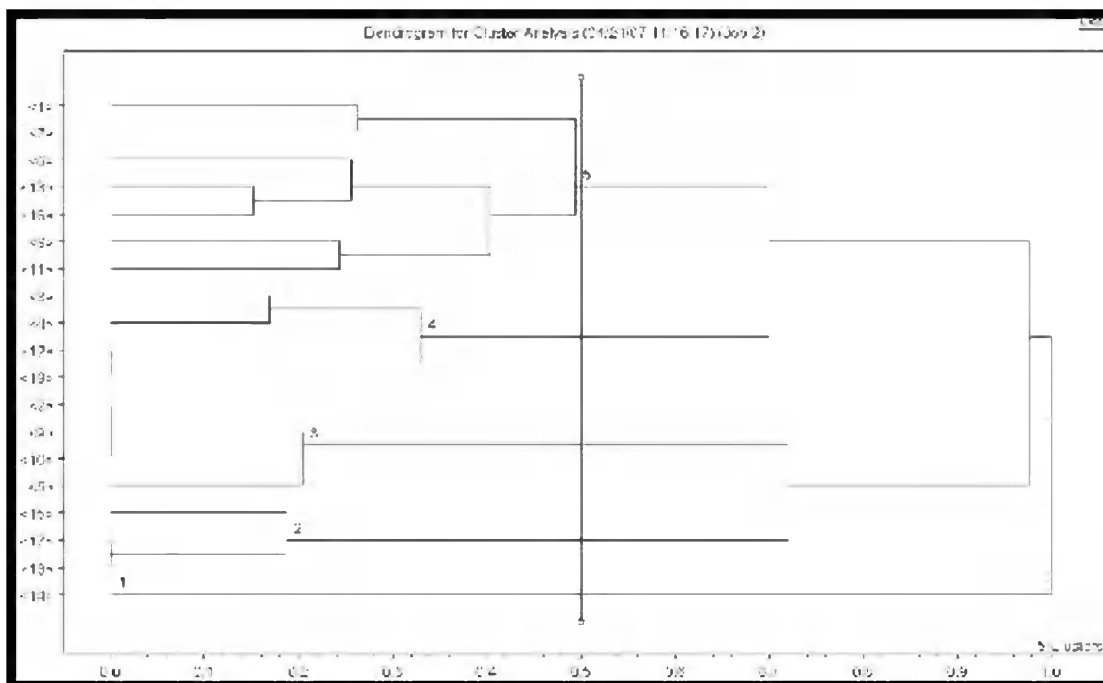


Fig. 1. Clustering of nmda receptor glycine site agonist molecules

	X0 Molecule Index	X1 Molecule Index	X2 Molecule Index	X3 Molecule Index	X4 Molecule Index	X5 Molecule Index	X6 Molecule Index	X7 Molecule Index	X8 Molecule Index	X9 Molecule Index	X10 Molecule Index	X11 Molecule Index
X0	0.0000	0.0000		0.0344	0.3278	0.1788	0.2087	-0.7634	0.0000	0.0000	0.0000	0.0000
X1	-0.018598	0.1939	0.16344		0.4647	-0.5063	-0.5738	0.13921	0.00000	-0.013381	0.21118	
X2	0.28735	0.39022	0.32878	0.49417		-0.28129	-0.38178	-0.28463	-0.27938	0.28777	0.22983	
X3	0.44779	0.12077	0.17088	-0.00000	-0.28128		0.00000	-0.56387	-0.42618	0.30178	0.098772	
X4	0.42028	0.22118	0.24947	-0.27341	-0.38178	0.00000		-0.00000	-0.48821	0.43711	0.14783	
X5	0.00000	-0.73980	-0.74014	0.13871	-0.39843	-0.66387	-0.68331		0.00000	-0.00384	0.08039	
X6	0.00000	0.00000	0.00000	0.03440	-0.37033	-0.42019	-0.48821	0.00000		0.00000	-0.00000	
X7	0.00000	0.00000	0.00000	-0.03440	0.28777	-0.28179	0.43711	-0.00384	0.00000		0.00000	
X8	0.76340	0.00000	0.00000	0.21118	0.13563	0.026375	0.14783	-0.00331	-0.00000	0.00000		
X9	0.00000	0.22384	0.02647	0.02883	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X10	0.00000	0.00000	0.00000	-0.21118	-0.00000	-0.00000	-0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X11	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X12	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X13	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X15	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X16	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X17	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X18	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X19	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X20	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
X21	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Fig. 2. Correlation matrix for nmda receptor glycine site agonist

Correlation between molecular mass and NMDA receptor agonist property

A positive and negative correlation is done between molecular properties and NDMA receptors against property are shown in the Table: 2.

Table 2: Correlation between NMDA receptor agonist activity and various molecular properties

Positive correlation	Negative correlation
Molecular volume logP	VAMP electronic energy
Hydrogen donor	Total molecular charge
Hydrogen acceptor	Heat of formation
VAMP molecular energy	Ionization potential
VAMP Mean polarizability	Total dipole

The selected compounds from *Aphylllophorales* can be divided into four major groups based on their Biological properties. NMDA receptor against are expected to promote the receptor function. The molecular properties such as Hydrogen donor and Hydrogen acceptor and VAMP molecular energy showed a positive correlation between certain molecular parameters and characteristic biological activity. This kind of analysis lends room for further modification of the molecules towards desired and improved biological activity.

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Genital morphology of some species of the subfamily Acronictinae (Lepidoptera: Noctuidae)

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Abstract

Genital morphology of five species of the sub family Acronictinae of Noctuidae was studied. Based on the structure of uncus, saccus and valva and juxta the species *Chasmina rejecta* Fabricius and *Chasmina tibialis* Fabricius shows resemblance and *Sasunaga tenebrosa* Moore, *Condica illecta* Walker and *Tarache nitidula* Fabricius were different. A key for the identification of species is also presented with eight figures.

Key words: Acronictinae Noctuidae, genital morphology

Introduction

Taxonomic segregation of Lepidoptera species has been on the basis of morphological characters, such as labial palpi, antennae structure, wing venation...etc. Recently the significance of external genital morphology in the Lepidopteran taxonomy and phylogeny has been well recognized¹. Several investigations have been made on the general external morphology of different Lepidopteran groups. Busck and Heinrich² discussed the systematic importance of the male genitalia in Microlepidoptera. Eyer³ discussed the morphological significance of juxta in the male genitalia of Lepidoptera and considered loss of juxta to mark a high degree of genital specialisation. Many authors^{4,5,6,7,8,9,10} stressed the importance of genitalia at the specific level and expressed the view that during revisional studies, greater dependence should be placed on the internal genitalia as well. Investigation by various workers had indicated that the structure of genitalia in association with other morphological and biological characters of the taxon would provide a satisfactory basis for taxonomic segregation. As far as the Indian fauna was considered detailed studies are yet to be made for many groups of Lepidoptera. Among Lepidoptera, Noctuidae are very important ecologically and economically. Hence an attempt was made to study the external genital morphology of this group of moths. In this study, the morphology of five species of Acronictinae collected from Kerala was carried out. For naming the various parts of the genitalia the terminology proposed by Klots¹¹ has been followed.

Materials and Methods

The moths were collected either from the fluorescent tubes or by using a portable light trap (fitted with

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mercury vapour lamp of 125 w)¹², for the collection of Lepidoptera. The collected specimens were killed using Benzene in a killing bottle. For preparing the permanent slide mounts of genitalia, the abdomen was detached using a microneedle and dropped into a test tube containing a small quantity of 10% KOH solution and the same was gently boiled in a water bath. As soon as the material became transparent enough, boiling was stopped. After giving two/three washing, the genitalia was dissected out with the help of fine needles. The material was then stained in Acid Fuschin and then cleared in carbol-xylol solution prepared in 3:1 ratio. After dehydration, the material was mounted in Canada balsam.

Result

In this study, genitalial morphology of five species of the subfamily Acronictinae was studied. Descriptions of which are given below:

Chasmina rejecta Fabricius

Male: Uncus very long, highly curved, sclerotized: tuba analis well developed and traingular. Tegumen and vinculum moderately long and sclerotized. Saccus saucer-shaped. Valva long, sclerotized, clothed with long hairs. Its anterior part broader than the posterior medially notched at the anterior part, sacculus sclerotized, Juxta sclerotized, cylindrical (Figs. 1 and 2).

Aedeagus: Short, stout and broad; vesica broad with a long spine-like outgrowth at the anterior end, highly sclerotized medially, posteriorly with a short spine on the right side; ductus ejaculatorius entering at the distal end.

Female: Ovipositor lobes short, well sclerotized and setosed. Apophyses more or less similar. Ostium bursae triangular in shape. Ductus bursae short, anterior part lightly sclerotized, posterior part triangular and funnel-like. Corpus bursae very long, saccular, bearing longitudinal and transverse striations. Ductus seminalis arise from the posterior end of corpus bursae, which has a long anteriorly directed process ending in a conical diverticulum (Fig. 3).

Chasmina tibialis Fabricius

Male: Not studied

Female: Ovipositor lobes short, sclerotized, fringed with long and short setae. Posterior apophyses short and rod-like. Anterior apophyses longer than posterior apophyses. Ostium bursae simple, ductus bursae short, its posterior part sclerotized. Corpus bursae very long, cylindrical, with long striations. Ductus seminalis highly coiled and arise from corpus bursae (Fig. 4).

***Sasunaga tenebrosa* Moore**

Male: Uncus curved, clothed with hairs and with its distal end flattened and bearing a spine. Socii and scaphium well developed. Tegumen broad and sclerotized. Tuba analis well developed. Vinculum longer than the tegumen. Saccus very long, conical, with its tip slightly drawn out. Valva long, leaf-like and with a well developed costa. Cucullus and valvula not clearly differentiated. Distal part of valvae narrow, bearing a series of fine setae, outer surface fringed with long, sparse hairs. Sacculus well marked, ampulla well developed and bifurcated distally. Harpe bearing a row of short hairs. Juxta well developed with no particular shape. Transtilla well marked and membranous (Figs. 5 and 6).

Aedeagus: Long and slender. Ductus ejaculatorius entering at the distal end. Vesica with a series of short spicules arranged in a circle.

Female: Not studied.

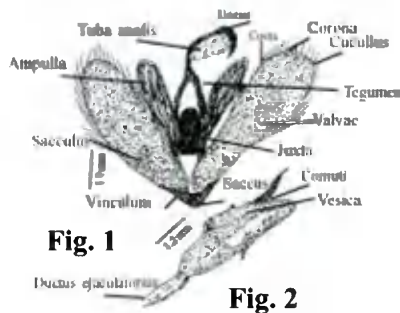


Fig. 1



Fig. 2

Fig. 3



Fig. 4



Fig. 5

Fig. 6



Fig. 7



Fig. 8

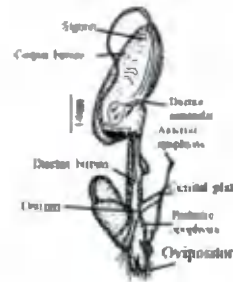


Fig. 9

- Figures
 1&2 Male genitalia of *Chasmodon refovea* Fabricius
 3 Female genitalia of *Chasmodon refovea* Fabricius
 4 Female genitalia of *Chasmodon tibialis* Fabricius
 5&6 Male genitalia of *Sasunaga tenebrosa* Moore
 7&8 Male genitalia of *Conotica illata* Walker
 9 Female genitalia of *Tarache aridula* Fabricius

***Condica illecta* Walker**

Male: Uncus very long and arched, apically pointed with a swollen sub-basal part. Scaphium broad and beak-shaped. Tegumen and vinculum long with narrow arms. Saccus U-shaped with a flat proximal part. Juxta Y-shaped, posteriorly tapering and anteriorly cup-like; valvae with long and narrow arms which are apically broad and setose. Saccus extending to about 1/3rd length of valva having a narrow blunt lobe (Figs. 7 and 8).

Aedeagus: Short, slightly constricted in the middle. Cornutii with two prominent spines, posterior part with two spines. Ductus ejaculatorius enters at the posterior end.

Female: Not studied.

***Tarache nitidula* Fabricius**

Male: Not studied.

Female: Ovipositor lobes long and well developed, setosed with long hairs. Posterior and anterior apophyses thin and elongate, the latter stouter than the former. Ostium bursae triangular, large and well developed. Ductus bursae cylindrical in shape, moderately long and highly sclerotized. Corpus bursae long, flattened, club-like, curved, ventrally constricted and longitudinally striated. Right upper side of corpus bursae highly sclerotized longitudinally, signum absent, ductus seminalis originates from the anterior middle tip of the corpus bursae and twisted backwardly to corpus bursae, into a long tubular curved process (Fig. 9).

Key for identification of species

Acronictinae

1. *Saccus U-shaped*.....2
 ---- *Saccus not U-shaped*3
2. *Costa well developed. Juxta well developed but without particular shape. Vesica with several short spicules arranged in a circle**Sasunaga tenebrosa*
 ---- *Costa not well developed, juxta Y-shaped*.....*Condica illecta*
3. *Juxta cylindrical, medially notched and sclerotized**Chasmina rejecta*
 ---- *Juxta not cylindrical or medially notched*4

4. *Corpus bursae very long and cylindrical**Chasmina tibialis*
---- *Corpus bursae long but flattened and club-like**Tarache nitidula*

Discussion

Uncus was well developed, long and curved at the above mentioned species of sub family Acronictinae. Uncus with terminal spines on *Sasunaga tenebrosa* Moore. Uncus was bluntly pointed in *Condica illecta* Walker. Sub basal part of uncus is swollen in *Condica illecta* Walker and the distal end flattened in *Sasunaga tenebrosa* Moore.

Saccus was U-shaped in *Sasunaga tenebrosa* Moore and saucer-shaped in *Chasmina rejecta* Fabricius. Valva showed distinct differentiation into costa, valvula and sacculus. It also presented a wide range of variations. It was usually long with characteristic aptical spines on cucullus. It was long in *Condica illecta* Walker and *Sasunaga tenebrosa* Moore.

Valvae were a symmetrical and leaf-like in *Sasunaga tenebrosa* Moore. Valvae were fringed with marginal hairs in *Sasunaga tenebrosa* Moore. Tegumen was long and narrow in *Condica illecta* Walker. Harpe was distinct, Ampulla was bifurcated distally in *Sasunaga tenebrosa* Moore.

Juxta was well developed and Y-shaped in *Condica illecta* Walker. Cylindrical and medially notched in *Chasmina rejecta* Fabricius. It is not cylindrical or medially notched in *Chasmina tibialis* Fabricius.

Aedeagus was usually long and well developed, short and stout in *Chasmina rejecta* Fabricius and *Condica illecta* Walker. Vesica was found to vary from species to species. Cornuti with several short spicules arrange in a circle in *Sasunaga tenebrosa* Moore.

Ovipositor was short and sclerotized in *Chasmina rejecta* Fabricius and *C. tibialis* Fabricius; Long and well developed in *Tarache nitidula* Fabricius. Ostium bursae was triangular as in *Chasmina rejecta* Fabricius, and *Tarache nitidula* Fabricius. Corpus bursae very long and cylindrical in *Chasmina tibialis* Fabricius but long and cylindrical in *Chasmina tibialis* Fabricius but long flattened and club-like in *Tarache nitidula* Fabricius. Ductus bursae short in *Chasmina tibialis* Fabricius; long and cylindrical in *Tarache nitidula* Fabricius.

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Comparing Odonate biodiversity, species richness and water quality at selected sites of Palakkad District

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Abstract

Odonata, comprising dragon flies and damsel flies, are the most fascinating and elegant group of insects essentially found near any wet lands. They lead a bimodal life: adults are aerial, where as larvae are purely aquatic. Larval Odonates form a high proportion of bio mass in fresh waters and thus occupy an important position in the energy flow pathway of the fresh water. As dominant members of the benthic and littoral fauna, they are considered as promising organisms for bio monitoring the organic pollution. Larval Odonates devour mosquito larvae as well as other harmful organisms and prove themselves as a friend of mankind. Since Odonates are at the top of the predatory food chain, their presence in any habitat shows that the conditions are varied, congenial and healthy, supporting diversified faunal components, which in turn are supported by complex flora. Seven wetland sites in Palakkad district were selected and a study on the abundance and diversity of Odonata was conducted. The goal of this study is to document species diversity, abundance and distribution across Palakkad district, and to examine the relationship among water quality parameters and Odonate assemblages at different wet land sites in Palakkad District. Site selection was made at random. Specimens were photographed and identified using field guides. Forty four species belonging to 11 families were collected, of which, 3 families coming under sub order Anisoptera (dragon flies) and 8 families coming under Zygoptera (damsel flies). The species richness is more for Anisoptera with 27 species followed by Zygoptera with 17 species. Libellulida family of suborder Anisoptera is the richest of the families enclosing almost 57 % the total identified species (25species) showing Libellulid dominance in the area. various water quality parameters showed that while a general measure of abundance has little potential for use in indicating water quality, a high diversity of species is strongly correlated with good quality aquatic environments containing low turbidity, moderate conductivity and high dissolved oxygen levels.

Keywords: Odonata, bioindicator, species abundance, turbidity.

Introduction

Odonate insects or dragon flies are one of the most conspicuous water insects that serve as bio indicators of hydrosystem quality because of the complex habitat requirements of individual species. As water quality is directly correlated to biodiversity, a degradation of water quality can be expected to result in a loss of biodiversity.

Odonata larvae inhabit water, while their adults live on earth. Therefore, the presence of vigorous and diverse Odonate population has been a reliable indicator of the health of an ecosystem¹. Fishes feed on

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larvae while insectivorous birds, reptiles and amphibians feed on both larvae and adults. The Odonata thus, form an integral part of aquatic as well as terrestrial ecosystems and are also the natural biological agents particularly the larvae, which are biological indicators of aquatic pollution. Furthermore Odonates are beneficial to outdoor enthusiasts because they prey on many pest insects including mosquitoes². Odonates are formidable predators as both larvae and adults². They are actively used in controlling causative agents of malaria and filariasis throughout the watery planet.

Dragon flies and damselflies make up nearly 5000 species². In India more than 500 species are seen. In Kerala 140 species of odonates are available. Studies of Odonata have included Odonata relationships with water quality³ biotope quality^{3,4} and general species richness^{2,3} and the use of Odonata as indicators for wetland conservation⁵, riparian management needs⁶, wetland buffer width requirements⁷ and shallow lake restoration⁸. This is largely because many of the criteria of good indicator species, such as being taxonomically well-known, relatively easy to identify, and having distinct habitat requirements^{2,3} are fulfilled by Odonata¹.

As a group of species that are especially sensitive to changes in their habitat, Odonata populations can also be indicative of the richness of other invertebrates and microphytes². Odonata can also act as umbrella species, facilitating the protection of habitat that is crucial for the survival of other species^{8,2}.

Wet lands can play a significant role in maintaining the ecological integrity of a region. While they cover less than 9% of the global land area, they provide a disproportionately wide range of functions, including support for Biodiversity, improvement of water quality, sequestration of carbon. However, it is estimated that approximately half of the world's wetlands have been lost through human disturbances such as increased urbanization and intensification of agriculture^{4,7}.

Materials and Methods

Study area and sampling

Seven wetland sites were chosen for the study. These sites included paddy fields, ponds, temporary water bodies and ditches. Sampling was conducted during 2010-2011 from June to December. Sites were visited frequently throughout the duration of field season. Sampling was conducted on warm, sunny days, since they thermo-regulate using solar heat. Many specimens were photographed for identification. Using field guide Subramanian, 2006¹¹) the specimens were identified.

Environmental data

Measures of pH, Temperature, Electrical conductivity, Turbidity and Dissolved oxygen were recorded.

Data analysis

In order to determine differences in water quality between the different sites, differences in pH, Temperature, Conductivity, Turbidity and Dissolved oxygen were evaluated using analysis of variance. As an assessment of the Odonate assemblages at each site Odonata diversity was computed using the Simpson's index and species abundance were calculated and compared. The formula for calculating Simpson's index is as follows:

$$\text{Simpson's index } (\lambda) = \sum_{i=1} n_i(n_i-1) / N(N-1)$$

Where, n_i is the number of individuals of the i^{th} species, and N is the total number of individuals in the sample. Simpson's index (λ) varies from 0 to 1. An increase in the values of the index indicates a decrease in diversity and vice versa.

Species richness or abundance is a measure of the number of species found in a sample. It can be measured using Mehinick's index as follows:

$$D = s / \sqrt{N}$$

Where, D is the measure of species richness, s equals the number of different species represented in the sample, and N equal the total number of individual organisms in the sample.

Results

Odonata diversity and abundance

A complete list of dragon flies and damsel flies species observed and photographed at the sampling sites can be found in Table 1 & 2. Throughout the seven sites, 17 species of damsel flies and 27 species of Dragon flies were collected totaling 44 species. Anisoptera dominated. Anisoptera was represented by 3 families, namely Gomiphidae, Libellulidae and Aeshnidae. Of them Libellulidae was represented by the maximum number of species (25), and individuals. Gomphidae and Aeshnidae are represented by single species. Among the Zygoptera, Coenagrionidae have highest number of species (5), followed by Lestidae (3), Platycnemididae (2) Protoneuride (2) and Synlestidae, Calopterygidae, Chlorocyphidae, Euphaeidae,

having single species each respectively (Figs. 1-3). Of the 44 species recorded, five of them, representing 11.4%, are IUCN red listed, one rare species and three are endemic species (Table 3 and 4).

Water quality

The comparison of different water quality parameters are shown in Fig. 4 and 5. The conditions of environmental quality of ecosystems around the Kallikad and Mannur are almost same. Slightly higher frequency of species compared to Mannur is seen in Kallikad. Low DO levels and increased temperature facilitate species diversity and species abundance. The maximum Odonata diversity is noticed in Kallikad. Highest abundance in Mannur is also due to the above mentioned environmental parameters. For the protection of aquatic life, pH should not measure below 6.5 or above 9.0. In all sites pH is between 7 and 8, except Dhoni where the pH is less. Dhoni showed minimum diversity.

The site 4 (Mannur) has TDS less than 120 mg L and electrical conductivity is less than 220 μ S cm. Kallikad also got low TDS electrical conductivity. Kallikad and Mannur got highest Odonate collections. In Dhoni TDS and Electrical conductivity is less but pH is also low, hence Odonate distribution is poor.

Discussion

Maximum number of Odonates are collected from Kallikad area. Mercy college campus provide the most favourable habitat for the Odonates. Availability of the ponds, heterogenous vegetations, grasslands, gardens and puddles and shade cover offered by the tall trees are the favourable conditions for the Odonates diversity. Mannur paddy fields are also favoured. Sixteen species were collected from here. Availability of water and vegetation provide suitable habitat for Anisoptera. Anisoptera was abundant in the most of the places sampled. This might be due to their high dispersal ability and their adaptability to wide range of habitats. High dispersal ability of the Anisoptera is the reason for their wide distribution across the Mannur paddy field. In Pirayiri area the number of the species is less. This may be due to the loss of wet lands. Here most of the paddy fields and other open areas have been filled for construction. Many areas are used as dumping ground for garbage. In Pudussary area the number is less. Urbanisation and pollution leads to the reduction of wetlands here. Dhoni also shows a reduction in the Odonata. Species, *Pseudagrion indicum* characteristic of hilly area is seen here.

Of the 44 species recorded, five of them, representing 11.4% are IUCN red listed and one a rare species. Three are endemic. They are respectively, *Pseudagrion indicum*, *Euphaea fraseri*, *Phylloneura westermanni*. *Rhyothemis variegata* was common during the sunny days of South-west monsoon. They are seen in swarms. *Pantala flavescens* can also be seen in large swarms before and after North-east

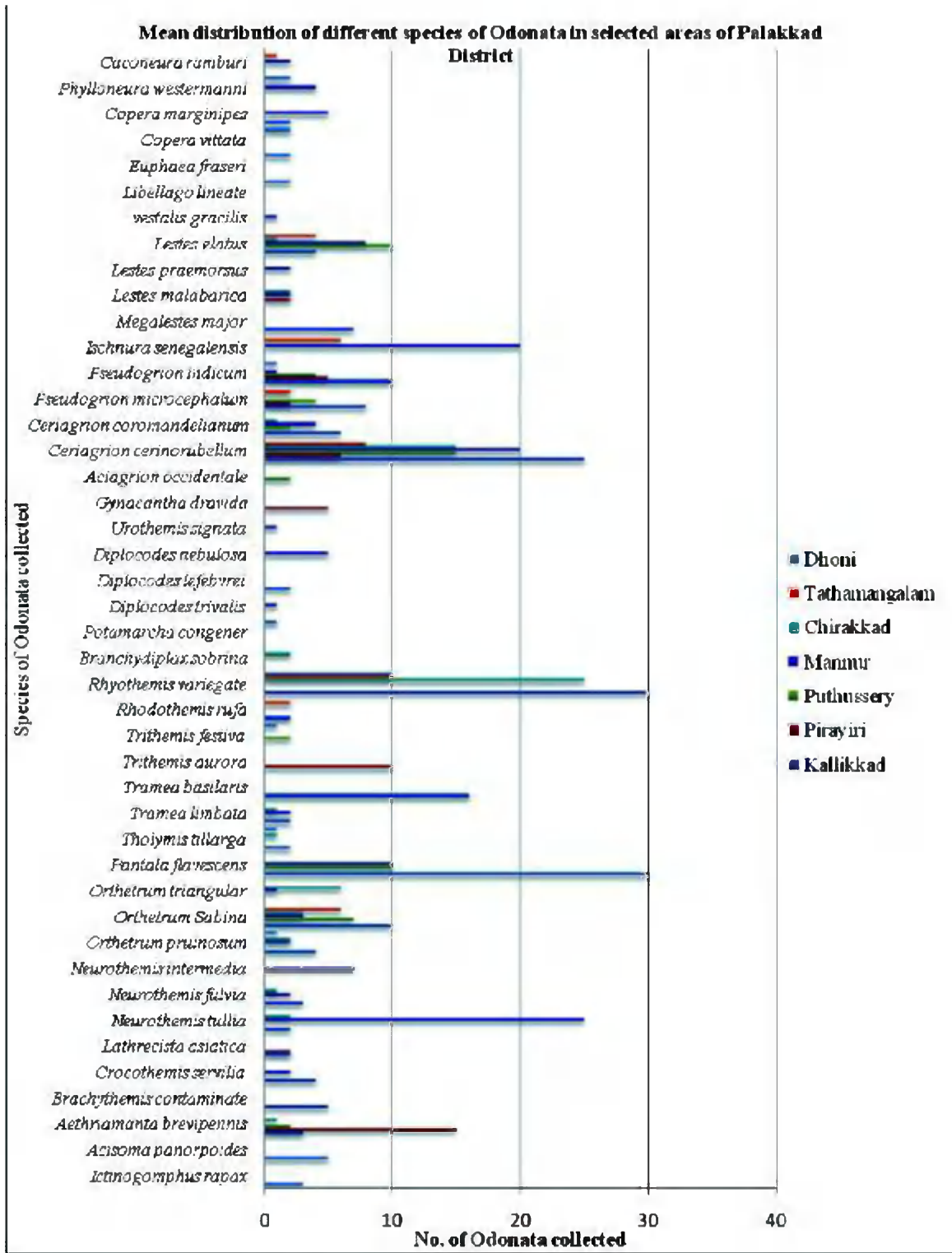


Fig. 1. Mean Distribution of different species in selected areas of Palakkad

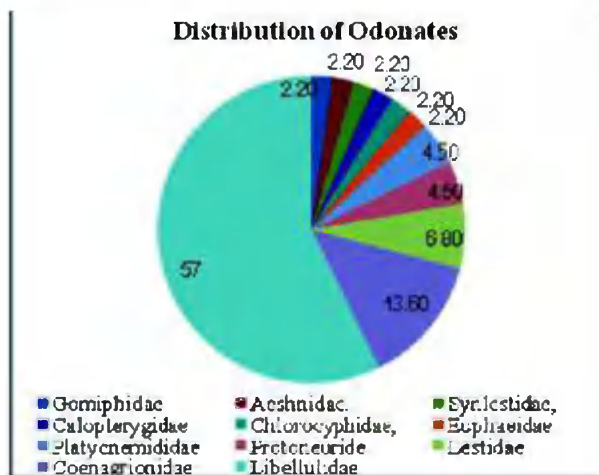


Fig.2. Percent Distribution of odonates in the seven selected sites of Palakkad district

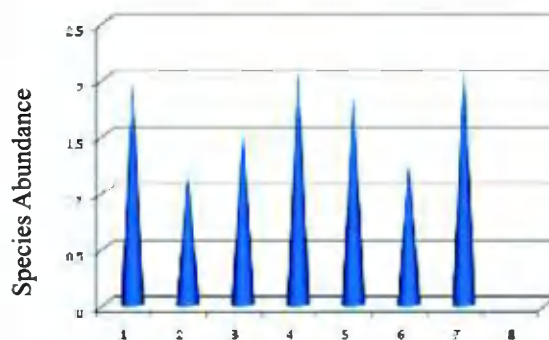


Fig. 3. Species abundance of odonates in seven selected sites of Palakkad

Note: 1-Kallikad,2-Pirayiri,3-Puthussery,4-Mannur,5-Chirakad,6-Tathamangalam,7-Dhoni.. Highest abundance is in Mannur, Least abundance is in Pirayiri

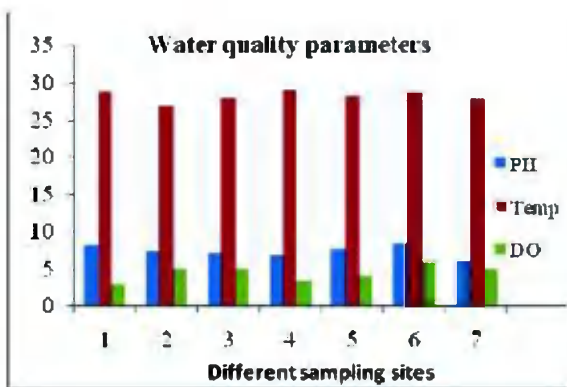


Fig. 4. Comparison of water quality parameters as pH, temperature and dissolved oxygen (DO).

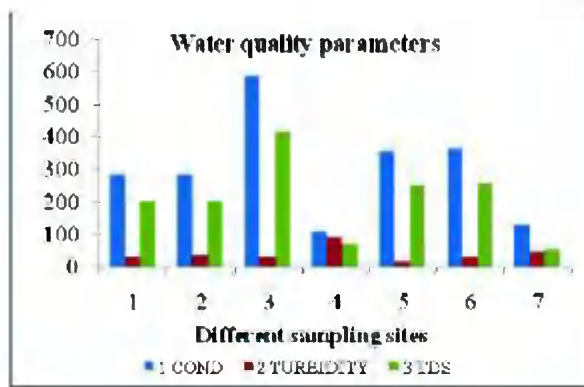


Fig. 5. Comparison of water quality parameters as conductivity, turbidity and total suspended solids.

Table 1. List of Odonates collected under suborder Anisoptera

Family	Species	Common name	Sampling sites
Ceratopogonidae (Clubtail)	<i>Ceratopogon chloroceryx</i>	Common clubtail	Ponds
	<i>Tramea panorpoides</i>	Trumpet Tail	Ponds
	<i>Amphicnemis brevicauda</i>	Scarlet Marsh Hawk	Ditches
	<i>Onychthemis communis</i>	Ditch Jewel	Ditches
	<i>Coenobremia australis</i>	Ruddy Marsh Skimmer	Paddy field
	<i>Cathorpeia asiatica</i>	Asiatic Broad Tip	Wetland
	<i>Neurothemis pulchra</i>	Pied Paddy Skimmer	Paddy field
	<i>Neurothemis filix</i>	Fulvous Forest Skimmer	Streams
	<i>Neurothemis intermedia</i>	Ruddy Meadow Skimmer	Gardens
	<i>Orthetrum pulcherrimum</i>	Cinnamon-Tailed Marsh Hawk	Paddles
	<i>Orthetrum rubellum</i>	Green Marsh Hawk	Gardens
	<i>Orthetrum triangulum</i>	Blue-Tailed Forest Hawk	Paddy field
	<i>Granta flavescens</i>	Wandering Glider	Paddles
	<i>Pantodon thagra</i>	Coral-Tailed Cloud Wing	Ponds
Libellulidae (Skimmers)	<i>Tramea laticornis</i>	Black Marsh Trout	Ponds
	<i>Trithemis aurora</i>	Cinnamon Musk Glider	Paddles
	<i>Trithemis jessieae</i>	Black Stream Glider	Paddy field
	<i>Microthemis rufa</i>	Rufous Musk Glider	Ditches
	<i>Myzothemis variegata</i>	Common Picture Wing	Paddy field
	<i>Macflydipia sobrina</i>	Little Blue Marsh Hawk	Ponds
	<i>Potamarchia erythrogastra</i>	Yellow-Tailed Ashy Skimmer	Paddy field
	<i>Diplocoelus ovalis</i>	Ground skimmer	Paddles
	<i>Diplocoelus tajikistanii</i>	Black Ground Skimmer	Wetlands
	<i>Diplocoelus ruber</i>	Black-Tipped Ground Skimmer	Wetlands
	<i>Urothemis signata</i>	Greater Crimson Glider	Wetlands
Aeshmidae (Damers)	<i>Gynacantha dravidica</i>	Brown Damer	Wetlands

Table 2. List of Odonates collected under sub order Zygoptera

Family	Species	Common name	Sampling sites
	<i>Aeschna occidentalis</i>	Slits	Ponds
	<i>Ceragrion</i>	Orange-tailed	Marsh Ponds
Coeragrionidae (Marsh dart)	<i>Coeragrion ballista</i>	Dart	
	<i>Ceragrion</i>	Commander Marsh Dart	Riparian vegetation
	<i>Coeragrionidiana</i>		
	<i>Pseudagrion microcerbatum</i>	Blue Grass Dart	Ditches
	<i>Pseudagrion thalicta</i>	Yellow Striped Blue Dart	Hill streams
	<i>Isochaeta paraguayensis</i>	Cold Dart	Grassland
Syolestidae (Giant spread wing)	<i>Syolestes malabaricus</i>	Giant Emerald Spread Wing	Riparian vegetation
Leucostictidae (Spread wing)	<i>Leucosticta malabaricus</i>	Malabar Spread Wing	Ponds
	<i>Leucosticta protracta</i>	Sapphirine Spread Wing	Ponds
	<i>Leucosticta</i>	Emerald Spread Wing	Grassland
Calopterygidae (Glories)	<i>Calopteryx</i>	Clear Winged Glory	Forest Hill streams
Chlorocyphidae (Stream jewels)	<i>Chlorocypha</i>	River Jewel	Hill streams
Euphaeidae (Torrent dart)	<i>Euphaea</i>	Malabar Torrent Dart	Hill streams
Platycenemidae (Bush dart)	<i>Platycenia</i>	Blue Bush Dart	Puddles
	<i>Platycenia</i>	Yellow Bush Dart	Puddles
Proconetidae (Bamboo tail)	<i>Proconeta</i>	Monister Bamboo Tail	Ponds
	<i>Proconeta</i>	Coorg Bamboo Tail	Riparian vegetation

Table 3. Species diversity index (Simpson) of

Odonates in seven sites in Palakkad district

Name of the site	Species diversity
Kallikad	0.05
Pirayri	0.06
Puthussery	0.15
Mannur	0.10
Chirakkad	0.17
Tathamangalam	0.15
Dhoni	0.20

Note: The maximum odonata diversity is noticed in Kallikad. Dhoni showed minimum diversity.

Table 4. Conservation status of odonata

Status	Species	Percentage (on 44 species)
Rare Species	<i>Phylloneura westermanni</i>	2.2%
IUCN	<i>Pseudogryon indicum</i>	11.4%
Red List	<i>Megalestes major</i>	
	<i>Caconeura ramburi</i>	
	<i>Potamarcha congener</i>	
	<i>Diplocodes nebulosa</i>	
Endemic Species	<i>Pseudogryon indicum</i>	6.8%
	<i>Euphaea fraseri</i>	
	<i>Phylloneura westermanni</i>	

monsoon. *Brachythemis contaminata* is seen in polluted water bodies in the human impacted area. The dominant family Libellulidae is tolerant to aquatic pollution. Absence of pollution sensitive families indicates poor quality water flowing through human impacted area. Family Libellulidae (Anisoptera) are the dominant Odonata collected. Eurytopic (wide habitat tolerance) nature of Libellulidae² might be responsible for their abundance when compared to other families, namely Coenagrionidae and Lestidae recorded in the present study. Libellulidae are common in plains and their diversity decreases with increase in altitude because fast flowing streams and rivers are not suitable for Libellulidae naiads, which require sluggish and weedy pond³ Dhoni recorded poor Libellulidae population. The presence of shade cover and aquatic vegetation favoured Zygoptera population. Less abundance of damsel flies was probably due to their limited dispersal ability and absence of shade cover over the habitat from the trees around the water bodies. Higher species richness and diversity of Odonata in Palakkad District could be attributed to the vast area, variety of biotopes and high shade cover and nearness to western ghat.

Low DO levels and increased temperature facilitate species diversity and species abundance. The maximum Odonata diversity is noticed in Kallikad. Highest abundance is in Mannur is also due to the above mentioned environmental parameters. For the protection of aquatic life, pH should not measure below 6.5 or above 9.0. In all sites pH is between 7 and 8, except Dhoni where the pH is less. Dhoni showed minimum diversity. According to water quality index of biodiversity, the mean salinity of fresh water

aqua system is approximately 120 mg L⁻¹, total dissolved solids (TDS) which corresponds to an electrical conductivity of approximately 220µS cm⁻¹. However, conductivities in freshwaters can range between 10 and 1,000 µS cm⁻¹ and in highly polluted rivers conductivities can exceed 1000 µS cm⁻¹^{9,10}, found that when TDS increased from 270 to 1170 mg L⁻¹ (approximately 500 to 1500 µS cm⁻¹), some species of plants were eliminated. The site 4 (Mannur) has TDS less than 120 mg L and electrical conductivity is less than 220µS cm. Kallikad also got low TDS and electrical conductivity. Kallikad and Mannur got highest Odonate collections. In Dhoni TDS & Electrical conductivity is less but pH is also low and Odonate population is poor.

The survey of different sites revealed the occurrence of 44 species of Odonates. The Odonata belonging to the family Libellulidae dominated in all sites. *Caconeura ramburi*, *Lestes praemorsus*, *Diplocodes trivalis* are represented by single individuals. *Crocothermis servilla* can be seen in all the sites. *Ceriagrion coramandelianum* is also seen in good numbers. *Pseudogrion indicum*, *Euphaea fraseri*, and *Phylloneura westermanni* are endemic to Western ghats. *Phylloneura westermanni* is a rare species. Decline in the population of Odonates is an alarm signal on the status of the various habitats in the wet land. Wet lands are therefore among the most threatened habitats globally because of their over exploitation, pollution and non sustainable development.

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Phytochemical analysis and *in vitro* antibacterial activity of some spices on food associated bacteria

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Abstract

The phytochemical analysis of both the aqueous and ethanolic extracts of three Indian spices [*Murraya koenigii* (curry leaf), *Piper nigrum* (pepper) and *Coriandrum sativum* (coriander)] and their antibacterial activity against food associated bacteria - *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, Lactic acid bacteria were investigated. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, reducing sugar, steroids and tannins. The antibacterial activity was measured by disc diffusion method. Aqueous extracts of black pepper did not exhibit antibacterial activity against *Salmonella* spp, *S. aureus* and *B. subtilis*. *Staphylococcus aureus* showed highest susceptibility with ethanolic extract of pepper with 24mm zone of inhibition. Curry leaf ethanolic extract showed antibacterial activity against the test organisms with zone of inhibition ranging between 10mm to 20mm. Both the coriander extracts inhibited the growth of the test organisms. The antimicrobial activity exhibited by extracts of black pepper and curry leaf on food associated organisms showcased the preservative potentials these extracts possess in controlling the growth of such organisms in foods. This research supports that the black pepper and curry leaf extracts may be used as natural antibacterial preservative to reclaim the shelf-life of food.

Key words: Phytochemical, disc diffusion method, antibacterial activity, extracts.

Introduction

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Many plant derived products such as spices or extracts have been used for centuries for the preservation and extension of the shelf life of foods¹. Studies in the past decade confirm that the growth of both gram-positive and gram-negative food borne bacteria, yeast and mold can be inhibited by garlic, cinnamon, cloves and other spices. Effects of the presence of these spices can be seen in food products such as pickles, bread, rice, and meat products. The fat, protein, water, and salt contents of food influence microbial resistance. Thus, it is observed that higher levels of spices are necessary to inhibit growth in food than in culture media². In this study, three different spices have been used (*Piper nigrum*- berry, *Murraya koenigii*- leaf and *Coriandrum sativum*- berry).

Microorganisms are associated in a variety of ways with all the foods we eat. Food products serve as sources of nutrition for human and other animals and as substrates for the growth of microorganisms³. The

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uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizeable losses of food products that are critically needed to meet global food requirements⁴. Growth of pathogenic, toxigenic and spoilage causing microorganisms such as *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Pseudomonas spp.*, *Enterobacter aerogenes*, *Mucor spp.*, *Rhizopus spp.*, *Aspergillus spp.* etc need to be controlled in food as they pose the risk of causing food borne infections, food borne intoxications and decay of the food.

Control of pathogenic and spoilage causing microorganisms is achieved by sanitizing the food, to reduce its microbial load and ultimately extend its shelf life by application of chemical preservatives and physical methods. These methods have been reported to be detrimental to the health of consumers of preserved foods⁵. Currently, there is growing pressure from consumers on food industries to replace use of synthetic chemicals with natural alternatives in order to ensure safety⁶. As a result of this, a lot of researches are conducted to screen plant materials for bioactive compounds that could be used as preservatives in foods. A product of such researches is the Grape Seed Extract (GSE), which has been accepted and presently applied as a 'safe plant derived preservative' for use in foods⁷. A very important step in the screening of a plant material for preservative activity is to evaluate its antimicrobial activity against food borne microorganisms.

It is in view of this, that the present research was set up to evaluate the phytochemical constituents and antimicrobial activity of aqueous and ethanol extracts of black pepper berries and curry leaves against some food – borne microorganisms as well as standard antibiotics by disc diffusion method, thus to help in the selection of drugs for the preservation of foods. If these plant samples possess natural preservative properties, then their application might be helpful in elevating the shelf life of foods.

Materials and methods

Collection and processing of plant materials

The samples (*Piper nigrum* – berries, *Murraya koenigii* – leaves, *Coriandrum sativum* – berries) bought from a local market in Palakkad were washed and shade dried over a period of two weeks. The dried samples were milled into fine powder using mortar and pestle, stored in sterile vials in a cool dry place till further use.

Extraction of plant materials

About three hundred grams (300 g) of each spice ingredient powder was suspended in water, and in 99% ethanol (500 ml) in separate flasks. The suspensions were kept at room temperature and left 14 days with

regular shaking. The suspensions were then filtered, and the solvents were removed by evaporation to dryness at room temperature⁸. The dried extracts were weighed and kept in labeled sterile vials in deep freezer till phytochemical analysis.

Preliminary phytochemical investigations

The major secondary metabolites classes such as alkaloids, flavonoids, saponins, reducing sugars, steroids and tannins were screened according to the following methods.

Test for alkaloids

Test for alkaloids was carried out according to the method described by Ciulci⁹. To 1.0ml of each extract in two separate test tubes, 3 drops of Dragendoff's reagent was added. An orange red precipitate/turbidity with Dragendoff's reagent would indicate the presence of alkaloids.

Test for flavonoids

Presence of flavonoids was tested according to the method of Sofowora¹⁰. A piece of magnesium ribbon was added to 4 mg/ml of each extract. This was followed by the addition of concentrated hydrochloric acid (HCl), drop wise. Crimson to magenta colour indicated the presence of flavonoids.

Test for saponins

The method of Brain and Turner was used for testing saponins¹¹. Half gram (0.5 g) of each extract was placed in a test tube and then 0.5 ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15mins indicated the presence of saponins.

Test for reducing sugars

The presence of reducing sugars was detected by the method of Brain and Turner¹¹. One milliliter (1 ml) of stock solution of each extract was diluted with 2 ml of distilled water, followed by the addition of Fehling's solution (A+B) and the mixtures warmed. Brick red precipitate at the bottom of the test tubes indicated the presence of reducing sugars.

Test for steroids

Test for steroids was carried out according to the method described by Ciulci⁹. Two grams (2g) of each extract was evaporated to dryness. The residues were dissolved in acetic anhydride, and chloroform was then added. Concentrated sulphuric acid was then added by the side of the test tube. A brown ring at the interphase of the two liquids and the appearance of violet colour in the supernatant layer indicated the presence of steroids.

Test for tannins

The method described by Ciulci was adopted to check the presence of tannins⁹. Solutions of the extracts were made with distilled water and 3 drops of 5% ferric chloride solution was added. A green-black or blue-black colouration indicated the presence of tannins.

Microbial strains

The microbial strains used are *Salmonella spp*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactic acid bacteria*. Overnight broth cultures of the preserved food associated bacterial strains were used to screen against the plant extracts.

Bioassay

The disc diffusion method was used for antimicrobial activity. The dried extract was reconstituted with Dimethyl sulfoxide (DMSO) to obtain a stock solution of 200 mg/ml. Overnight broth cultures of the respective strains were prepared. Mueller Hinton Agar (Hi Media Laboratories Pvt. Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Several Whatman filter paper (No.1) discs of 6mm diameter were impregnated with 10µl of the solution of each crude extracts (200 mg/ml) prepared using DMSO as solvent. The discs were evaporated at 37°C for 24h. Discs prepared with only the corresponding volume of DMSO was used as negative control. Gentamycin and Ampicillin discs were used as positive control for all test bacteria and gram negative bacteria respectively. The plates were incubated at 37°C for 24h. Antibacterial activity was evaluated by measuring diameter of the inhibition zone (IZ) around the discs.

Results and discussion

A qualitative phytochemical analysis performed for the crude extracts of pepper, curry leaf and coriander revealed the presence of alkaloids, flavonoids, saponins, reducing sugars, steroids and tannins (Table 1). Phytochemical investigation of ethanol and aqueous extracts of all the three drugs revealed differences in their phytoconstituents. Extracts of the same drug obtained using different solvents (ethanol and water) also exhibited differences in their constituents.

Mainly, five food associated bacteria were chosen for the present antibacterial study. Antibacterial activity of crude extracts of pepper, curry leaf and coriander were evaluated by measuring the diameters of zone of inhibition on bacterial strains and the results were obtained as shown in Table 2. Fig.1 and Fig. 2 show the graphical representation of antimicrobial activity of solvent extracts. Fig. 3 and Fig. 4 are the photographs of antimicrobial activity of solvent extracts.

Table 1. Result of phytochemical screening of crude extracts of spices

Extract	Alk.	Flav.	Sap.	R.sugar	Ste.	Tan.
Pepper Ethanol	+	+	+	+	-	-
Pepper Water	+	+	+	+	+	+
Curry leaf Ethanol	-	+	-	+	+	-
Curry leaf Water	+	-	-	-	-	-
Coriander Ethanol	-	+	-	+	-	-
Coriander Water	-	-	-	+	-	-

Note: Alk- alkaloids, Flav- Flavanoids, Sap – saponins, R.sugar- reduced sugar, Ste – steroids, Tan- tannins, + = detected, - = not detected

Table 2. Antibacterial activity of different solvent extracts of spices

Bacteria	DMSO 5%	Gen. 25 µg/ml	Amp. 25 µg/ml	Zone of inhibition (mm)					
				Pepper		Curry leaf		Coriander	
				E	W	E	W	E	W
<i>Salmonella</i> spp	-	29	22	0	0	10	0	0	0
<i>Escherichia coli</i>	-	28	24	22	12	12	0	0	0
<i>Staphylococcus aureus</i>	-	22	-	24	0	20	0	18	0
<i>Bacillus subtilis</i>	-	25	-	23	0	20	0	10	9
<i>Lactic acid bacteria</i>	-	19	-	11	9	10	0	9	0

Note: DMSO– Dimethyl sulphoxide (negative control), Gen- Gentamycin and Amp-Ampicillin (positive control for all test bacteria and gram negative bacteria respectively), 0-No activity.

Present study indicated great variation in antimicrobial activities of aqueous and ethanol decoctions against selected spices. The most promising spice was black pepper. The results showed that the ethanol decoction of black pepper possesses great antibacterial potential as compared to ethanol decoction of curry leaf while ethanol decoction of coriander did not show much antibacterial effects. In the present study, the ethanol decoction of black pepper exhibited maximum effect against *Staphylococcus aureus* (24 mm average diameter of inhibitory zone) and found to be most active antibacterial agent against the test organisms.

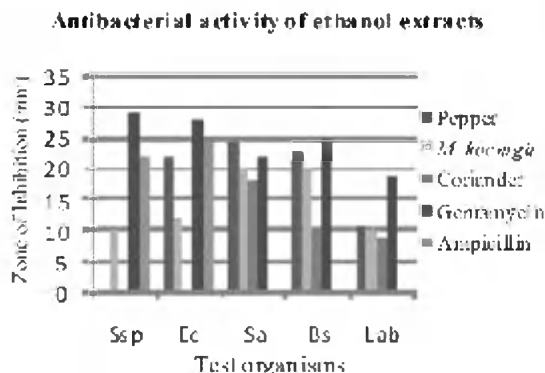


Fig. 1. Graphical representation of antibacterial activity of ethanol extract of spices

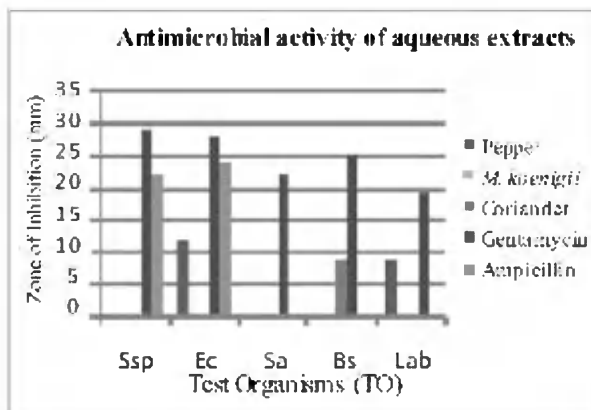
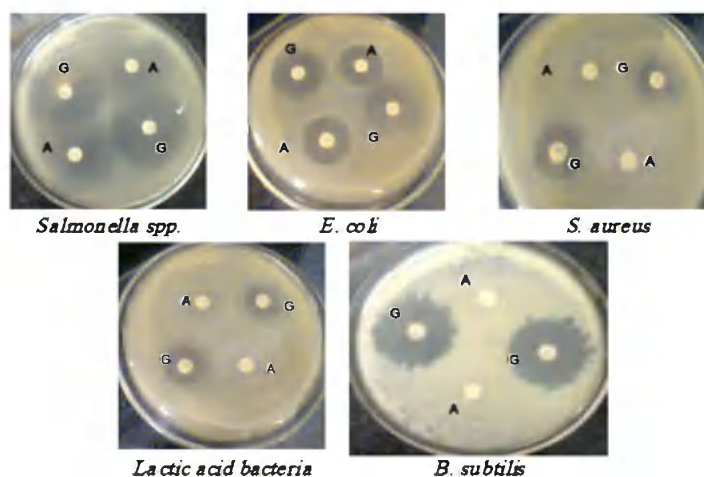


Fig. 2. Graphical representation of antibacterial activity of aqueous extract of spices

Note: Test Organisms (TO) – (Ssp- *Salmonella spp*, Ec – *Escherichia coli*, Sa – *Staphylococcus aureus*, Bs – *Bacillus subtilis*, Lab – *Lactic acid bacteria*)

Piper nigrum (black pepper) extracts showed presence of alkaloids, flavonoids, saponins, reducing sugars, steroids and tannins. Findings of the present phytochemical analysis of pepper are similar to those reported by Shamsuddeen et al¹². They observed the antimicrobial effect of black pepper and found remarkable inhibition against variety of tested bacteria including *S. aureus* and *Salmonella spp*. In another study, inhibition of the growth of meat spoilage bacteria has been reported by Ouattara et al¹³. Later, both aqueous and ethanolic extracts of black pepper have been screened for antibacterial activity against a penicillin G resistant strain of *S. aureus*¹⁴, *Bacillus cereus* and *B. subtilis*¹⁵ in two different studies. The major constituent of black pepper is Piperine. It is bioactive compound and has been reported to be the major contributors to the antimicrobial activity of spices.



Note: A – Ampicillin, G – Gentamycin

Fig. 3. Antibacterial activity of Standards

Murraya koenigii (curry leaf) exhibited the presence of alkaloids, flavonoids reducing sugars and steroids in its extracts. In the present research, the antibacterial effect of ethanol decoction of curry leaf was next to black pepper. It exhibited maximum antibacterial potential against *S.aureus* and *B.subtilis* (20 mm). Of the three tested spices, only ethanol decoction of curry leaf inhibited the growth of *Salmonella spp.* The results of present investigation are in correlation with the study in which methanol extracts of leaves of *M. koenigii* inhibited *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corynebacterium gravis* and *Bacillus cereus*. Also the hexane extract inhibited all microorganisms except *Staphylococcus epidermidis*, *Streptococcus uberis* and *Bacillus cereus* ¹⁶. Another study was carried out by Bhaskar K. Gupta et al., which investigated the antibacterial effect of curry leaf and their extracts on *S. aureus*, *P. aureginosa*, *B. subtilis*, *S. typhi*, *E. coli*, *P. vulgaris*, and *P. aeruginosa*. As per the result, plant extract showed a broad spectrum of very significant antibacterial activity of producing a clear zone of inhibition (19-20 mm) against *S. aureus*, *P. aureginosa* and *B. subtilis* whereas, *S. typhi*, *E. coli*, *P. vulgaris*, and *P. aeruginosa* were found to be moderately sensitive, which showed an inhibition Zone of 10-16 mm ¹⁷.

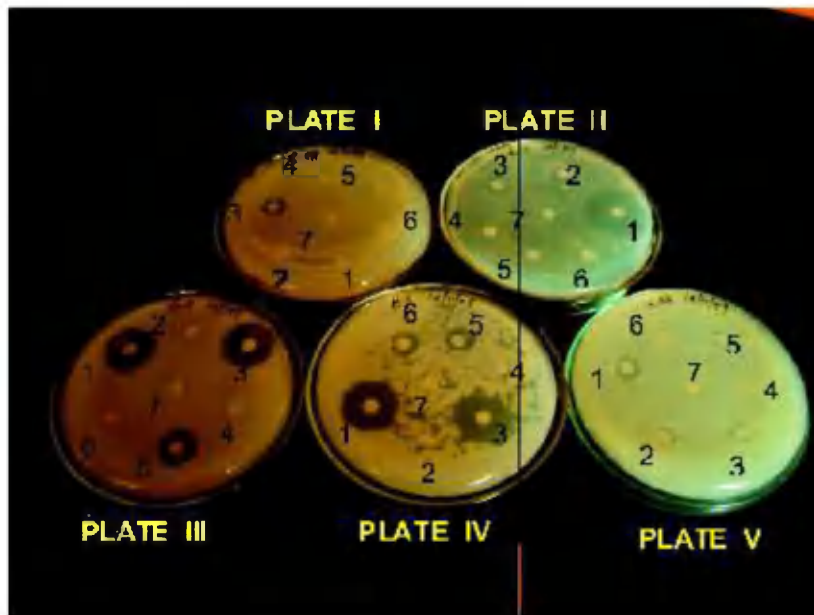


Fig. 4. Antibacterial activity of Test Samples

Note: Plate I – *Salmonella spp.*, Plate II – *E. coli*, Plate III – *S. aureus*, Plate IV – *B. subtilis*, Plate V – *Lactic acid bacteria*, 1- pepper ethanol, 2- pepper aqueous, 3- curry leaf ethanol, 4- curryleaf aqueous, 5- coriander ethanol, 6- corriander aqueous, 7- DMSO

Phytochemical compounds such as flavonoids and reducing sugars were detected in *Coriandrum sativum*. In the present study, antibacterial effect of aqueous decoction of coriander was also evaluated. The aqueous decoction of coriander did not possess any remarkable antibacterial potential. In contrary, some workers have found that coriander has strong antibacterial activity against *S. aureus*, *S. typhi* and *E. coli*¹⁸.

The result of the present study showed that the extract of pepper and curry leaf contains many phytochemical components. The present investigation in *Piper nigrum*, *Murraya koenigii* and *Coriandrum sativum* for *in vitro* antibacterial properties confirms that the *P. nigrum* and *M. koenigii* plants contain bioactive components that exhibit measurable *in vitro* antimicrobial activity against the test organisms in this study. The results of the various screening tests indicate that the berries of *P. nigrum* possess good inhibitory action against majority of the test bacteria, whereas leaves of *M. koenigii* possess only a measurable inhibitory action against gram positive *S. aureus*, *B. subtilis* and *Lactic acid bacteria*.

Although the antimicrobial activity of some spices and herbs is documented, the normal amounts added to foods for flavor is not sufficient to completely inhibit microbial growth. The antimicrobial activity varies widely, depending on the type of spice or herb, test medium, and microorganism. For these reasons, spice antimicrobials should not be considered as the only preservative method¹⁹. However, the addition of herbs and spices can be expected to aid in preserving foods held at refrigeration temperatures, at which the multiplication of microorganisms is slow. As a conclusion, it is clear that some of the bioactive compounds produced by plants in this study serve to protect it against microbial attacks and to certain extent they may be used as natural antibacterial preservatives to reclaim the shelf life of food.

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Histopathological effects of short term administration of sub-lethal concentration of endosulfan on the brain and alimentary canal of *Periplaneta americana*

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Abstract

The effect of the organochlorine insecticide, endosulfan was studied on the brain, gizzard, hepatic caecae and initial portion of the mid gut. The experimental specimens were treated with the 0.0004% endosulfan for a period of twenty seconds twice a day for 4 days. No notable changes were observed in the brain. However, gizzard, hepatic caecae and mid-gut showed marked histopathological changes. In the gizzard chitinized teeth and acanthae were distorted. The secretory nature of hepatic caecae was lost and formation of new villi was noticed. The absorptive villi of the mid-gut were found to be distorted and reduced in size and content.

Key words: histopathology, endosulfan, cockroach, mushroom bodies, gizzard, hepatic caecae, mid gut

Introduction

Since 2000 BC, humans have utilized pesticides to protect their crops. The first known pesticide was elemental sulphur dusting which was used about 4500 years ago in ancient Mesopotamia. By 5th century, toxic chemicals such as arsenic, mercury and lead being applied to crops to kill pests. In 17th century, nicotine sulphate extracted from tobacco leaves were used as insecticide. The 19th century saw the introduction of natural pesticides like Pyrethrum, derived from *Chrysanthemum*¹. Herbicides became common in 1960s, led by triazine and other nitrogen based compounds, carboxylic acids as 2, 4, dichlorophenoxyacetic acid and glyphosphates. During, 1940's manufactures began to produce large amount of synthetic pesticides and their use became widespread.

Endosulfan developed in early 1950's emerged as a leading chemical used against a wide variety of insects and mites. It acts as a contact and stomach poison. Endosulfan is one of the pesticides responsible for many fatal pesticide poisoning incidents around the world. It is fatal even to plants. Rate of photosynthesis in lettuce was found to get reduced after the application of endosulfan². In human beings delay in sexual maturity and interference in sex hormone synthesis has been reported on exposure to endosulfan³. Increased incidences of cryptochildism have been cited by Damgaard⁴. Studies on the effect of endosulphan on other lower animals are on an increase. Mortality rate among amphibian larvae as a result of exposure to endosulphan has been studied by Jones⁵. Guptha and Guptha⁶ summarizes

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information on the nomenclature and chemistry, metabolism and toxicity in mammals, birds, fishes and insects, degradation and metabolism of endosulfan in plants along with brief reference to the degradation in water and light, in their review. Environmental Justice Foundation, in their summary report, presents compelling evidences of the considerable threat endosulfan poses to human health and environmental integrity⁷. Because of its toxic effects it has been banned in April, 2011 by Stockholm convention. This insecticide is toxic to almost all living things. In this context, it was thought worthwhile to find out how this chemical would affect the cockroach, *Periplaneta americana*, which is one among the most sturdy insects which are capable of developing resistance against most pesticides.

Materials and methods

Periplaneta americana were brought to the lab and maintained in meshed wooden cages. Adult female cockroaches raised from the above colony were used for the present investigation. Concentrations of endosulfan to be tested were determined after trials with 0.0004%, 0.003% & 0.05% solutions in distilled water, for 4 days. Calculated LC₅₀ of endosulfan on cockroach for 48 hours was found to be 0.0004%.

The selected female cockroaches were grouped into two sets, set A & set B, each containing 10 specimens. Set A was kept as control. The experimental specimen of set B were tied carefully on glass slide and head was completely dipped in 0.0004 % endosulfan for 20 seconds. This was done twice a day and the procedure was repeated for 4 days. The behavioural changes in the cockroach before and after treatment was noted and recorded. All the cockroaches died within 80-96 hours. The dead cockroaches were collected and their alimentary canal and brain were dissected out and preserved in 10% formalin solution for making serial sections. Cockroaches from the control group were anaesthetised with chloroform before dissection. The experiment was repeated in 4 sets.

Preparation of permanent slides for histopathology

The preparation of the slides for histopathology was done adopting the procedure of Rajkumar and Saranya⁸. The posterior part of the foregut and the anterior part of mid-gut of the control and experimental animals were dissected out and fixed in 10% formalin. After washing in running tap water, it was dehydrated by passing through 70% and 95% alcohol three changes each. Each changes run for 1 hour. Then, they were kept in mixture of 95% alcohol and 95% chloroform for 1 hour. Slides were then passed through 3 changes of pure chloroform for 5 hours, one and half hours for the first two changes and two hours for the third one. The two sets of alimentary canal were then transferred to molten wax kept at 60 °c for two and half hours. They were then embedded in paraffin wax in moulds for sectioning. Blocks holding specimens were then labelled to differentiate between the experimental ones from the control.

Serial sections of 5µm thickness were obtained using a rotary microtome. Sections were double stained with Haematoxylin and Eosin and mounted in DPX. The permanent slides were then examined under compound light microscope. Microphotographs were taken at various magnifications for comparison. The above procedure was repeated to obtain sections of brain.

Results

The examination of the serial sections prepared showed no structural differences in mushroom bodies when compared to the sections of normal cockroach (Fig.1 & 2).



Fig.1. Mushroom body of normal cockroach (P - Pars intercerebralis).



Fig.2. Mushroom body of experimental cockroach (K – Kenyon cells,C - Calyx,P - Pars intercerebralis)

Sections of gizzard

In the section of gizzard of the control cockroach, the central region contained six chitinized intima also called chitinous teeth on which acanthae were present. These chitinized acanthae work against each other and help to grind the food into small particles (Fig.3). In the section of experimental group, the definite shape of chitinized intima were lost and distorted (Fig.4).

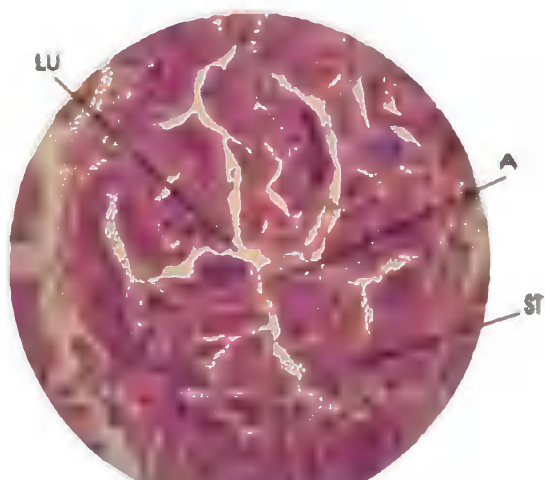


Fig. 3. Section of the gizzard of control specimen (LU-Lumen, A-Acanthae, ST-chitinous teeth)

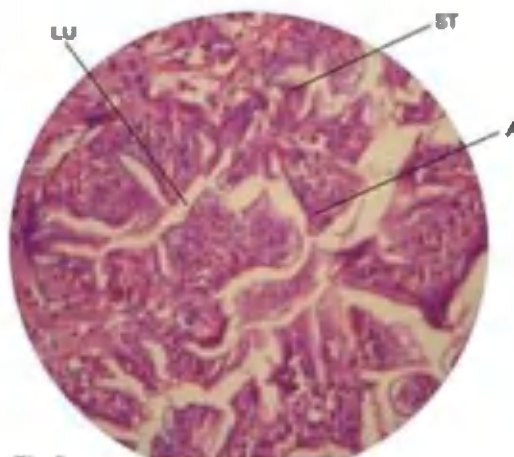


Fig. 4. Section of the gizzard of experimental specimen showing acanthae and chitinized teeth which are distorted. (LU-Lumen, A-Acanthae, ST-chitinous teeth)

Sections of hepatic caecae

In the cross sections of hepatic caeca of control specimens large number of secretory cells was found projecting into the lumen and the space of lumen was much reduced. Well developed peritoneal membrane was found. The columnar epithelial cells and longitudinal muscle fibres were present in the region (Fig. 5). In the sections of the experimental group, the peritoneal membrane and columnar epithelial cells appeared similar to that in the control sections but a considerable degeneration of the secretory cells was observed. The secretory cells were disintegrated and lost and new villi were found forming and projecting into the lumen of hepatic caeca. (Fig.6).

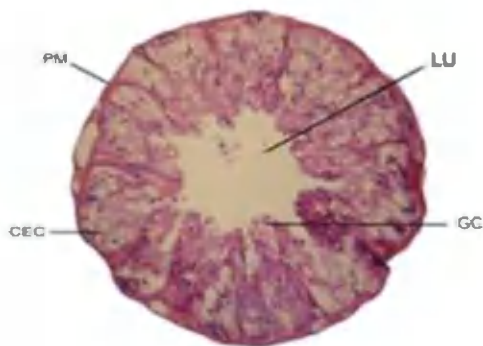


Fig. 5. Section of hepatic caecae of Control specimen (GC-Gastric cells, PM-Peritoneal membrane, LU-Lumen, CEC-Columnar epithelialcells, V-Villi)

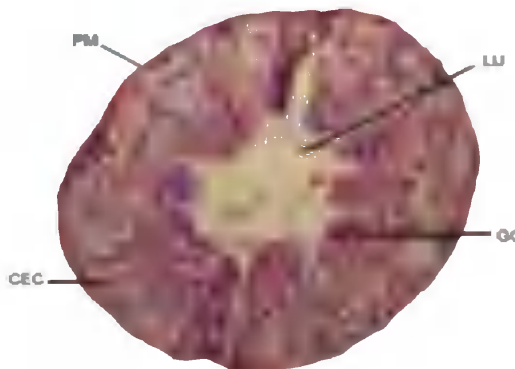


Fig. 6. Section of hepatic caecae of control cockroach (GC-Gastric cells, PM-Peritoneal membrane, LU-Lumen, CEC-Columnar epithelial cells,)

Sections of initial part of midgut

The cross section of the initial part of the midgut showed the presence of well shaped villi on the basement membrane (Fig.7). Degeneration and reduction in size of the villi were observed in the sections of experimental animals. Definitive shape of the villi was also lost (Fig.8).



Fig. 7. Section of the initial part of midgut of control cockroach (BM-Basement membrane, V-Villi)



Fig. 8. Section of the initial part of midgut of treated cockroach with degeneration of villi (BM-Basement membrane, V-Villi)

The present investigation showed vividly that short term exposure of sub-lethal concentration of endosulfan does not cause any considerable change in the structure of mushroom bodies of *Periplaneta americana*. The internal structure of the gizzard of the control specimen fits well with the description given by Shu –Hai⁹. However, literature on the damage of the sclerotized intima and the acanthae due to endosulfan or any pesticide has not been reported. Present report seems to be the pioneer one.

In the present study, a change of structure from secretory to absorptive nature has been observed in the hepatic caecae. Examination of the sections of control specimens presented a picture indicating secretory function with well packed large vacuolated cells leaving only a small lumen in the centre. But the treated specimens showed caecae with villi protruding into the lumen. Gresson¹⁰ has recorded that the hepatic caecae and anterior region of the mid-gut are chiefly secretory while the posterior part of the mid-gut is mainly absorptive in function. However, Woodruff¹¹ has opined that secretory role is doubtful and described the cellular appearance as artefacts. Gresson¹⁰ has also stated that in the caecae and anterior part of the mid-gut, the periods of secretion alternates with the periods of absorption. It is probable that the presence of endosulfan has reversed the function and changed the structure accordingly producing appropriate absorptive cells from the stem cells.

In the case of the initial part of the mid-gut, the villi were found to be degenerated in the treated animals. Literature relating to mid-gut is not available although toxic degeneration of the liver, lung, brain and

thyroid of human beings has been reported in the endosulfan fact sheet produced by IPEN¹². However, mention has been found on a variety of other acute and chronic toxic effects on insects and other animals.

The present study supports the previous information available in literature regarding acute and toxic effects of endosulfan on a variety of animals including insects. Its action particularly in the digestive sphere is evident in cockroach, which is a sturdy insect developing resistance to almost every pesticide. This demands serious attention because if cockroaches are affected seriously with such a low dilution and in a very exposure, the wide use of endosulfan would put all the other non-target insects and animals, especially man and other mammals at risk. Therefore, the use of endosulfan should be discouraged to prevent further tragedies in human settlements.

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A comparative study of antibacterial activity in leaf and leaf derived callus extracts of *Indigofera tinctoria*

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Abstract

The antibacterial property of the leaf and leaf callus extracts of *Indigofera tinctoria* was assayed against eight strains of human pathogenic bacteria, namely, *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* by disc diffusion method using crude methanol extracts. The methanol extract of the leaf has exhibited maximum inhibition against *E. coli* bacteria followed by *B. cereus* with. The inhibitory effect of the callus extract was found to be less. Identification of the presence of antibacterial activity in the callus demonstrates its possibility for using it as an alternative source to intact plants. The screening tests against the eight strains of bacteria with the leaf callus extract gave the observation that the highest inhibition zone was obtained in *E. coli* followed by *B. cereus*. Minimum inhibitory concentration study of the methanol leaf extract showed that the minimum concentration effecting inhibition is 62.5 µg/ml, while leaf callus extract exhibited inhibition with the MIC value of 125 µg/ml against both *K. pneumoniae* and *B. cereus*. Identification of the presence of antibacterial activity in the callus demonstrates its possibility for using it as an alternative source to intact plants.

Key words: *Indigofera tinctoria*, leaf, leaf callus, antimicrobials, minimum inhibitory concentration, phytochemicals.

Introduction

Traditional herbalists in India use a variety of herbal preparations to treat different kinds of ailments including several microbial infections. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented¹. The demand for plant based medicines, health products and pharmaceuticals is increasing in both developing and developed countries due to the growing recognition that the natural products are non-toxic, have least side effects and easily available at affordable prices². It is estimated approximately that 20% of the plants found in the world alone have been submitted to pharmacological or biological tests³. Medicinal plants are the richest bioresource of drugs in traditional systems of medicines, modern medicines, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs⁴. The present enthusiasm in the search for natural sources of biologically active compounds from plants has all the more enhanced the importance of medicinal plants.

Several workers throughout the world have carried out antibacterial studies on large number of medicinal plants. Out of the floral population of approximately 10,000 plant species in Kenya, 1200 plant species

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were reported to have medicinal properties and phytochemical evaluation of some of them have been reported⁵. Some research has succeeded in producing a wide range of valuable secondary phytochemicals in unorganized callus or suspension cultures, in other cases; production requires differentiated microplant or organ cultures⁶. Even though in some cases the callus cultures are a good source of all the secondary metabolites produced in the mother plant, in other cases only one or few phytochemicals are produced in the *in vitro* culture. For example, in the case of *Opium poppy* (*Papaver somnifera* L.) the mother plant contains a number of pharmaceutically important alkaloids of the benzylquinoline type including morphine, codeine, papaverine and sanguinarine in high concentration, only the phytoalexin sanguinarine has been found at significant level in *Opium poppy* cell cultures⁷. Conversely, the extracts of *in vitro* induced callus of *Alternanthera maritima* showed bioactivity against the same strains in which adult plant extracts showed bioactivity. But the intensity is quite low in the callus extracts due to lesser concentration of antibacterial compounds in the callus⁸.

The *tinctoria* species has been extensively used in folklore and traditional medicine systems for treatment of several disorders. In Ayurveda and Siddha it is used for Tikita Rasam, Katu rasam, Ushna veeryam, Katu, Vipaka etc. The plant demonstrates anthelmintic, analgesic and antiperiodic properties. Roots are used for anti poison, giddiness, colic and gonorrhoea and as a hair tonic. Leaves are used for jaundice, vatha, fever etc. Decoction of the leaves is used in blennorrhagia and treatment of hydrophobia. Roots are used for treatment of urinary complaints and hepatitis. It is also used as a sedative and for treatment of piles and ulcers⁹. The use of indigo (dye extracted from the plant) and its constituents, indirubin and indigotin prevents allergic contact dermatitis. The different types of secondary metabolites are the main components of the phytochemicals that are bioactive, demonstrating antimicrobial, antioxidant, antiinflammatory and anti-allergic activities. In our previous experiments conducted with the antibacterial activity of the crude leaf extracts in six solvents namely Petroleum ether, Chloroform, Acetone, Ethyl acetate, Methanol and Water, it was observed that methanol leaf extract was the most effective one to inhibit the growth of the pathogenic strains tested¹⁰. Hence this study was conducted to compare the antibacterial activity of crude methanolic leaf extract and leaf derived callus extract of *Indigofera tinctoria* and also to detect the antimicrobial principles in the leaf derived callus extract.

Materials and Methods

Collection and preparation of plant materials

Intact plants of *Indigofera tinctoria* (Fig.1) were collected from the Botanical Garden of St. Thomas College, Pala, Kerala. The leaves were excised, washed thoroughly in tap water, shade dried and powdered using mortar and pestle.

In vivo plant materials

Powdered plant material (50 g) was extracted in 300 ml Methanol using soxhlet apparatus for 72 h. After 72 h. the extract was filtered through Whatman No.1 filter paper and the filtrate was concentrated in vacuum rotary evaporator at 60 °C. The extracts so prepared were stored in labelled screw capped bottles in the refrigerator at 4 °C. The extract was reconstituted using minimal amounts of the extracting solvents prior to use. On completion of extraction, the extract was filtered using Whatman No.1 filter paper and the filtrate was concentrated in a vacuum evaporator at 60 °C in order to obtain almost complete evaporation of the solvent. The extract was collected in sterile screw capped bottles, labelled and stored in the refrigerator for further experiments. The extract was dissolved in minimal amount of methanol prior to use in antibacterial screening study.

Callus induction

Small young plant twigs with healthy, disease free semi- matured leaves were collected from one year old plants of *I. tinctoria*. The twigs were washed thoroughly in tap water for 30 min., transferred and kept in the systemic fungicide 5% Bavastine solution to which 4-5 drops of Tween 20 was added. The explants were constantly shaken for 15 min., rinsed with sterile distilled water and transferred to 70% alcohol for sixty seconds. Finally, to remove all surface contaminants, the explants were washed in 0.1 % aqueous mercuric chloride solution followed by three rinses of five min. each in sterile distilled water with constant shaking. The leaves of *I. tinctoria* was trimmed to a size of 5×7.5 mm size in the aseptic conditions of the laminar flow chamber and inoculated on solid medium prepared and kept in the laboratory conditions. The medium employed for the tissue culture studies is the modified MS medium. To culture the leaf disc explants of *I. tinctoria* MS¹¹ media containing 0.5-3mg l⁻¹ NAA alone or in combination with BAP 0.5–2mg l⁻¹ were utilized. Similarly, MS media containing 0.5-3 mg l⁻¹ NAA and 0.5-2 mg l⁻¹ Kin were also used. The media were sterilized by autoclaving at 1.05 kg/cm² at 121 °C for 20 min.

Incubation, maintenance and subculture of cultures

One set of explants was cultured on medium without plant growth regulators that served as control. Each treatment consisted of 12 explants and all the experiments were repeated three times. The cultures were maintained in the dark for 10 days and then transferred to photoperiodic conditions (16h. light: 8h. dark) with irradiance of 42 μm mol m⁻²s⁻¹ by cool fluorescent tubes. The temperature was maintained at 25± 2°C.

In vitro induced callus

The leaf callus of *I. tinctoria* grown on solid MS medium containing 1mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA were removed from the culture in the stationary phase of growth. The calli were air dried in the laboratory

conditions and powdered using mortar and pestle. 25 g. of the dried powder was extracted in 200 ml of the methanol using soxhlet apparatus for 72 h. Already prepared and stored powdered extract was dissolved in methanol was used for the screening.

Antimicrobial assays

Bacterial strains tested

The test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH), Chandigarh, India. *Escherichia coli* (MTCC 443), *Bacillus cereus* (MTCC 430), *Klebsiella pneumoniae* (MTCC 2405), *Salmonella typhi* (MTCC 531), *Enterobacter aerogens* (MTCC 111), *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 779) and *Proteus vulgaris* (MTCC 426) were the bacterial strains tested. The bacteria were subcultured on nutrient agar slants, incubated at 37 °C for 24 h. and stored at 4 °C in the refrigerator for further experiments. The cultures were maintained by periodic subculture in nutrient agar slants.

Bioassay

The procedure adopted is that of Barry and Thornsberry¹². Muller Hinton agar medium was used for the disc diffusion method. Blank sterile discs (Hi-Media Laboratory Pvt. Ltd. Bombay) of 6 mm diameter were used in the study. Each disc was impregnated with 25 µl of the extract solution containing about 100-500µg (0.1-0.5 mg) of plant extract. For each set of experiments, controls were maintained where pure solvents were used instead of the extracts. Streptomycin discs (25µg/disc)) were used as negative control so as to compare the degree of inhibition exhibited by the extracts. The dried discs were carefully placed over the spread cultures. The cultures were incubated overnight at 37 °C. On completion of the required period of incubation, the plates were examined for the presence of zone of inhibition. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values were taken.

Determination of Minimum Inhibitory Concentration

The Minimum inhibitory concentration measures the lowest concentration of the plant extract that will inhibit the growth of known strains of bacterium. MIC was determined using the broth dilution technique¹³. Mean value for all the assay was taken from the three repeated tests. The values are expressed in Mean ± SD.

Results and Discussion

Callus induction

The leaf disc explants of *I. tinctoria* cultured on MS medium supplemented with 0.5mg l^{-1} each of NAA and BAP showed initiation of the callus within 20 days of culture. The callus so initiated showed rather good proliferation within 30 days of culture (Fig. 2). Raising the concentration of BAP to 1mg l^{-1} in the above media demonstrated callus initiation within 10 days of culture. The callus proliferated further within 20 days of culture and gave rise to a well proliferating callus within 30 days of culture. The callus thus obtained cut into segments and transferred to the same medium showed very good proliferation. Proliferating callus so obtained by repeated subculture were collected after 15 days of culture, dried, powdered and used for testing of antibacterial activity.

From the results obtained in the *in vitro* studies on induction and proliferation of callus from *I. tinctoria*, it is clearly found that the entire process is controlled by the growth substances (hormones) present in the medium¹⁴. Specific concentration of plant growth regulators are needed to initiate callus formation and effect. Further, the requirement of the concentrations and combinations of the hormones varies from species to species and even depends on the source of the explants¹⁵ as well as the internal concentration of hormones in the explants. Lower concentration of auxin and higher concentration of cytokinin was required for the development of callus.



Fig. 1. *Indigofera tinctoria*



Fig. 2. Callus proliferation from the leaf explant

Antibacterial study

The methanol extract of the leaf has exhibited maximum inhibition against *E. coli* bacteria with an inhibition zone diameter of 24.17 ± 0.15 mm (Fig.3) followed by *B. cereus* with mean inhibition zone diameter of 23.06 ± 0.052 mm.

Comparison of the diameter of the inhibitory zone developed in cultures in the screening tests against the eight strains of bacteria with the leaf callus extract gave the observation that the highest diameter of inhibition zone was obtained in *E. coli*. with the diameter of 12.0 ± 0.0 mm (Fig.4) followed by *B. cereus* with a zone diameter of 10.17 ± 0.06 mm. Table-1 demonstrates a comparison of the antibacterial activity of the crude methanol leaf callus extract and methanol leaf extract of *I. tinctoria*.

Table 1. Antibacterial activity of the methanol leaf and leaf callus extracts of *I. tinctoria* (inhibition zone diameter in mm Mean \pm SD)

Bacteria	Crude extract	Callus extract	Streptomycin
<i>E. coli</i>	24.17 ± 0.15	12.0 ± 0.0	18.2 ± 0.1
<i>B. cereus</i>	23.06 ± 0.052	10.17 ± 0.06	20.5 ± 0.1
<i>K. pneumoniae</i>	18.42 ± 0.03	10.03 ± 0.06	15.42 ± 0.2
<i>S. typhi</i>	20.5 ± 0.01	8.17 ± 0.06	22.1 ± 0.11
<i>E. aerogenes</i>	22.2 ± 0.01	9.37 ± 0.06	16.06 ± 0.01
<i>S. aureus</i>	22.1 ± 0.01	8.06 ± 0.05	22.0 ± 0.1
<i>P. aeruginosa</i>	12.8 ± 0.06	0 ± 0.0	22.0 ± 0.02
<i>P. vulgaris</i>	20.68 ± 0.073	0 ± 0.0	15.6 ± 0.02

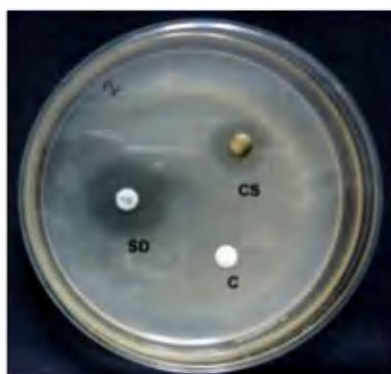


Fig. 3. Inhibitory effect of methanol leaf extract of *I. tinctoria* on *E. coli*.



Fig. 4. Inhibitory effect of methanol leaf callus extract of *I. tinctoria* on *E. coli*.

The MIC values of crude methanol leaf callus extract and methanol leaf extract of *I. tinctoria* obtained in treatment with eight bacterial strains gave the data depicted in Table-2. $62.5 \mu\text{g/ml}$ methanol leaf extract was found sufficient to induce inhibition in *E. coli* and *S. aureus*. Similarly $62.5 \mu\text{g/ml}$ was found effective in inducing inhibition in *E. aerogenes* and *P. aeruginosa*. The minimum inhibitory concentration of methanol leaf derived callus extract showed a rather high value towards each strain (Table-2).

Table 2. MIC for crude methanol extract of *I. tinctoria* (values are given in µg/ml) Bacterial strains

Extracts	<i>E. coli</i>	<i>B. cereus</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>	<i>E. aerogenes</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>
Leaf extract	62.5	62.5	125	125	250	62.5	125	125
Leaf callus extract	250	125	125	250	250	500	---	---

Notes : ---: no inhibition

The study brought out the fact that the methanol extract of the callus was effective in inducing inhibition on the same strains of bacteria on which the methanol leaf extract induced inhibition but the intensity and extent of inhibition was less. Earlier similar results were also reported in *Saraca asoca*¹⁶, four species of *Nigella*¹⁷, and three endemic wild species of *Ephedra*¹⁸. The reduction in the accumulation of bioactive phytochemicals in the callus may be due to the optimum *in vitro* environmental conditions in which the callus was maintained¹⁹. In spite of the fact that the callus developed in *I. tinctoria* and *I. enneaphylla* demonstrated a reduced antimicrobial activity, our results indicated the ability to utilize the callus culture technique towards development of desired bioactive metabolites in *in vitro* culture instead of using wild plants for pharmaceutical purposes.

The bioactive principles present in the plant are responsible for the antibacterial activity. In recent years keeping in mind the limited natural resource of plants, the scientists are in search of alternate sources of phytochemicals. Plant tissue culture has been identified as the most important one among them. Several groups of phytochemicals, like Alkaloids, Rotenoids, Flavonoids, Isoflavonoids. Nevertheless, our results indicate the ability to utilize plant cell culture techniques towards developments of desired bioactive metabolites in *in vitro* culture instead of using wild plants for pharmaceutical purposes. The identification of the presence of phytochemicals in the callus highlights the possibility of using plant cell cultures as an alternative system for the production of biologically active compounds.

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Synthesis, characterization and antimicrobial study of Cobalt (II) complex of hippuric acid

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Abstract

The cobalt (II) complex $[\text{Co}(\text{C}_6\text{H}_5\text{CONHCH}_2\text{COO})_2 \cdot 2\text{H}_2\text{O}] \cdot x\text{H}_2\text{O}$ formed with hippuric acid has been synthesized. The mode of coordination and its structure have been determined by elemental analysis, spectral (IR and UV) studies and magnetic measurement studies. Hippuric acid acts as a bidentate ligand with the carboxylate oxygen and the nitrogen of the amido group as the coordinating sites. The fifth and sixth coordination sites are satisfied by water molecules. Antimicrobial studies of the complex were done with Gram positive and Gram negative bacteria. It is then compared with standard antibiotic discs. The Co (II) complex has been suggested to show an octahedral geometry.

Key words: synthesis, characterization, Cobalt (II) complexes, hippuric acid.

Introduction

Coordination chemistry has registered rapid growth since the beginning of the second half of the twentieth century. Earlier, most of the ligands which came into the scene were inorganic. Later chelated complexes with organic donors with coordination centers Nitrogen, Sulphur, Oxygen etc. were investigated. Mixed ligand complexes play essential role in biological systems¹. Synthesis and characterization of some bivalent simple and mixed ligand transition metal complexes of hippuric acid have been reported²⁻⁴. Hippuric acid⁵, also called N-benzoyl glycine ($\text{C}_6\text{H}_5\text{CONHCH}_2\text{COOH}$) is an α -amido acid containing an acidic COOH, basic NH and substituent benzoyl group. It is thus capable of forming metal chelates. The present work includes the synthesis, characterization, and antimicrobial study of cobalt (II) complex formed with hippuric acid.

Materials and Methods

Solution of hippuric acid was prepared by dissolving it in one equivalent of sodium hydroxide. The cobalt chloride solution was prepared in one equivalent of hydrochloric acid. To prepare the metal complex, the hippuric acid solution was mixed with 0.1 M metal ion solution in 1:1 molar ratio at room temperature. The pH of the resulting solution was adjusted to 5.2 by adding sodium hydroxide drop by drop. The clear pink solution was then concentrated over a water bath and allowed to crystallize. The crystalline precipitate was then filtered and washed with 50 % ethanol-water mixture. It is then dried in an air oven and heated at

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100-110 °C for two hours, so that the color of the complex changes from pale pink to deep violet, indicating the loss of water molecule from the complex. The antibacterial activity of the prepared complex against *Bacillus subtilis* and *Escherichia coli* was assessed by Kirby-Bauer method⁶ which involves the rapid determination of the efficiency of the sample by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Comparing the zone of inhibition obtained from the sample, with that contained in a standardized chart, the test organism is determined to be resistant, intermediate or susceptible to the sample or antibiotic.

The IR spectra of the ligand and the metal complex were recorded on a Shimadzu FT-IR spectrometer in the 4000-400 cm⁻¹ range with KBr discs. The magnetic susceptibility of the complex was measured at room temperature using Gouy magnetic susceptibility balance. Systronics UV-VIS spectrometer -117 was used to record electronic spectrum of the complex in methyl alcohol in the range 200-800 nm. The elemental analysis (CHN) was carried out on a Vario CHNO/S elemental analyser. The cobalt content of the complex was determined by the Atomic Absorption Spectrometer.

Results and Discussion

Elemental Analysis

Dihippuratodiaquacobalt (II) trihydrate. Pink crystal. Anal. Calcd for [Co(C₆H₅CONHCH₂COO)₂.2H₂O].3H₂O

Co = 11.68%, C = 42.77%, H = 5.14%, N = 5.54%

Found; Co = 11.01%, C = 42.22%, H = 5.96%, N = 5.53%

IR Studies⁷

Hippuric acid show characteristic ν (C=O) absorption band for the COOH group at 1739.27 cm⁻¹, which vanishes in the case of metal complex. Instead, asymmetric and symmetric COO⁻ stretching frequencies are obtained. The metal complex shows ν_{as} COO⁻ and ν_s COO⁻ frequencies at 1644.67cm⁻¹ and 1433.78cm⁻¹ respectively. Hippuric acid shows ν (CN) absorption frequency at 1334.63cm⁻¹ which lowers on coordination in metal complex. The ν (NH) vibration in hippuric acid is observed at 3337.15cm⁻¹, which in the complex appears at 3158.85cm⁻¹. Hippuric acid shows amide I (ν C=O) band at 1598.77cm⁻¹, amide II (δ NH+ ν CN) band combined with benzene ring vibrations in the range(1556 – 1415)cm⁻¹ and amide III (ν CN+ δ NH) band at 1334.63,1317.30 cm⁻¹. In the complex amide I band is observed at 1574 cm⁻¹. Amide II band, benzene ring and ν_s COO⁻ vibrations are mixed together and give a broad band at 1548.87cm⁻¹. The amide III band is mixed with ν (CN) vibrations and is obtained at 1319.56 cm⁻¹. The appearance of rocking

ν_r (HOH) frequency⁸ at 720.37 cm⁻¹ in the complex shows the presence of coordinated water molecules. The IR frequencies of the ligand and the metal complex are shown in the table 1.

Magnetic Studies⁹ and Electronic Spectra¹⁰

The high-spin octahedral cobalt (II) complexes have the magnetic moment in the range 4.50–5.20 B.M. and the tetrahedral complexes have magnetic moment generally in the range 4.10–4.80 B.M. They may have higher magnetic moment than spin-only value due to higher orbital contribution. Complexes show magnetic moments, corresponding to three unpaired electrons, suggesting octahedral geometry around the complexes. Magnetic moment value of the prepared Co (II) complex is 4.73 B.M, which indicates an octahedral geometry for the complex. The magnetic measurements are given in table 2.

In octahedral Co (II) complexes, three transitions namely, $4T_{1g}(F) \longrightarrow 4T_{2g}(F)$ (1); $4T_{1g}(F) \longrightarrow 4A_{2g}(F)$ (2) and $4T_{1g}(F) \longrightarrow 4T_{1g}(P)$ (3) are possible.. The 1 is generally broad and 3 is a set of multiple bands and may be mixed with spin-forbidden transitions. Electronic spectra of the present Co(II) coordination compound(1) displays three absorption bands nearly at frequencies 14084.5cm⁻¹, 19607.84cm⁻¹ and 28571.42cm⁻¹ which may be ascribed to the transitions: $4T_{1g}(F) \longrightarrow 4T_{2g}(F)$ (1), $4T_{1g}(F) \longrightarrow 4A_{2g}(F)$ (2), and $4T_{1g}(F) \longrightarrow 4T_{1g}(P)$ (3) respectively, characteristic of octahedral geometry.

Antibacterial Studies

The results of antimicrobial studies of the ligand, the complex and a series of standard antibiotic discs such as Erythromycin, Gentamicin and Amikacin are given in table 3. From the results it is seen that the complex show high antimicrobial activity compared to the corresponding ligand. The complex is very sensitive towards both Gram positive and Gram negative bacteria. This increase in the activity on chelation¹¹ might be due to the partial sharing of the positive charge of the metal in the chelated complex with the ligand's donor atoms so that there is electron delocalization over the whole chelate ring. This may increase the lipophilic character of the metal chelate and thus enabling it to permeate the lipid layers of the bacterial membrane. Also the cobalt complex of hippuric acid shows high antimicrobial activity when compared to the antibiotic discs namely Erythromycin, Gentamicin, Amikacin etc.

Thus the evidences obtained from IR, electronic spectra and magnetic measurements suggest a six fold octahedral structure for the complex in which two molecules of hippuric acid satisfies four coordination sites of cobalt and the remaining two sites are occupied by two water molecules. The complex shows high sensitivity towards microorganisms such as *Bacillus subtilis* and *Escherichia coli* when compared to the ligand as well as the antibiotic discs namely Erythromycin, Gentamicin, Amikacin etc.

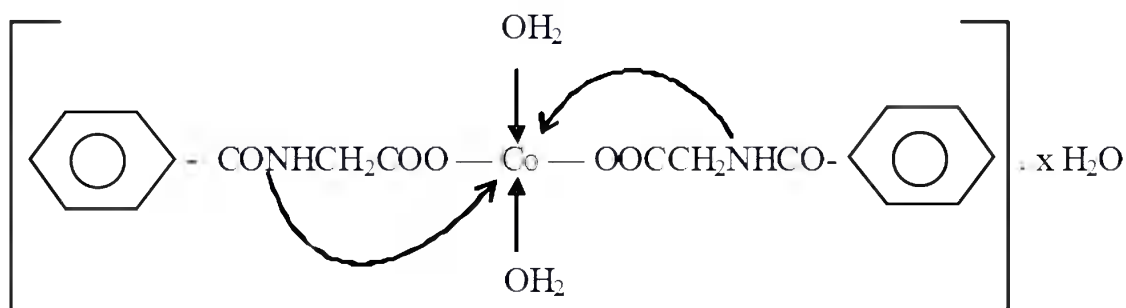

Fig. 1. Structure of the cobalt (II) complex of hippuric acid($x = 3$)

Table 1. IR frequencies of hippuric acid and the cobalt complex

Hippuric acid (cm^{-1})	$[\text{Co}(\text{IIA})_2 \cdot 2\text{H}_2\text{O}] \cdot 3\text{H}_2\text{O}$ (cm^{-1})	Band assignment
3337.15	3158.85	$\nu(\text{NH})$
1739.27	-	$\nu(\text{C}-\text{O})$ carboxylic acid
-	1644.67	$\nu_{\text{as}} \text{COO}^-$
-	1433.78	$\nu_s \text{COO}^-$
1598.77	1574	$\nu(\text{C}=\text{O})$ amide I band
1556.90, 1489.37, 1415.31	1548.87	(δNH νCN) amide II band -benzene ring
1334.63, 1317.30	1319.56	(νCN δNH) amide III band
-	720.37	ρ_s (H_2O) coordinated water

Table 2. Magnetic measurements of the cobalt (II) complex

Complex	T(K)	$\chi_s \times 10^6$ (cgs)	$\chi_M \times 10^6$ (cgs)	$\chi_{M(\text{corrected})} \times 10^6$ (cgs)	μ_{eff} (BM)
$[\text{Co}(\text{IIA})_2 \cdot 2\text{H}_2\text{O}] \cdot 3\text{H}_2\text{O}$	299	17.9639	9071.787	9355.627	4.736

Table 3. Antimicrobial Study Results

Organism	Antibiotic disc used	Inhibition zone diameter	Remark
<i>Bacillus subtilis</i>	Gentamicin	23	Sensitive
	Erythromycin	20	Intermediate
	Amikacin	16	Intermediate
	[Co(HA)2.2H ₂ O].3H ₂ O	27	Sensitive
	Hippuric acid	14	Intermediate
<i>Escherichia coli</i>	Gentamicin	22	Sensitive
	Erythromycin	15	Intermediate
	Amikacin	No zone	Resistant
	Hippuric acid	13	Intermediate
	[Co(HA)2.2H ₂ O].3H ₂ O	27	Sensitive

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Phytoremediation studies using turmeric plants

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Abstract

The current remediation technique of heavy metal from contaminated soil-water is expensive, time consuming and environmentally destructive. Phytoremediation is a novel strategy for the removal of toxic metals from the environment using plants. It is an emerging technology for contaminated soils, ground water and waste water that is both low tech and low cost is defined as the engineered use of green plants (including grasses, herbs and woody species) to remove or render harmless environmental contaminants such as heavy metals, trace elements, organic compounds etc in soil. *Curcuma longa* and *Curcuma aromatica* are the most available varieties of curcuma, required in large quantities because of its wide variety of usage. Thus the present study focused on phytoremediation efficiency of heavy metals like Zn and Pb using above mentioned species of curcuma. The experiment was designed using different concentration of both the heavy metals with Turmeric tubers to observe the absorption efficiency with respect to the growth of the tubers. The result showed that the curcuma has better capacity to remove metal ions, Lead and Zinc of which Lead is removed greater than Zinc. The experiment also revealed that curcuma species can accommodate these metal ions beyond their permitted range.

Keywords: Phytoremediation, pollution, heavy metals

Introduction

In natural environments, the heavy metals occur at low concentrations. However at high concentrations as is the case in contaminated environments, they result in public health impacts. Heavy metals may be released into the environment from metal smelting and refining industries, scrap metal, plastic and rubber industries, and various consumer products and from burning of waste containing these elements. On release to the air, the elements travel for large density¹. Once deposited, these metals are not degraded and persist in the environment for many years poisoning humans through inhalation, ingestion and skin absorption. Acute exposure leads to nausea, anorexia, vomiting, gastrointestinal abnormalities and dermatitis^{2,3}.

Heavy metals, such as cadmium, copper, lead, chromium, zinc, and nickel are the major environmental pollutants^{4,5,6}. Among them, Lead is one limited class of element that can be classified as purely toxic. Many other elements included in heavy metals are required as nutrients at least in low level. In many decades of research, no nutritional value or positive has been shown to result from lead exposure. The toxicity of lead is mild anemia, brain damage, vomiting etc. Zinc is also an element of great concern due to

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their potential toxicity to man and animal⁷. Zinc is considered to have low toxicity in human being up to a certain limit. Beyond this it can cause dehydration, stomach pain, nausea, lethargy etc^{3,8}.

The current remediation techniques of heavy metal from contaminated soil-water are expensive, time consuming and environmentally destructive⁹. Phytoremediation is the name given to a set of technologies that use plants to clean contaminated sites. Phytoremediation consist of mitigating pollutant concentrations in contaminated soil, water, or air with plants¹⁰. Phytoremediation applications can be classified based on the contaminant fate: degradation, extraction, containment or a combination of these^{11,12,13}. Turmeric is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. Turmeric contains a specialized compound known as terpenoid. The turmeric releases plant signal molecules like terpenes in to rhizosphere. These compounds have a signal function for inducing co metabolism of organic xenobiotics and heavy metals. This authenticates the importance of turmeric in phytoremediation and the competence of it in removing the most hazardous heavy metals from contaminated environments. In this regard the present study focuses on the efficiency of turmeric in phytoremediation of Lead and Zinc.

Materials and methods

Preparation of pots for the cultivation experiment

Two types of plants were selected for the phytoremediation experiment, *Curcuma aromatica* and *Curcuma longa*. The tubers of both plants were sowed in 2 groups of 9 earthen pots. About 10 kg of garden soil were filled in each pot. All the 9 different pots of 2 set of plants were labeled based on the concentration of heavy metals added on it. One of the pots in each group of 9 plants was kept without the addition of any heavy metals and was labeled as blank. All the sowed pots were kept for growth in same environmental condition with proper watering.

Preparation of heavy metal solutions

Four different concentrations of Zinc nitrate and lead nitrate were prepared from 1M solutions. Concentrations were prepared like 0.3 M, 0.5 M, 0.7M And 0.9 M for Lead and Zinc. The solutions were prepared in 100ml standard flasks. Different experiment pots were labeled as in Table 1.

Table 1. Sample codes and concentration of metal ions

Metals	Zinc (Zn)					Lead (Pb)				
Concentrations	0.3	0.5	0.7	0.9	Blank	0.3	0.5	0.7	0.9	Blank
<i>Curc. aromatica</i>	A1	B1	C1	D1	BL1	F1	F1	G1	H1	BL1
<i>Curc longa</i>	A2	B2	C2	D2	BL2	E2	F2	G2	H2	BL2

The sowed pots were subjected to watering every day. 50 ml of different concentrations of heavy metals were added in to pots in all days from the first day of sowing onward. The frequency of addition of heavy metals was then reduced as thrice in a week after the development of shoots and leaves.

Collection of samples for analysis

After three months of experiment both the soil and germinated turmeric tubers were collected for heavy metal analysis (Zn, Pb).

Soil sample collection

Soil from different experiment pots were collected and air dried in shade. The dried soil samples were powdered using mortar and pestle. The soil then sieved with 2mm sieve and kept in dry container for chemical analysis.

Turmeric sample collection

The turmeric tubers were taken out of soil and air dried in shade. The dried sample were weighed, digested in concentrated nitric acid (HNO₃) and concentrated up to 30ml and kept in containers for sample analysis.

Heavy metal analysis of soil and turmeric samples

Soil sample (40g) was dissolved in DTPA (Diethylene Triamene Penta Acetate) and kept in shaker incubator for one hour. From the soil samples the metal ion concentrations are found out using AAS (Atomic Absorption Spectroscopy).

The turmeric tubers were taken out, washed with distilled water and dried. The dry weight was taken and the plants were cut in to pieces and dissolved in the mixture of concentrated nitric acid and hydrogen peroxide by boiling. This was then filtered, washed and concentrated to 30 ml each. These solutions were then utilized to detect the metal ion concentration using AAS. A blank was also prepared by dissolving the respective plants which was not watered with metal ion solutions¹⁴.

Results and Discussion

After the completion of experiment the soil and tubers from different experiment pots were analyzed for the heavy metals Zinc and Lead using AAS. The results obtained can be used as indicators for the evaluation of phytoremediation efficiency of both the *curcuma* species. The parameters for the analysis of each individual samples are mentioned in Table 2.

Table 2. Metal ion concentrations in turmeric and soil samples

Sample	Turmeric*		Soil**		Sample	Turmeric*		Soil**	
	Dry wt	con(ppm)	con(ppm)			Dry wt	con(ppm)	con(ppm)	
A1	21.26	2.0006	804.03		E1	17.93	132.1996	1816	
A2	29.04	9.1998	773.78		E2	4.9	17.4998	1636	
B1	13.45	3.7001	709.72		F1	9.83	118.3	1676	
B2	9.4	7.5999	750.56		F2	12.08	160.3	1644	
C1	51.06	2.9972	936.42		G1	4.54	203.2998	1954	
C2	8.03	7.3996	800.8		G2	7.29	180.9997	1796	
D1	23.15	1.9006	822		H1	5.18	141	1882	
D2	13.37	7.1997	814.04		H2	10.17	207.1	1740	

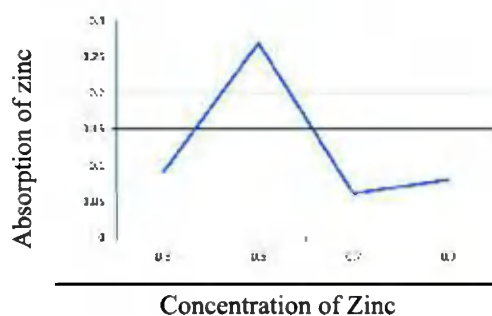
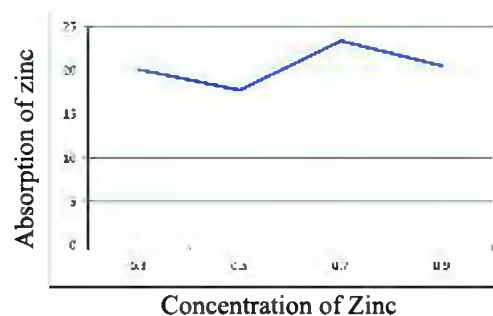
Note: *Aspirated volume = 5 ml, **Sample weight = 40g

Study of phytoremediation of zinc by *Curcuma aromatica* species

The quantitative data corresponding to the absorption of zinc by both the tubers of *curcuma longa* and also by the soil is given in table 3 and Figs. 1 and 2.

Table 3. Zinc ion concentrations in *Curcuma aromatica* and soil

Sample	<i>Curcuma aromatica</i>			Soil	
	Dry weight	Conc (ppm)	Conc (ppm/g)	Conc (ppm)	Conc (ppm/g)
A1	21.26	2	0.0941	804.32	20.1080
B1	13.45	3.7	0.2751	709.72	17.7430
C1	51.06	3	0.0587	936.42	23.4105
D1	23.15	1.9	0.0821	822	20.5500

**Fig. 1.** Zinc ion concentrations in *Curcuma aromatica***Fig. 2.** Zinc ion concentrations in Soil

On comparing the above result it is assumed that as concentration increases the tuber absorption of zinc increases and there is a corresponding decrease in the soil absorption. But at 0.7ppm concentration the absorption of metal ions in tuber decreases and in soil it increases. This may be because of the reason that when accumulation of metals in tubers attains a maximum value it then starts to distribute these metal ions to shoots and leaves and at that time the absorption of heavy metals from soil is minimum. Therefore at this optimum concentration of heavy metals in tubers the soil absorption value increases. After this point at .9ppm concentration the soil absorption decreases to lower values and also the increase in tuber absorption is very slow. This result shows that after attaining optimum concentration at tuber the shoots and leaves starts to accumulate metal ions from soil and the tuber absorption becomes slow.

Study of phytoremediation of lead by *Curcuma aromatica* species

The quantitative data and corresponding plot of absorbance against the concentration is given in Table 4 and Figs. 3 and 4.

Table 4. Lead ion concentration in *Curcuma aromatica* tuber and soil.

Sample	Sample		Soil		
	Dry weight	Conc.(ppm)	Conc.(ppm/g)	Conc.(ppm)	Conc.(ppm/g)
E1	17.93	132.2	7.3731	1816	45.4
F1	9.83	118.3	12.0346	1676	41.9
G1	4.54	203.3	44.7797	1954	48.85
III	5.18	141.0	27.2201	1882	47.05

On studying the above data it can be concluded that the plant tuber absorption of Lead increases as concentration increases and it attain a maximum value at 0.7ppm concentration and then it decreases. And in the case of soil the absorption decreases first, then it increases and finally it again decreases as concentration increases. From this information we can say that from 0.3 to 0.5 ppm concentration the plant tuber starts to absorb lead and because of that there is a corresponding decrease in the soil absorption and when it reaches to 0.7 ppm concentration both in tuber and soil absorb maximum lead. This result shows that the absorption of lead by the plant tuber of *curcuma aromatica* increases as concentration increases and there is corresponding decrease in the soil of the same pot. But when concentration reaches to 0.7 ppm there is maximum absorption in plant tuber and also in the soil. Above this concentration both tuber and soil absorption decreases .This may be because of the reason that there should have a range of maximum absorption by tuber and when this optimum range absorption reaches the tuber may start to accumulate metal ions in plant shoots and leaves. Therefore the concentration of heavy metals in tuber as well as in soil decreases due to the accumulation at shoots and leaves at 0.9ppm concentration.

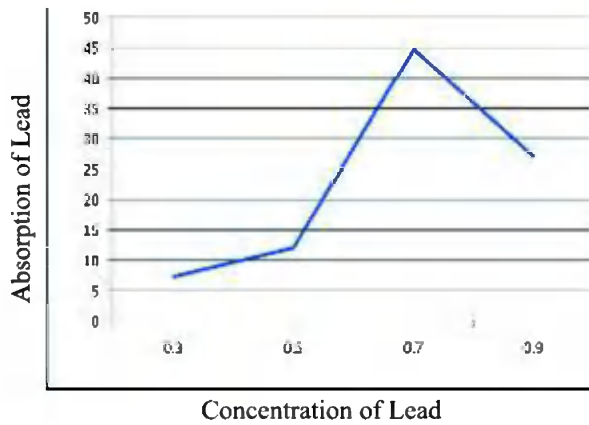


Fig. 3. Lead ion concentration in *Curcuma aromatica* tuber

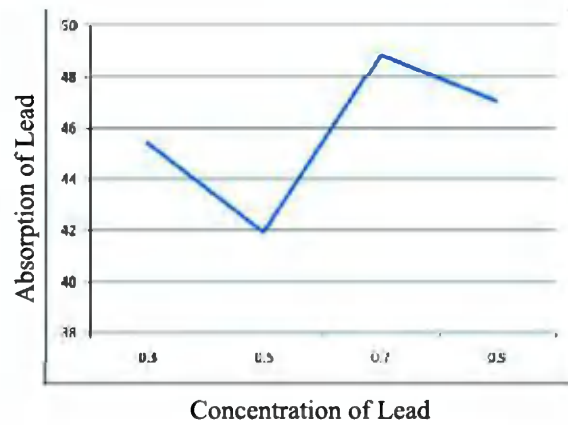


Fig. 4. Lead ion concentrations in Soil

Phytoremediation of Zinc by *Curcuma longa*

The quantitative data obtained from the analysis of phytoremediation studies of Zinc by *curcuma longa* tubers and also the soil is given in Table 5 and Fig. 5 and 6.

Table 5. Zinc ion concentration in *Curcuma longa* tuber and soil

Sample	<i>Curcuma longa</i> tub			soil	
	Dry weight	Conc. (ppm)	Conc. (ppm/g)	Conc. (ppm)	Conc. (ppm/g)
A ₂ (0.3)	29.04	9.2	0.3168	773.78	19.3445
B ₂ (0.5)	9.4	7.6	0.8085	750.56	18.7640
C ₂ (0.7)	8.03	7.4	0.9215	800.8	20.02
D ₂ (0.9)	13.37	7.2	0.5385	814.04	20.351

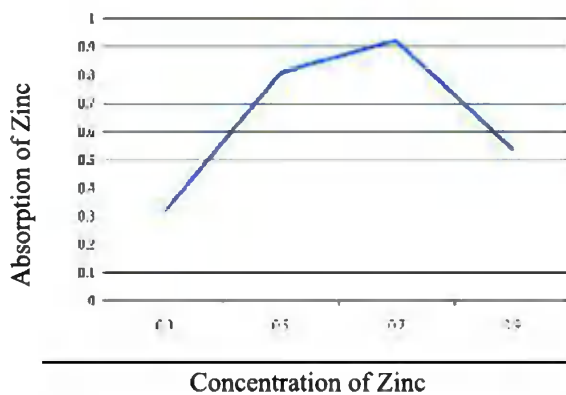


Fig. 5. Zinc ion concentration in *Curcuma longa* tuber

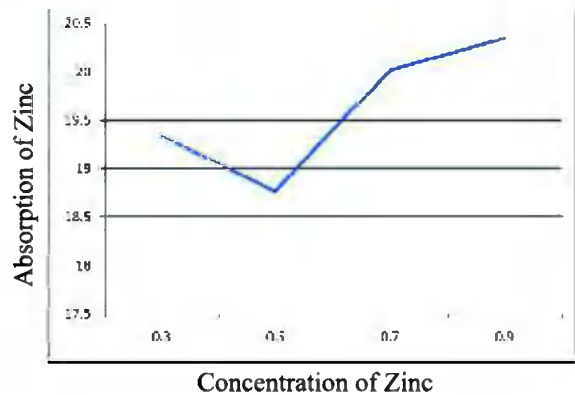


Fig. 6. Zinc ion concentration in soil

This shows that as concentration increases from 0.3 to 0.5 ppm the absorption by plant tuber increases and there is a corresponding decrease in the soil. At 0.7 ppm concentration the tuber absorbs maximum of zinc but in soil also there is an increase in absorption. This may be because of the reason that there is an optimum concentration in tuber at these concentrations and further increase in concentration leads to the decrease in absorption at tubers and increase in absorption in soil. This shows that after attaining maximum (optimum) absorption at tubers it cannot bear higher concentrations of metal ions and the decrease in absorption may be because of the deterioration of the plant due to high concentration of heavy metals.

Phytoremediation of lead by *Curcuma longa* species

The quantitative data corresponding to the absorption of lead by both the tubers of *Curcuma longa* and the soil of the same pot is given in table 6 and Figs. 7 and 8.

Table 6. Lead ion concentration in *Curcuma longa* tubers and soil.

Sample	<i>Curcuma longa</i> tuber			Soil	
	Dry weight	Conc. (ppm)	Conc. (ppm/g)	Conc. (ppm)	Conc. (ppm/g)
E2	4.9	17.5	3.5714	1636	40.9
F2	12.08	160.3	13.2699	1644	41.1
G2	7.29	181.0	24.8285	1796	44.9
H2	10.17	207.1	20.3638	1740	43.5

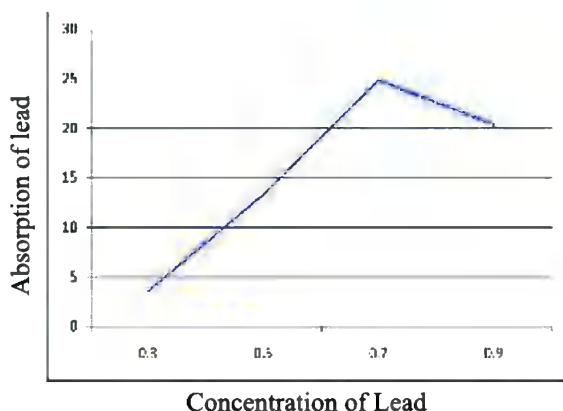


Fig. 7. Lead ion concentration in *Curcuma longa* tubers

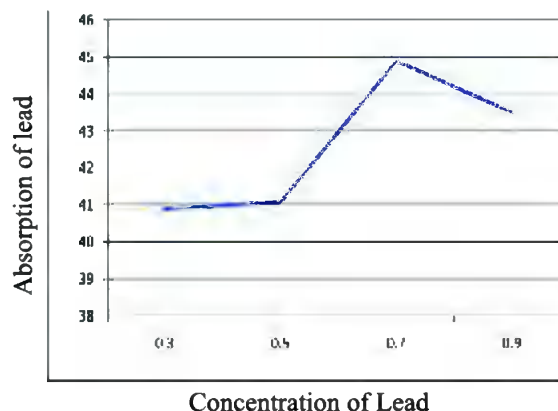


Fig. 8. Lead ion concentration in soil

This result shows that as concentration increases the plant tuber absorbs more heavy metals and correspondingly there is only a small increase in soil absorption. But when it reaches to 0.7ppm concentration it attains optimum absorption where there is large increase in corresponding soil absorption also. This may be because of the reason that at this concentration the soil gets maximum absorption due to low intake of metal ions by plant tuber due to maximum accumulation of metal ions in it. Further increase in concentration shows decrease in absorption in both tuber and soil. This may be due the intake of metal ions by plant shoots and leaves after attaining optimum absorption in tubers.

The *curcuma aromatica* and *curcuma longa* showed efficient phytoremediation properties with reference to the most toxic heavy metals like Zinc and Lead. The *curcuma aromatica* and *curcuma longa* is the most suitable plant for the phytoremediation of Lead. The tubers of both the species of *curcuma* takes comparatively less time and more efficient absorption of heavy metals into its fleshy tuber tissues. Since the heavy metals observed to be accumulated in the tubers and vegetative part of both the two species of *curcuma*, the treated *curcuma* is not recommended to use as food additive.

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Climate change scenario over Cauvery Delta Zone

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Abstract

Evident change in climate is occurring due to the enhanced green house gas concentration in the atmosphere and it has major impact on all forms of life. To access the impact of future climate change on different organisms and to mitigate the effect, it is necessary for us to have future knowledge about the changing climate, which can be obtained from climate models under different scenarios. In this study to assess the climate change over Cauvery Delta Zone (CDZ) of Tamil Nadu, two regional climate models PRECIS and RegCM3 were employed under A1B scenario. The results from both the models showed an increase in maximum temperature, minimum temperature, rainfall and slight decrease in solar radiation at the end of 21st century. The rate of increase per decade was found to be 0.33 C and 0.27 C in maximum temperature, 0.38 C and 0.32C in minimum temperature, 6.92 and 16.10 mm in rainfall and rate of decrease was 0.02 and 0.07 MJm⁻² in solar radiation under PRECIS and RegCM3 respectively.

Key words: climate change, PRECIS, RegCM3, Cauvery Delta Zone.

Introduction

Climate change occurs both by natural and anthropogenic means. Due to anthropogenic increase in greenhouse concentration, climate change is occurring at faster rate than earlier days. Due to this, evident effects are experienced around the world like average global surface temperature increase by nearly 1°C over the past century and also projected to increase by 1.4 to 5.8 °C¹. This unprecedented increase is expected to have severe impacts on the global hydrological system, ecosystems, sea level, crop production and related processes.

So, it is necessary for scientific community to have a tool for assessing the climate change impact on different ecosystem, which was given by IPCC², 2000 to carry out research on impacts of climate change using Special Report on Emission Scenarios (SRES) with four storylines, which yields four sets of scenarios called “families” A1, A2, B1 and B2. Global climate models (GCMs) are most credible tools available for simulating the response of global climate system³ which use differential equations based on basic laws of physics, fluid motion and chemistry but they cannot assess the spatial scales that are required for climate impact and adaptation studies due to their coarser resolution.

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Therefore, several downscaling methods have been developed to derive fine-scale information from GCM simulations. One of the best methods is dynamical downscaling using high-resolution regional climate models (RCMs) nested in GCMs⁴. In this study we have assessed the climate change impact on Cauvery Delta Zone of Tamil Nadu, which is the major rice growing area of the state, using two regional climate models PRECIS and RegCM3 with the objective to develop the climate change scenario over Cauvery Delta Zone (CDZ).

Materials and methods

Scenario

In this study A1B scenario was selected for both the regional climate models (PRECIS and RegCM3). The A1B emission scenario falls under A1 storyline that describes a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and the rapid introduction of new and more efficient technologies and assumes balance energy flow across all sources which takes into account fossil and non-fossil energy.

PRECIS

PRECIS (Providing Regional Climates for Impacts Studies) is a regional climate model that can be run over any area of the globe, which is easy-to-use and is designed to provide detailed climate scenarios. The Hadley Climate Centre of the UK Met office has developed PRECIS regional climate modeling system to assess the vulnerability due to climate change. Among many GCM boundary conditions, HadCM3Q0 is one among the boundary used in this experiment.

PRECIS modeling software was run in Open SuSE, which is a Linux operating system for the model as recommended by the Hadley centre. Run was made for 129 years from 1971 to 2099 under A1B scenario. The domain was fixed with sufficient buffer zone over Tamil Nadu state as in Fig. 1.

RegCM3

RegCM3 is a Regional Climate Model (RCM), which was developed by the Abdus Salam International Centre for Theoretical Physics

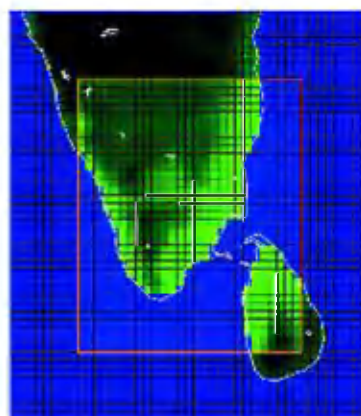


Fig. 1. Domain of PRECIS

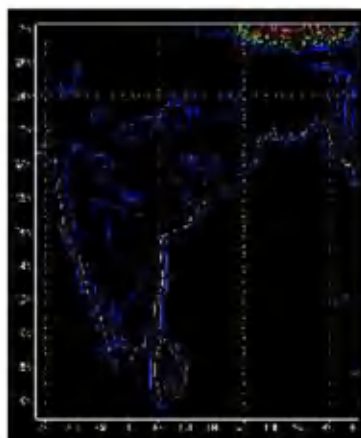


Fig. 2. Domain of RegCM3

(ICTP) in Italy and has been mostly applied to studies of regional climate and seasonal predictability around the world. It is an open source RCM⁵ available on the public domain, which can be used for climate simulation. In this study boundary data considered was EH5OM GCM output.

Model was run on the Macintosh Operating System. MAC X serve was used for RegCM3 compilation and running. Run was made for 130 years (1971-2100) for A1B scenario. The domain was fixed with sufficient buffer zone covering most part of India as in Fig. 2.

Resolution

Cauvery Delta Zone comprising nine districts, which is the rice bowl of Tamil Nadu, was considered for making impact study for the changing climate. Both the RCMs were run at 0.22° X 0.22° or 25 Km X 25 Km resolution.

Parameters retrieved

The daily data were derived from the models for the following four weather variables viz., maximum temperature, minimum temperature, rainfall and solar radiation.

Trend analysis

To assess the climate change over Cauvery Delta Zone (CDZ) yearly conversion was done using weather cock software and the decadal values were calculated in excel spread sheet. The mean of decade was used for analysis. Trend analysis was done using the Microsoft excel spreadsheets. Trend was drawn for decadal data for CDZ using linear regression in excel spreadsheet.

Results and Discussion

The results of the projected climate change over Cauvery Delta Zone for A1B scenario using PRECIS and RegCM3 regional climate models are described below

Trend analysis

Maximum temperature

The rate of increase of maximum temperature in all districts was found to be similar. Overall maximum temperature range projected by PRECIS was 3.4-3.8°C and in RegCM3 it was 3.0-3.2°C shown in Fig. 3, the result was similar to the IPCC report, which has projected an average mean warming of 3.0-4.0C for south Asia at the end of 21st century. The slope of the regression equations predicting the trend of maximum temperature in Cauvery Delta Zone showed an increasing trend of 0.33C decade⁻¹ in PRECIS and 0.27C decade⁻¹ in RegCM3 (Table 1).

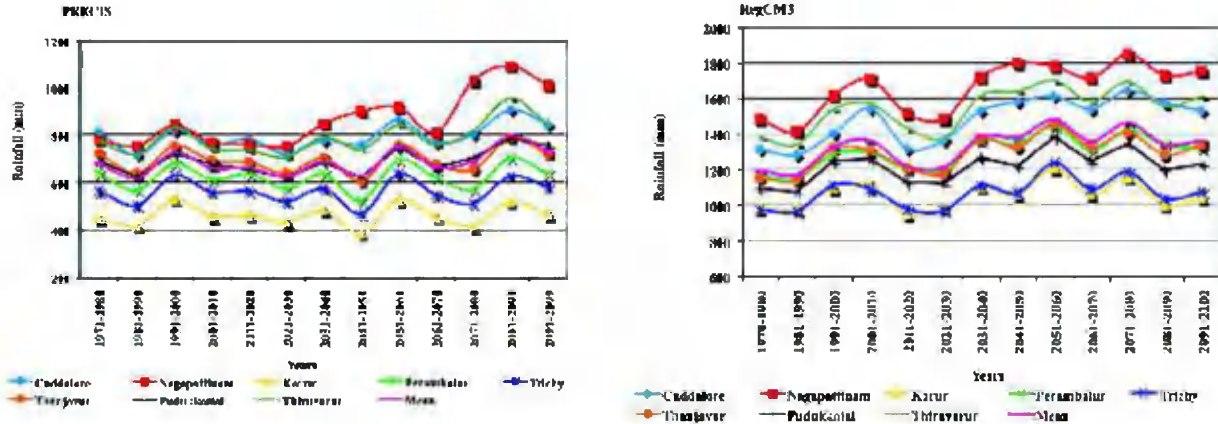


Fig. 5. Mean decadal rainfall

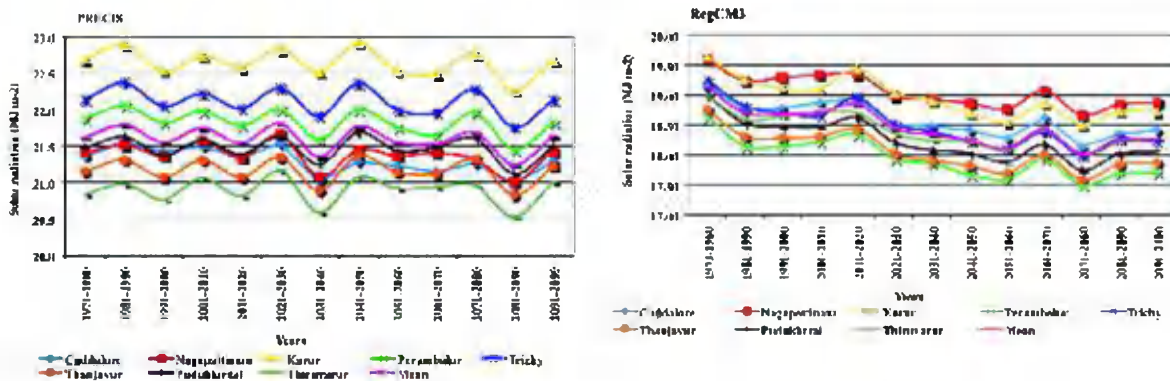


Fig. 6. Mean decadal solar radiation

Solar radiation

In both PRECIS and RegCM3 decrease in solar radiation over CDZ for all eight districts was observed as given in Fig. 6. In case of PRECIS the rate of decrease per decade was projected as -0.02 MJm^{-2} and with regard to RegCM3 the decrease was expected to be -0.07 MJm^{-2} (Table 1).

Table 1. Slope values decade⁻¹ recorded for different weather parameters

Parameters	Slope values	
	PRECIS	RegCM3
Maximum temperature (°C)	0.33	0.27
Minimum temperature (°C)	0.38	0.32
Rainfall (mm)	6.92	16.10
Solar radiation (MJm^{-2})	-0.02	-0.07

The A1B scenario over Cauvery Delta Zone showed that rate of increase in maximum temperature was 0.33 C and 0.27 C per decade, in case of minimum temperature the increase was 0.38 C and 0.32C per decade. This showed that the increase in minimum temperature was more compared to maximum temperature. In case of rainfall the increase was found to be 6.92 and 16.10 mm per decade and decreasing trend was projected for solar radiation with 0.02 and 0.07 MJm⁻² per decade under PRECIS and RegCM3 respectively.

Acknowledgements

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Comparative phytochemical analysis of *Syzygium caryophyllatum*(L.) Alston and *Syzygium densiflorum* Wall.

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Abstract

The study of ethnomedicine keenly represents one of the best avenues in searching new economic plants for medicine. Keeping this view in mind, the present investigation is carried out to investigate the preliminary phytochemicals on *Syzygium caryophyllatum* (L.) Alston and *Syzygium densiflorum* Wall. root, bark and leaf extract. The results suggested that the plant contains most of the bio active components, but the methanol extracts of leaves of both the plants contains maximum compounds than the root and bark extracts.

Key words: *Syzygium caryophyllatum*, *Syzygium densiflorum*, acetone, aqueous, methanol

Introduction

Medicinal plants are of great importance to health of individuals and communities and also it is one of the wealth of our country. Among ancient civilizations, India has been known to be a rich repository of medicinal plants which were collected from forests by the tribal villagers. More than 550 tribal communities have acquired considerable knowledge on use of plants for their livelihood, healthcare and other purposes through their long association with forests, inheritance, practices and experiences. The ethnic and rural people of India have also preserved a bulk of traditional knowledge and this is transferred to generations through the word of mouth and the same has been extensively used for the treatment of common diseases and ailments. The medicinal value of these plants lies in some chemical substances known as bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds¹. These bioactive compounds are also known as phytochemicals.

“Phyto” is the Greek word for plant and there are many “families” of phytochemicals which help the human body in a variety of ways. These are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans from diseases. *Syzygium caryophyllatum* (L.) Alston (*Syn:* *Syzygium caryophyllaeum*; Vernacular name Malayalam - Kattunjavai) and *Syzygium densiflorum* Wall. (*Syn:* *Eugenia arnottiana*; Vernacular name Malayalam - Ayuri, Vellanjavai) are endemic to the Southern Western Ghats- Anamalai, Palani and Nilgiri Hills. According to IUCN these are placed under The Red

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List of Threatened species 2006 and belongs to the family Myrtaceae. *S. caryophyllatum* has been valued in Ayurveda of medication for possessing variety of therapeutic properties such as the bark, leaves, fruits has been used for vata, pitta, diarrhea, diabetes, leucorrhoea, fever, skin diseases and general debility. According to the traditional knowledge, the decoction from the stem bark is used for diabetes in Srilanka². Flower buds have antioxidant property³. There are not much therapeutic informations available for the *S. caryophyllatum* and *S. densiflorum*. Hence, the present study has been made to investigate the phytochemicals of *S. caryophyllatum* and *S. densiflorum*.

Materials and methods

Selected plants

Root, bark and leaves of *Syzygium caryophyllatum* (L.) Alston and *Syzygium densiflorum* Wall. were collected from Kodai hills of Tamil Nadu, India, during the month of October 2009. The plants were identified and authenticated by Dr. Arumugasamy, Professor, Department of Botany, KonguNadu Arts and Science College, Coimbatore and the voucher specimens (T/1 and T/2 respectively) were deposited. The parts of the plants were air dried under the shed at room temperature. The dried plant material was manually powdered and it was kept in a polyethylene bags until used.

The plant parts (root, bark and leaves) were washed with distilled water, dried at room temperature and powdered in a mechanical grinder. About 100 gm of powdered samples were extracted with acetone, aqueous and methanol (250ml) solvents using soxhlet apparatus. Extraction process was continued until the color of the final drop of the extract became colorless. All the above extracts were conc. to a small volume by rotary evaporator. Each time before extracting the next solvent, powdered materials were dried in an oven at a temperature of 40- 50° C for 8 hrs. Later the extracts were used for the qualitative phytochemical studies.

Preliminary Phytochemical Screening

Standard screening test of the extracts were carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures⁴.

Test for Alkaloids

a) *Preliminary test*: 100 mg of the extracts were dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendroff's and Mayer's reagents. The treated solutions were observed for any precipitation.

b) Confirmatory test: Five grams of each extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated *in vacuum* to about 5 ml. Chloroform extract was then spotted on thin layer plates. Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. An orange or dark coloured spots against a pale yellow background was confirmatory evidence for presence of alkaloids.

Test for Flavonoids

a) Lead acetate test: To a solution of 0.5 g of the extract in water, about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

b) Reaction with sodium hydroxide: Dilute sodium hydroxide solution was added to a solution of 0.5 g of the extract in water. The mixture was inspected for the production of yellow colour which is considered as positive test for flavonoids.

c) Test for free flavonoids: 5 ml of ethyl acetate was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle and inspected for the production of yellow colour in the organic layer which is taken as positive for free flavonoids.

Test for phenolic compounds

a) To 2 ml of filtered solution of the aqueous macerate of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1% ferric chloride and 1 ml of potassium ferrocyanide was added to detect phenolic compounds. Formation of bluish-green colour was taken as positive.

Test for Tannins

Ferric chloride test: A portion of the extracts were dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black for the presence of tannins.

Test for Saponins

Froth test: 0.5 g of the extract was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minute period of time. If "honey comb" froth above the surface of liquid persists after 30 min., the sample is suspected to contain saponins.

Test for steroidal compounds

a) *Salkowski's test*: 0.5 g of the plant extracts were dissolved in 2 ml chloroform in a test tube. Conc. sulphuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

b) *Lieberman's test*: 0.5 g of extract was dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. Conc. sulphuric acid was then carefully added. A colour change from purple to blue to green indicated the presence of a steroid nucleus i.e. aglycone portion of the cardiac glycosides.

Test for Anthraquinones glycosides (Modified Borntrager's test)

For combined anthraquinones, 5 g of the plant extract was boiled with 10 ml 5% sulphuric acid for 1 hour and filtered while hot. The filtrate was shaken with 5 ml benzene; the benzene layer separated and half its own volume of 10% ammonia solution added. The formation of a pink, red or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract.

Test for triterpenoids

Ten μ ml of the extracts were dissolved in 1 ml of chloroform and 1 ml of acetic anhydride with the addition of 2 ml of Conc. Sulphuric acid. Formation of reddish violet colour indicated the presence of triterpenoids.

Test for Fats and Fixed oils

Few drops of 0.5 N alcoholic potassium hydroxide solution was added to small quantity of the extract along with a drop of phenolphthalein. The mixture was heated on water bath for two hours. Formation of soap or partial of alkali indicates the presence of fixed oils and fats.

Results and discussion

The results obtained in the present investigation (Table 1&2), acetone, aqueous, and methanol extracts of root, bark and leaves of *S. caryophyllatum* and *S.densiflorum* showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, triterpenoids, fats and fixed oils and the absence also noted in the extracts. Further the leaf methanol extract of *S. caryophyllatum* showed maximum presence of the tested compounds and also the presence of maximum of tannins and minimum of glycosides. But in *S.densiflorum* leaf methanol extract contains maximum compounds, at the same time the presence of maximum tannins and fixed oils were noted.

Plants contain different phytochemicals with different biological activity and therapeutic index. Phytochemicals are non-nutrient plant compounds, which protect us from chronic diseases. For example,

glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities^{5,6}. Saponins possess hypocholesterolemic and antidiabetic properties⁷. The terpenoids have also been shown to decrease blood sugar level in animal studies⁸. phytochemicals such as saponins, terpenoids, flavonoids, tannins and alkaloids have anti-inflammatory effects^{9,10,11,12}. The saponins are responsible for central nervous system activities¹³. Phytochemical screening of the acetone, aqueous and methanol extracts of *S. caryophyllatum* and *S.densiflorum* used in this study revealed that the crude extract contained most of the biologically active components.

In the present study, it was found that most of the biologically active phytochemicals were present in the methanol extract, due to these reasons further studies are in progress for the identification of phytoconstituent activity in *Syzygium caryophyllatum* and *Syzygium densiflorum* methanol leaf extracts.

Table 1. Qualitative Phytochemical analysis of *Syzygium caryophyllatum*

Phyto constituents	Reagents	Acetone			Aqueous			Methanol		
		Root	Bark	Leaf	Root	Bark	Leaf	Root	Bark	Leaf
Alkaloids	Dragendorff's	++	-	--	++	-	+	-	-	++
	Mayers	++	-	--	++	-	+	-	-	++
	10% lead acetate	±	-	-	++	-	-	±	-	-
Flavonoids	Sodium hydroxide	+	+	+	-	++	-	+	+	+
	Ethyl acetate	++	-	--	-	++	-	-	+	++
Phenols	Ferric chloride & Potassium ferrocyanide	±	+	+	-	++	±	-	+	+
	Tannins	Ferric chloride	++	-	--	--	-	+	+	++
Saponins	Froth test	-	-	±	++	-	+	-	-	+
Steroids	Acetic anhydride & Conc. Sulphuric acid	+	-	-	--	-	++	-	-	++
	Chloroform & sulphuric acid	-	-	+	+	-	+	-	-	+
	Glycosides	Borntrager's test	++	-	-	++	-	-	++	-
Triterpenoids	Acetic anhydride & Conc. Sulphuric acid	+	+	-	+	-	-	++	-	
	Fats and fixed oils	Phenolphthalein	-	-	+	-	±	+	-	+

Note: ++ = Strongly positive, + = Strongly positive, - = Negative, ± = weakly positive

Table 2. Qualitative Phytochemical analysis of *S.densiflorum*

Phyto constituents	Reagents	Acetone			Aqueous			Methanol		
		Root	Bark	Leaf	Root	Bark	Leaf	Root	Bark	Leaf
Alkaloids	Dragendroff's	-	-	—	-	+	—	-	-	—
	Mayers	-	-		-	⊥		-	-	⊥
Flavonoids	10% lead acetate	—	++	+	-	-	-	-	-	—
	Sodium hydroxide	-	-	—	—	-	±	++	-	+++
	Ethyl acetate	-	-	—	++	-	—	±	-	—
Phenols	Ferrie chloride	++	-	—	-	-	—	+	++	—
Tannins	Ferrie chloride	-	-			-			-	⊥
Saponins	Froth test	-	-	—	—	-	—	—	-	±
Steroids	Acetic anhydride & Conc. Sulphuric acid	++	+	—	-	-	—	++	+	—
	Chloroform & sulphuric acid		⊥		-	-			⊥	
	Borntrager's test		-		-	-				
Triterpenoids	Acetic anhydride & Conc. Sulphuric acid	-	-	-	—	-	+++	-	-	+++
Fats and fixed oils	Phenolphthalein	-	-		-		-	-		

Note: —: Very strong positive, —: Strong positive, +: Trace, -: Negative

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Biosorption of heavy metals in tannery effluent using agricultural wastes

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Abstract

Heavy metal contamination is caused by natural process or by human activity and it is one of the serious ecotoxicological problems. Contamination of soil by heavy metals is a serious concern in environmental perspective for safe utilization in agriculture. Tanning is essentially the reaction of collagen fibers in the hide with tannins, chromium and other chemical agents. The leather tanning effluent was collected and was analyzed for various physiochemical parameters. It was found that pH and electrical conductivity of the effluent were high. The concentration of metals such as chromium, zinc, nickel, copper, cadmium and the average levels of magnesium, calcium, potassium, sodium, phosphorus and nitrogen were found to be higher in effluent contaminated soil due to the presence of metals in the effluent than in control soil. Biosorption of tannery effluent was carried out using various adsorbents such as neem seed powder, tea waste, saw dust, egg shell and wheat bran under varying conditions of pH (3-9), temperature (4°C, 20°C, 37°C, 50°C) and contact time (4-24 h). From the results it was found that saw dust could adsorb the metals at pH 3. Neem seed powder could adsorb maximum quantity of metals at pH 5; Tea waste, egg shell and wheat bran could adsorb at pH 7. Maximum adsorption of metals occurred at 37°C than at other temperatures. Increase in contact time increased the adsorption of heavy metals up to 24 h of incubation.

Key words: tannery effluent, biosorption, heavy metals

Introduction

India is one of the largest producers of leather in the world and there are at present more than 3000 tanneries, with annual processing capacity of 0.7 million tons of hides and skins. The semi-soluble protein, collagen is converted in the process called tanning into tough flexible, insoluble and highly durable leather in a succession of many complex stages, consuming high quantities of water¹. Tanning is essentially the reaction of collagen fibers in the hide with tannins, chromium and other chemical agents.

Most of the pollutants and heavy metals discharged in industrial effluents ultimately find their way to aquatic ecosystem such as rivers, ponds and lakes. The presence of heavy metal pollutants in water bodies poses risk to the health of humans and ecosystem². Several heavy metals are required as essential micronutrients for a number of living organisms at very low concentrations; however at higher concentrations they can be toxic and sometimes fatal. The heavy metals affect the essential metabolic

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processes through protein denaturation by the blockage of functional groups displacing an essential metal, modification of the active confirmation of the molecule or by rupture of cellular and organelle membrane integrity³. Biosorption is a novel technique for decreasing metal ion content in waste water. It is considered as an alternative process for the removal of heavy metals, metalloid compounds and particles from aqueous solution by biological materials⁴. Adsorbent material derived from low cost agricultural wastes can be used for the effective removal and recovery of heavy metal ions from waste water streams⁵ since it is cost effective and the wastes are available in plenty.

Materials and Methods

Collection of tannery effluent

The pooled effluent from all the units of the tanning industry as let down by the factory was collected from a leather processing industry in Dindigul district (Tamil Nadu) at regular intervals and stored at 4 °C for analysis.

Characterization of the effluent

The collected tannery effluent was analyzed for physicochemical properties like colour, odour, turbidity, pH, total suspended solids, total dissolved solids, chemical oxygen demand, biochemical oxygen demand, chromium, copper, cadmium, nickel and zinc¹, carbonate and bicarbonate, sodium and potassium⁶.

Collection of the adsorbents

The adsorbents like wheat bran, tea waste, egg shell, saw dust and neem seed powder were collected and stored. The adsorbents such as wheat bran and egg shell were washed with distilled water, dried at 105 °C separately. After drying, the adsorbents were ground and sieved using a 50 micron sieve. Neem seed powder, tea waste and saw dust were used as such since they did not require any pretreatment.

Effect of adsorbents

Different pH: Fifty ml of the effluent was taken in a conical flask and 2g of each adsorbent was added. Controls were also prepared without the addition of adsorbents. Then the conical flasks were kept in shaker incubator at 100 rpm for 24 h. After incubation, the effluent was analyzed for the metals such as Cu, Cd, Cr, Ni and Zn⁷. The experiment was carried out at different pH (3, 4, 5, 7, and 9) values.

Different contact time: Fifty ml of the effluent was taken in a conical flask and 2g of adsorbent was added. After incubation for 4 h the concentration of metals (Cu, Cd, Cr, Ni and Zn)⁷ in the sample was analyzed by spectrophotometry. The experiment was repeated for incubation periods of 8, 12, 16, 20 and 24 h.

Different temperature conditions: Fifty ml of the effluent was taken in various conical flasks and 2g of each adsorbent *i.e.* wheat bran, tea waste, egg shell, saw dust and neem powder were added to separate conical flasks. It was incubated at 4 °C for 24 h in an incubator shaker. After incubation the concentration of metals (Cu, Cd, Cr, Ni and Zn)⁷ in the sample was analyzed. The incubation temperature was repeated for 20 °C, 37 °C and 50 °C.

Statistical Analysis

All the analyses were carried out in triplicates and the mean values are given in Table and figures.

Results and Discussion

Characterization of tannery effluent

The collected leather tanning industrial effluent was assessed for its physiochemical properties and its toxic metal levels. Table 1 shows the physiochemical characteristics of the selected tannery effluent. As per BIS (2009)⁸ water should be colourless, odourless and clear. The presence of colour and odour in any water sample indicates the unpleasant nature of water. The leather tannery effluent was found to be light brown coloured, turbid and also had an offensive odour. Normal pH range of water according to BIS (2009) is between 6.0 and 9.0. The effluent sample had a high pH value of 11.2, which is due to the presence of high concentrations of heavy metals and alkaline metal ions in the effluent⁹.

Table 1. Physiochemical characteristics of the tannery effluent

Parameters	Sample [†]	BIS limits (2009)
Colour	Light brown	Absent
Odour	Offensive	Absent
Turbidity	Turbid	Absent
pH	11.2	6.0- 9.0
Total suspended solids (mg/l)	2600	100
Total dissolved solids (mg/l)	13900	2100
Chemical oxygen demand (mg/l)	1950	250
Biological oxygen demand (mg/l)	180	30
Carbonate (mg/l)	6900	NM
Bicarbonate (mg/l)	10,750	NM
Sodium (mg/l)	63	NM
Potassium (mg/l)	213	NM

Note: * - Tolerance limits for tannery effluent discharged into inland water source as per BIS (2009); # - Mean of duplicate analysis; NM - Not mentioned.

The total suspended solids and total dissolved solids in sample were found to be high when compared with BIS standards. The presence of high level of total suspended solids and total dissolved solids might be due to the insoluble and soluble organic and inorganic matter present in the effluent¹⁰. Chemical oxygen demand and biochemical oxygen demand in the selected effluent sample were found to be 195 mg/l and 18 mg/l respectively which is lower than the BIS limits. The use of large amounts of salts, dissolved lime and acids in different stages of the tanning process leads to an effluent with very high concentrations of heavy metals¹¹.

The effluent chosen for the present study was analyzed for the presence of metals and the concentrations of chromium, zinc, cadmium, nickel and copper were found to be 173 mg/l, 143 mg/l, 140 mg/l, 130 mg/l, 85 mg/l respectively, which were significantly higher than the BIS limits.

The tanning industry is one of the major users of chromium salts. During leather processing, the conversion of putrefactive proteinaceous matter (skin) into non-purifiable matter occurs during the treatment with chromium sulphate solution. According to an estimate, 32 tons of chromium sulphate salts are used annually in Indian tanneries. As a result of unplanned disposal of spent tannery wastes, 2000-3000 tons of chromium as element escapes into the environment¹².

Biosorption studies

Biosorption is a novel method for removing heavy metals from the contaminated sites by using biomaterials. The study was carried out to find out the metal adsorbing capacity of various bioadsorbents like neem seed powder, egg shell, saw dust, wheat bran and tea waste. Two gram of each bioadsorbent was added to 50ml of raw effluent and was subjected to varying pH, temperature and contact time. After incubation, the adsorbents were filtered and the concentrations of metals such as zinc, nickel, cadmium, copper and chromium in the filtrate were analyzed.

Differing pH values

The metal adsorbing capacity of various bioadsorbents was analyzed under various pH values such as 3, 4, 5, 7 and 9 using phosphate buffer. Figure I-VC showed that saw dust had a higher metal adsorbing capacity at pH 3. Neem powder could adsorb the metals better at pH 5. Tea waste, egg shell and wheat bran could effectively adsorb the metals at pH 7. By comparing the results it was found that saw dust and neem seed powder had a higher adsorbing capacity than other wastes. The pH of the metal solution usually plays an important role in the biosorption of metals. The dried leaves of *Tridax procumbens* adsorbed maximum chromium at a pH of 3.0¹³ whereas groundnut husk showed maximum absorption at pH 2⁹ for chromium and at pH4 for lead. When the pH value of the medium was raised from 1 to 4.8 the adsorption capacity of plant materials was found to be enhanced⁵.

Different temperatures

The metal adsorbing capacity of various bioadsorbents were analyzed at different temperatures like 4°C, 20°C, 37°C and 50°C. Figure I-VA showed that maximum adsorption of metals such as chromium, cadmium, nickel, zinc and chromium by the bioadsorbents occurred only at 37°C than at 4 °C, 20°C and 50°C. Chromium adsorption by groundnut husk and *T. procumbens* leaves was maximum at a temperature of 40°C^{2,13}.

Varying contact time

The concentration of metal levels in the bioadsorbents was analyzed at intervals of 4 h up to 24 h after incubation. Figure I-VB showed that maximum adsorption occurred only at 24 h. This is due to the fact that increase in contact time increased the adsorption of heavy metal ions. Similar increased absorption of copper ions by metal resistant *Pseudomonas* was observed other workers¹⁴. The adsorption of chromium was maximum in 150 min. using dried leaves of *T. procumbens*².

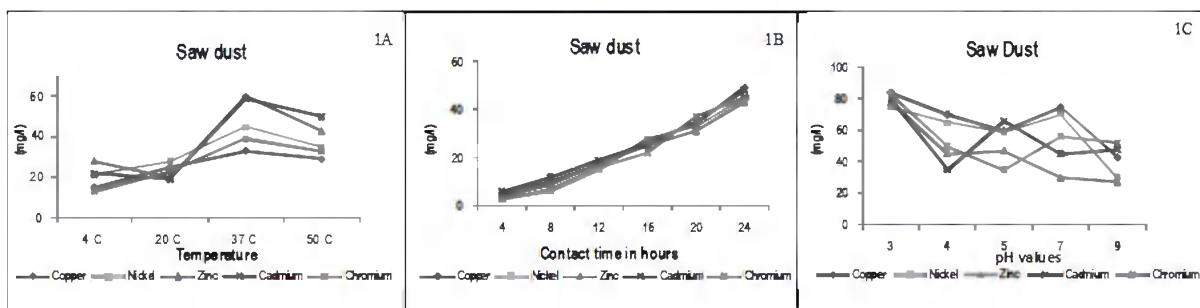


Fig. 1. Biosorption by saw dust at different temperatures (A), different contact times (B) and different pH values (C)

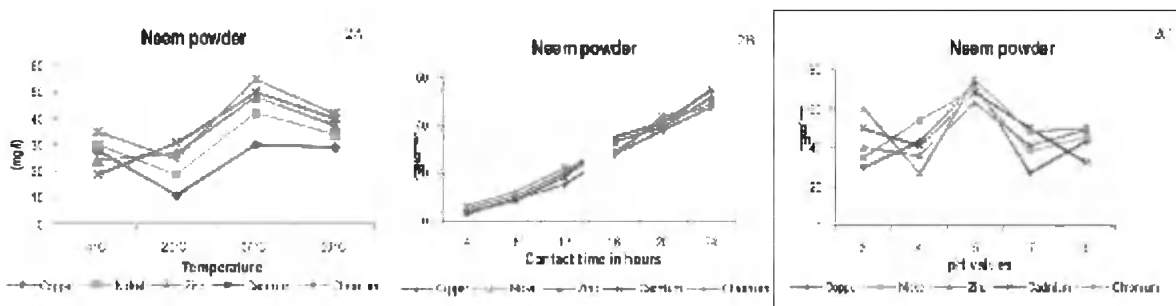


Fig. 2. Biosorption by neem powder at different temperatures (A) different contact times (B) and different pH values (C).

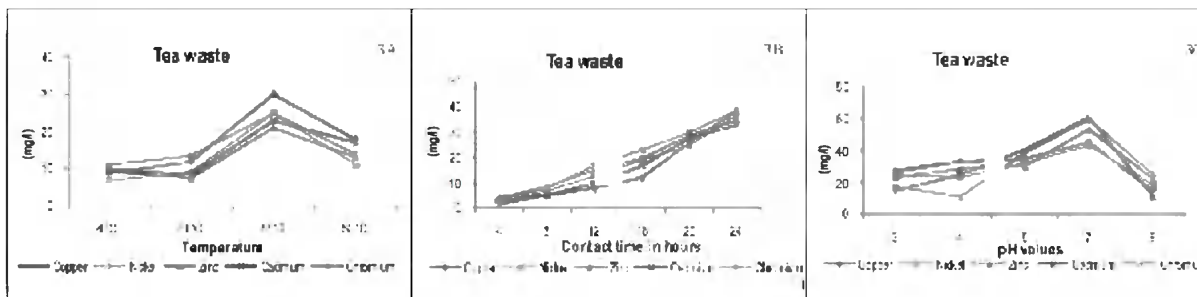


Fig. 3. Biosorption by tea waste at different temperatures (A) and different contact times (B) and different pH values (C).

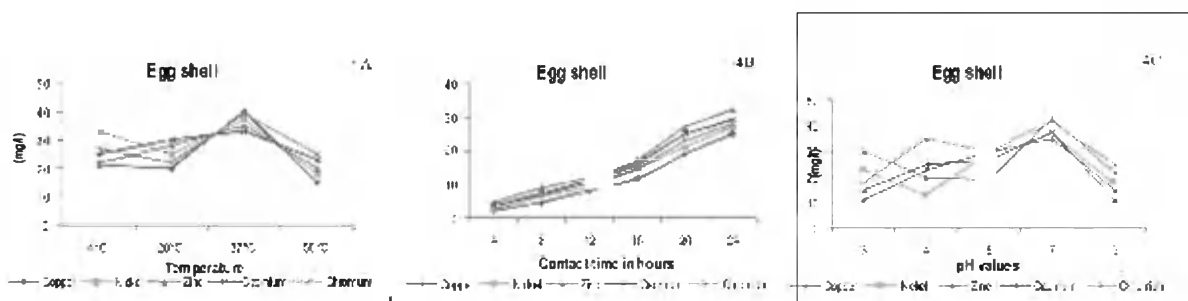


Fig. 4. Biosorption by egg shell at different temperatures (A) and different contact times (B) and different pH values (C).

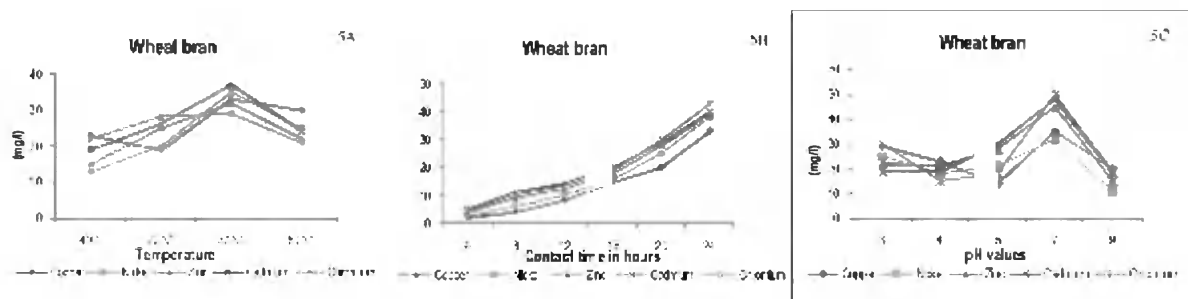


Fig. V. Biosorption by Wheat bran at different temperatures (A) and different contact times (B) and different pH values (C).

Biosorption of tannery effluent was carried out using various adsorbents such as neem seed powder, tea waste, saw dust, egg shell and wheat bran under different conditions such as different pH, temperature and contact time. From the results it was found that saw dust could adsorb the metals at pH 3. Neem powder could adsorb at pH 5. Tea waste, egg shell and wheat bran could adsorb at pH 7. Maximum adsorption of metals occurred at 37°C. Increase in contact time increased the quantum of adsorption and hence maximum adsorption occurred at 24 h of incubation. Incubation for longer duration might still increase the adsorption of metal ions and this need to be further verified.

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Diversity of 'Pests' of Toddy Tapped from *Borassus flabellifer* in Thenkurissi, Palakkad, Kerala State

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Abstract

Toddy tapping, collection, preservation and distribution forms one of the major sources of income to the State of Kerala. Present study showed that several insects *Apis mellifera*, *Apis dorsata*, *Camponotus* spp., *Monomorium* spp., *Myrmecaria* spp., *Galleria mellonella*, *Forficula auricularia*, *Periplaneta* spp. and also several other non-insect pests get attracted to toddy and get trapped in the collection pot. They caused economic loss through consumption and contamination. Loss incurs through loss of time of tappers spent for fighting the stinging honey bees and also for filtering out the pests falling into the product. Reason for the variation in the prominence of various insect pests during different months is discussed. Present study forms the pioneer attempt to work out the role of insects as pests of toddy.

Key words: Toddy tapping, biodiversity, pests, economic loss, wind.

Introduction

Toddy tapping, collection, preservation and distribution forms one of the major sources of income to the State of Kerala. Scientific investigations on the quantity, quality especially pest problems, or any other parameter related to toddy industry appears to be meager. The chemical nature of toddy yielded from *Borassus flabellifer* has been elucidated¹. The fresh sap is reportedly a good source of vitamin B complex². The unfermented coconut sap (sweet toddy) and some simple alcohols viz. ethyl alcohol, isopropyl alcohol and isoamyl alcohol show a significant activity in attracting the pest *Rhynchophorus ferrugineus*³.

A number of pests are attracted towards toddy and they get trapped inside the collection pot. The present investigation seems to be the pioneer work on the 'pests of toddy' as no literature could be traced. In this work, variation in toddy production, diversity and density of insect and non-insect pests of toddy tapped from palmyra palm have been elucidated.

Materials and Methods

For the present study, 20 healthy well yielding palms in Mannalur, a small village in Thenkurissi panchayat in Palakkad district, Kerala were selected. The trees were 15 to 40 years old and about 12-15 meters in height. Each palm yielded up to 10 liters. The tapping was performed by skilled laborers.

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The first collection was made on 15th November, 2009, as every year collection begins in November. Collections were taken with the help of a tappers in the middle of consecutive months till May when the production ends. The insects were filtered out from toddy using filtering sieves of 1mm mesh size, in the early morning just after the toddy was brought down.

The insects were separated with a muslin sieve. Collections were put into plastic vials and brought into laboratory. They were anesthetized using chloroform and killed with ethyl acetate. They were then preserved in 70%ethyl alcohol and kept in labeled bottles and small vials for further studies. Insects were identified and classified according to ⁴. Expert Taxonomists were also consulted whenever necessary.

Results and Discussion

In the present study, the insects and other animals which were isolated from the toddy collection are referred to as pests based on the assumption that any animal that causes economic loss to humans are considered as pests ⁵.

Average yield per palm varied from month to month (Table.1, Fig. 1) from November to May of next year, which the tappers call as a 'season'. For the seven months the quantity measured were 1, 5, 6, 5.5, 4, 3, 2.5 liters respectively. Insects were sieved out and counted from all the collections as a single unit. Density of insects was calculated and represented as number of insects/lr of toddy.

Table. 1. Showing the number of insects pests in the samples of toddy.

Insect	Nov	Dec	Jan	Feb	Mar	Apr	May
Avgn toddy collected/month	20 ltrs	100 ltrs	120 ltrs	110 ltrs	80 ltrs	60 ltrs	50 ltrs
<i>Apis dorsata</i>	120	3880	80	1680	20	40	0
<i>Apis mellifera</i>	0	3280	240	4120	8040	6860	280
<i>Campoplex</i> sps.	0	7900	480	2295	5720	0	0
<i>Monocentrus</i> sps.	0	760	0	345	680	1240	1080
<i>Mesochorus</i> sps.	0	0	0	0	0	100	280
<i>Facileola curvicauda</i>	0	120	0	70	40	20	0
<i>Gellaria mellonella</i>	0	80	60	70	0	0	0
<i>Pezomachus</i> sps.	0	40	20	0	20	0	0
Other insects	0	160	40	40	90	40	40
TOTAL INSECTS	120	16240	1040	2870	14480	8500	1880
Toddy collected per mday	1 ltr	5 ltrs	6 ltrs	5.5 ltrs	4 ltrs	3 ltrs	2.5 ltrs
Insects/lr	6	1624	87	71.1	181	121.6	37.6

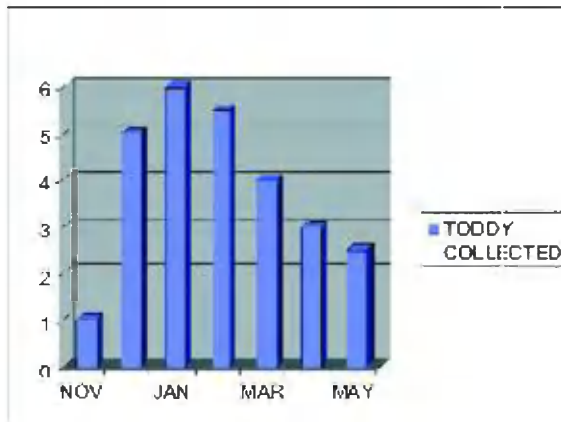


Fig.1 Toddy yield per palm per day

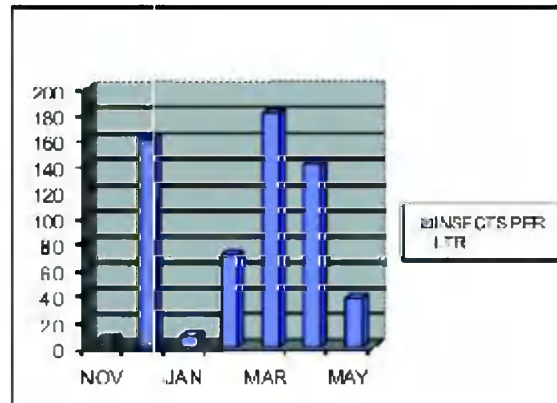


Fig.2. Total number insect per litre of toddy per month

The major insects collected were Honey bees and ants (Table.1). Their numbers varied in each collection in each month and also in varying seasons (Table.1 & Fig.2). Two species of honey bees were identified. They were *Apis mellifera* and *Apis dorsata*. Ants in the collection belonged to three genera viz. *Camponotus*, *Myrmecaria* and *Monomorium*. Wax moths (*Galleria mellonella*), cockroaches (*Periplaneta americana*), Earwigs (*Forficula auricularia*) and flies from Homoptera, Hymenoptera and Diptera also were found in collections, which fall into the collecting pot (local name 'Muttipaani') often. In the month of November 2009 only *Apis dorsata* (120) were found in toddy. In December 2009 seven types of insects were identified (Table & Graph) in the collection viz., *A. dorsata* (3880), *A. mellifera* (3280), ants belonging to *Camponotus* spp. (7900) and *Myrmecaria* (760), earwig *Forficula auricularia* (120), wax moth *Galleria mellonella* (80), and cockroach *Periplaneta americana*. (40). In January 2010, five types of insects were present. However, their number was found to be much lesser than in the previous month. *A. dorsata* (80), *A. mellifera* (240), ants *Camponotus* spp. (480), wax moth *Galleria mellonella* (60), and *Periplaneta americana*. (20) The six types of insects found in February are *A. dorsata* (1080), *A. mellifera* (4120), ants *Camponotus* spp. (2295) and *Myrmecaria* spp.(245), *F. auricularia* (20), and wax moth *G. mellonella* (20), *A. dorsata* (20), *A. mellifera* (8040), ants *Camponotus* spp. (5720), *Myrmecaria* (680), and *F. auricularia* (40), also were found in March 2010. A new genus of ant *Monomorium* started to appear in the collections from March 2010 and went on up to the end of the tapping season i.e., May 2010. From the month of April 2010 *Camponotus* was totally absent (Table. 1). The number of other insects were *A. dorsata* (40), *A. mellifera* (6860), ants *Myrmecaria* (1440), *Monomorium* (100), and *F. auricularia* (20) were also observed during this month. During May 2010, the last month of the tapping season, no *A. dorsata* were found in the collection. *A. mellifera*, ants

Myrmecaria, *Monomorium* were present in numbers 280, 1080, 280 respectively. Maximum yield of toddy was found to be in the month January from where it starts to decline slowly. The total number of insects gets shot up with the increase in the toddy production. However a marked decrease in the total number in reverse with the toddy production is prominent in the month of January (Table. 1).

Non-insects pests

Invertebrate Pests

During the present study, in addition to the insect pests, non-insect pests were also found in toddy collections. During the period of November 2009 to February 2010 sixty spiders belonging to the genus *Nuphilia* were filtered out. After that they were found only occasionally. Two land snails were also found from the beginning of the season to the end.

Vertebrate Pests

Geckos, Calotes, rats and bats were the vertebrate pests which fell into the pot and necessitated the rejection of the collection resulting in economic loss. It was found that the abundance and variety of insects do not correspond exactly to the increase or decrease in the yield of toddy. All the insects except *A. mellifera* and *Monomorium* increase corresponding to the increase in the production (Table.1). However, there was observed a sudden fall in the number of all the insects during the month of January 2010. This may be due to the strong 'East Wind' coming through 'Palakkad Gap' which blows through the entire region of Palakkad. In Palakkad, Palmyra (*B. flabellifer*) is the main plant used as the wind breaker along with coconut trees (*Cocos nucifera*), to protect the paddy cultivation – the main crop in Palakkad. The strong wind prevents the free flying of all the flying insects and hence the decline in number during the month of January 2010. Reduction in the activity of flying insects due to wind action has been reported^{6,7}. Alcohol has been used as an attractant to make insect collections^{8,9}. The attraction, consumption and contamination of toddy by a variety of insects, including honey bees and ants, other invertebrate and vertebrate pests support this fact. They get attracted by the scent of toddy and after consumption they get intoxicated and fall in to the pot. The particular shape of the pot prevents them from escaping.

Various species of ants like *Camponotus* sps. and *Monomorium* sps. are reported to be the common predators of honey bee⁴. The wax moth *Galleria mellonella* are also documented as serious predators of honey bee¹⁰. The behaviour and occurrence of *Camponotus*, *Monomorium* and *G. mellonella* in association with the collections is to be studied in depth to discern the possibility that they come to the toddy pot mainly to predate on bees rather than to consume alcohol. Though both are honey bees, the appearance of *A. mellifera* and *A. dorsata* in the collections varied widely during the tapping season, A.

dorsata was the first and only insect to appear in the collections of the month of November 2009. *A. mellifera* appeared from December onwards. The peak appearance of the former was in December while that of the latter was in March. According to the toddy tappers, from late December onwards *A. dorsata* leaves the open places and settles down in nearby forests as the 'East Wind' gets stronger, and hence the reduction in number. The observation that the activity of flying insects get reduced due wind action supports this possibility^{6,7}. After February, availability of flowers becomes much reduced in Thenkurussi, in the area of study, and *A. mellifera* relies on toddy for food.

Conclusions

The present study is an attempt to work out the diversity and density of pests related to toddy industry. The study showed that several insects and other animals form pests causing economic loss through consumption and contamination. Loss incurs through loss of time of tappers spent for fighting the stinging honey bees and also for filtering out the pests falling into the product. Rejection of collection when rats and bats get drowned in toddy brings about greater loss. Most of the pests identified were well explored vectors of fatal diseases like Haepatitis and Rabies. Detailed and in-depth study is necessary to prevent economic loss in toddy industry and also to improve the quality of the product so that the possibility of spread of diseases through toddy consumption is reduced.

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Study of Amylase and Invertase Activity at optimal pH in the guts of *Danus chrysippus* Linn., *Iphita limbata* Stal. and *Tabanus striatus* Fab.

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Abstract

Activity variation of amylase and invertase were carried out in the salivary glands and guts of *Danus chrysippus* Linn., *Iphita limbata* Stal. and *Tabanus striatus* Fab. The presence of amylase and invertase activity in the digestive system of the three insects, indicates that they are adapted to utilize starch and sucrose. The activity of amylase was powerful in the digestive system of *Iphita* and was weak in *Tabanus*. The activity of invertase was vigorous in the digestive system of *T. Striatus* and less active in *I.limbata* particularly in the hindgut. The pH optimum for amylase was 9.0-9.5, 8.0-8.5 and 9.0 respectively. A pH optimum of 6 was noticed for invertase activity in all the cases.

Key words: amylase, invertase, pH, gut, *Danus chrysippus*, *Iphita limbata*, *Tabanus striatus*.

Introduction

Carbohydrases of insects have been reviewed by many authors^{1,2,3,4,5}. But, comparative studies on the activity of enzymes like amylase and invertase in insects having different food habits⁶ are few. So, the activity variation of the above enzymes in the salivary glands, foregut, midgut and hindgut of the following insects having different diet pattern were studied. The last instar larva of danaid butterfly, *Danus chrysippus* Linn. (Lepidoptera: Danaidae) at its 48th h of development, adults of *Iphita limbata* Stal. (Heteroptera; Pyrrhocoridae) and *Tabanus striatus* Fab. (Diptera: Tabanidae) adults were selected for the study. The larva of danaid butterfly prefer solid food in the form of leaves from the plant *Calotropis* sp., adults of *I. limbata* prefer plant sap from *Cebia* sp. and *T. striatus* adults prefer blood of domestic animals. The optimum pH level for activity of the enzymes was determined, keeping other conditions constant. The study gives a conclusive nature of amylase and invertase distribution in the guts of insects having different food habits and their preferred pH for activity.

Materials and Methods

Sampling of the tissues, preparation of homogenates of salivary glands, foreguts, midguts and hindguts were prepared⁷ and detection of the amylase and invertase were conducted^{8,9}. A homogenate of a tissue is,

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by definition, a suspension of the formed elements of the cells in a diluted solution, which is isotonic with the cell contents and which is inert. What was attempted in the study was a cell-free preparation, with maximum exposure of intracellular enzymes and for this purpose water was used as the dispersion medium. Salivary glands were ground in a homogenizer type of apparatus, while the gut parts were ground in glass mortar with pestle. During grinding the homogenizer / mortar was kept surrounded by ice and the medium was added intermittently, which lasted for about 10 minutes. The slurry was transferred to 10 ml tubes with bakelite caps and the homogenizer / mortar was washed twice with minimum ice cold distilled water and the washing added to the main preparation. The material was quantitatively transferred, with washings, to a 10 ml graduated cylinder and the volume adjusted appropriately and the preparation transferred back to the container. The ground preparation thus obtained was stored in a refrigerator (0-4 °C) and used within 30-60 minutes. The suspension was mixed properly before each pipetting of aliquot.

The assay system for amylase was consisted of 0.50 ml glycine – NaOH buffer, 50 mM, at pH 9.1, 0.20 ml of soluble starch, 5% (w/v), 0.10 ml enzyme preparation of appropriate concentration, any supplements and distilled water to 1.0 ml. The incubation was for 1 h at 37 °C. The reaction was terminated with ZnSo₄ . Ba (OH)₂ added in equivalent amounts. The suspension was centrifuged and reducing sugar in aliquot from the supernatant was determined. Maltose (BDH) was used as the standard.

The assay system for invertase was consisted of 0.5 ml buffer, phosphate, 50 mM, at pH 6.0, 0.10 ml sucrose solution, 300 mM, 0.10 ml enzyme preparation of appropriate concentration, any supplements contained in 0.10 ml and made up to 1.0 ml with water. Following incubation at 37°c for half hour, the reaction was stopped with ZnSo₄ . Ba (OH)₂ . The reducing sugars were determined using glucose as standard. Controls were run simultaneously with heat – denatured enzyme preparations in both the cases.

The pH at which the maximum enzymatic hydrolysis of a given substrate occurred was found out by performing the assay at different hydrogen – ion concentrations, keeping the other conditions constant. Phosphate buffer (50 mM) was employed from pH 6.5 to 8.0 and glycine-NaOH buffer (50 mM) from pH 8.5 to 10.5.

Results and Discussion

The amylase and invertase enzymes were assayed under the experimentally determined optimal conditions. The results of amylase and invertase activity in the different regions of the alimentary system in *D. chrysippus*, *I. limbata* and *T. striatus* are given in Table 1 and 2 respectively.

Table 1. Amylase activity in the digestive areas of *D. chrysippus*, *I. limbata* and *T. striatus*.

Region	Enzyme Activity (Units/mg Tissue)		
	<i>D. chrysippus</i>	<i>I. limbata</i>	<i>T. striatus</i>
Salivary Gland	6.1	7.3	4.7
Foregut	20.6	24.1	18.3
Midgut	293.2	312.7	186.2
Hindgut	17.8	5.4	7.6

Note: The values are means of five determinations each.

The presence of amylase and invertase activity in the digestive system of the three insects, indicates that the insects are well adapted to utilize starch, sucrose and any other poly-glucan occurring in their food. The activity of amylase was more powerful in the digestive system of *Iphita limbata*. It indicates that the plant sap is a rich source of starch and soluble sugars. Maximum activity of the enzyme was noticed in the midgut region of all the three experimental insects. In *T. striatus*, the activity of amylase is weak, compared with the other two. The presence of less starch in the blood meal may be one of the reasons for this.

Table 2. Invertase activity in the digestive areas of *D. chrysippus*, *I. limbata* and *T. striatus*.

Region	Enzyme Activity (Units/mg Tissue)		
	<i>D. chrysippus</i>	<i>I. limbata</i>	<i>T. striatus</i>
Salivary Gland	55.9	32.6	22.7
Foregut	34.4	36.4	62.1
Midgut	134.1	202.7	158.3
Hindgut	67.4	14.5	87.6

Note: The values are means of five determinations each.

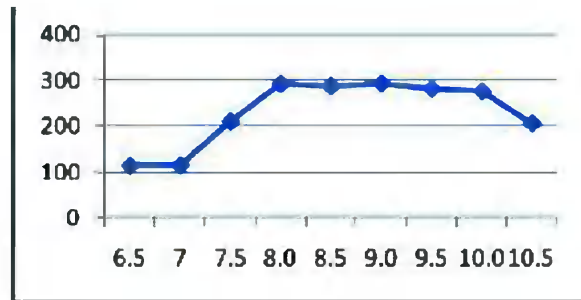
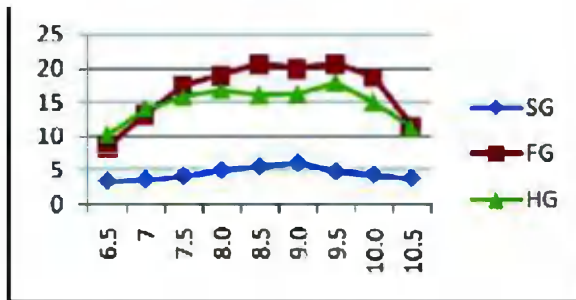
The activity of invertase was more vigorous in the digestive system of *T. striatus* and was less active in *I. limbata*, particularly in the hindgut. The food of *D. chrysippus*, was expected to contain sucrose. Sucrose is the major transport form of carbohydrate. In the case of *I. limbata*, the plant sap usually contains high sucrose content. The blood meal of *T. striatus* is a rich source of sucrose.

The digestive enzymes in an insect are generally correlated to the constituents in its diet¹⁰. The amylase is more active in the midgut of the insect gut^{7,9,11}. Invertase has been reported in the digestive system of several insects, whose feed material was likely to contain sucrose^{10,12}.

Optimum pH of Activity

Amylase

A pH optimum of 9.0-9.5 found for *D. chrysippus* larvae (Fig. 1A, 1B) was keeping with the optima reported for other lepidopterous larvae. Strong midgut alkalinity and high pH optimum for amylase are characteristics of leaf eating lepidopterous larvae^{6,13}.



Note: X axis represents pH, Y axis represents units of activity/mg tissue.

Fig. 1A: Optimum pH for amylase activity in salivary gland, oregut and hindgut of *D. chrysippus*

Fig. 1B: Optimum pH for amylase activity in midgut of *D. chrysippus*

In *Iphita limbata* the pH optima was found to be 8.0-8.5 (Fig. 2A, 2B). A pH optimum 9.5 was noticed in *Tabanus striatus* (Fig. 3A, 3B). The optimal pH for amylase activity from various insects was found to be in the range of 5.5-9.5⁴. Available data on the optimum pH of amylase in various insects has been summarised by Hori⁸.

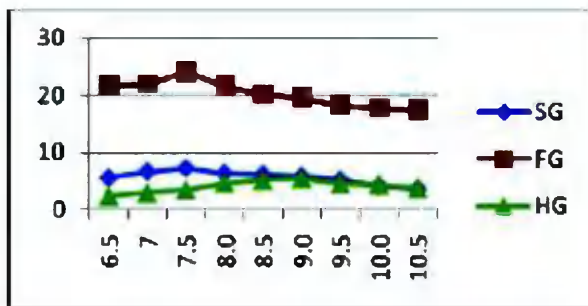


Fig. 2A: Optimum pH for amylase activity in salivary gland, foregut and hindgut of *I. limbata*.

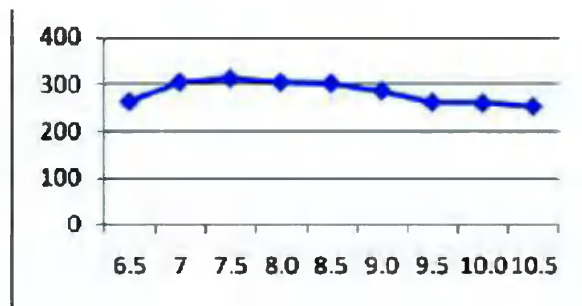


Fig. 2B: Optimum pH for amylase activity in midgut of *I. limbata*.

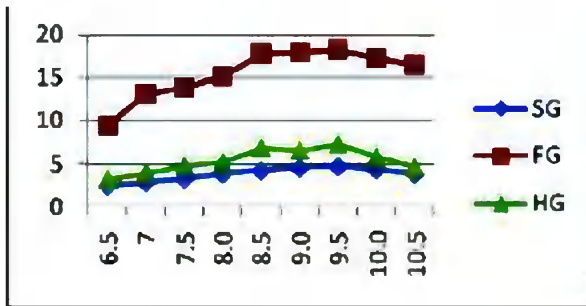


Fig. 3A Optimum pH for amylase activity in salivary gland, foregut and hindgut of *T. striatus*

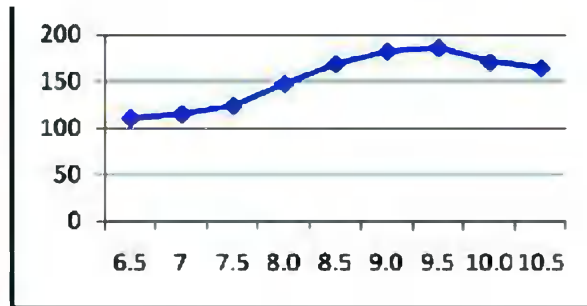


Fig. 3B Optimum pH for amylase activity in midgut of *T. striatus*.

Invertase

In *D. chrysippus*, *I. limbata* and *T. striatus*, a pH optimum of 6 (Fig. 4A & 4B, 5A & 5B, 6A & 6B) for invertase activity was noticed, irrespective of the fact that, they were feeding on different diets.

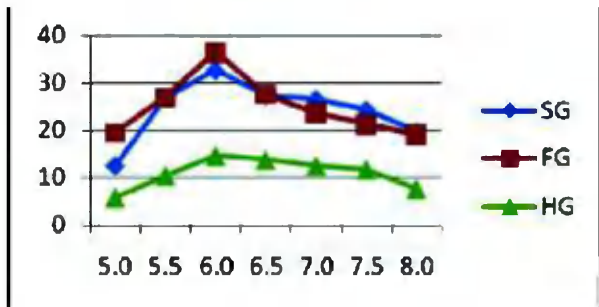


Fig. 4A. Optimum pH for invertase activity in salivary gland, foregut and hindgut of *D. chrysippus*.

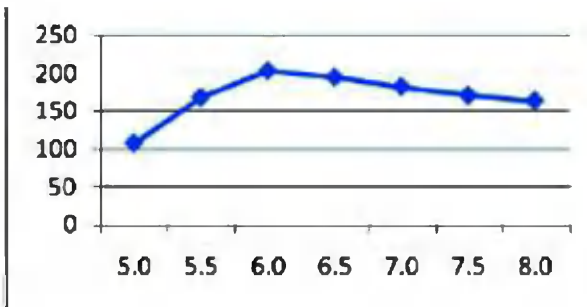


Fig. 4B. Optimum pH for invertase activity in midgut of *D. chrysippus*.

The amylase and invertase were more active in the midgut region of all the insects studied compare to other regions. The preferred pH for amylase was slightly alkaline and was slightly acidic for invertase.

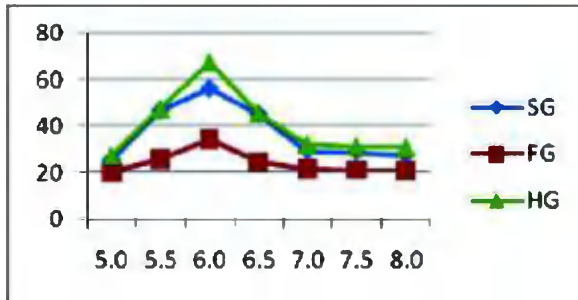


Fig. 5A. Optimum pH for invertase activity in salivary gland, foregut and hindgut of *I. limbata*

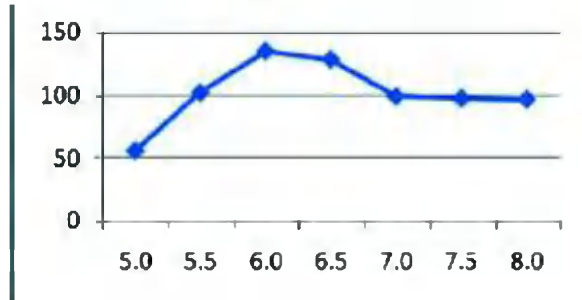


Fig. 5B. Optimum pH for invertase activity in midgut of *I. limbata*.

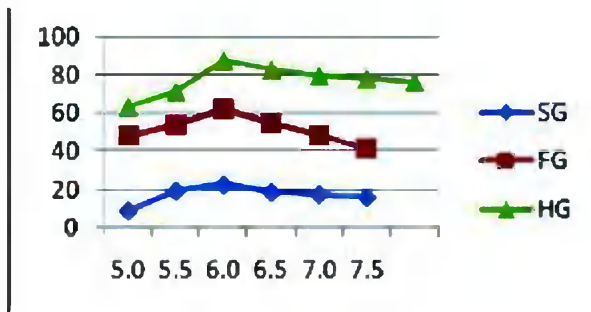


Fig. 6A. Optimum pH for invertase activity in salivary gland, foregut and hindgut of *T. striatus*.

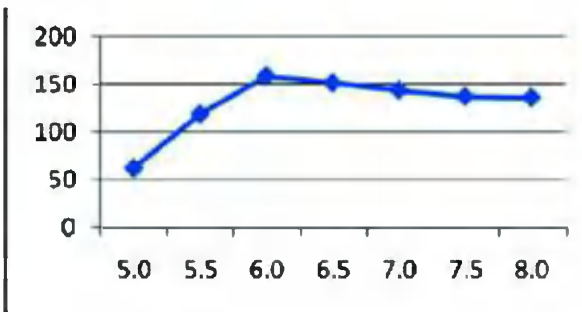


Fig. 6B. Optimum pH for invertase activity in midgut of *T. striatus*.

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A study on the diversity of Heterocera (moths) from Kakkani region near Dhoni forest

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Abstract

The Kakkani region near Dhoni forest was monitored for diversity in Heterocera (Moth) community during February 2011 to June 2011. A total of 14 species of moths belonging to 8 different families were collected by using simple light trap operated from dusk to dawn daily. Families Noctuidae, Arctiidae, Lymantriidae, Geometridae, Sphingidae, Hyblaeidae, Hypsidae and Pyralidae were present in the collection samples. Noctuidae family had the maximum representation in the total collection. Only few moths belonging to families like Sphingidae, Hyblaeidae, Hypsidae and Geometridae were obtained in the collection. During the five months of collection maximum number of species representation was found during the month of March.

Key words: Diversity, Heterocera, Noctuidae, Dhoni, Western Ghats.

Introduction

Kakkani region near Dhoni forest situated in the district of Palakkad in Kerala enjoys a tropical climate with a very hot season extending from March to June. The temperature of the district ranges from 20 °C to 45 °C during these months. The most important rainy season is the South-West monsoon which sets in the second week of June and extends up to September. About 75% of the annual rainfall is received during the South-West Monsoon period. During the period from December to May, practically no rain is received. Dhoni forest near the study area forms a part of Western Ghats. Several previous studies have shown that Western Ghats is a place of high Lepidopteran diversity. Hampson¹ had recorded 1136 species from Western Ghats. Similar work was done by Moore² and Swinhoe³ which included the biology of all the species then known from the Western Ghats. Studies by George Mathew (1987 to 1990) identified 400 species of moths belonging to 19 families from Silent valley National Park. A majority of forest lands of the area was transformed into largely monoculture plantations. As man makes willful manipulation of forest land converting them into monoculture plantations, these changes advertently affect the fauna of the original ecosystem. Moths are a group of insects which show high habitat specificity. Monitoring of moth population can be a good indicator for change in ecosystem condition that affects the faunal biodiversity.

Materials and Methods

From previous studies it is evident that moth shows maximum diversity during summer season. Hence, the

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sampling period was confined from February to June 2011. The moths were collected by using a simple light trap consisting of a light on a cable hanging down. CFL lamp was used as a light source and the moths were also collected using sweep net or are hand picked. The lamp was suspended beside a white wall to enhance the brightness. Sampling was done mainly thrice a month. Insects thus collected were sorted and moths were separated by their characteristic features like antennae and scaly wings.

Result and Discussion

During this study a total of 128 specimens of moths were identified. They belong to families *i.e.*, Noctuidae (6species), Arctiidae (2species), Lymantriidae, Geometridae, Sphingidae, Hyblaeidae, Hypsidae and Pyralidae (1 species each).

Noctuidae formed dominant family in the collection represented by *Spodoptera litura* (Fb.), *Spodoptera maurita* Boisduval, *Othreis materna* Lin., *Othreis fullonica* Lin., *Helicoverpa armigera* and *Earias vitella*. Around 166 species of this family was recorded from the Western Ghats in 1896⁴. In several recent biodiversity studies carried out in Western Ghats, Noctuidae again formed a dominant part of the collection^{5, 6}. From family Arctiidae, *Pericallia ricini* Fb. and *Syntomis* sp., *Euproctis fraterna* Moore (Lymantriidae), *Thalassodes* sp. (Geometridae), *Hippotion rosetta* (Sphingidae), *Hyblaea puera* Cram.(Hyblaeidae), *Asoto* sp.(Hypsidae) and *Orthaga exvinacea* Hampson(Pyralidae) were also able to identify from the collection.

Among the total collected heterocerans very few had representations in all the five months of collection (Table 1). Members of family Noctuidae were present in all the five months of collection even though the species represented varied (Fig. 1). Arctiidae family represented by two species formed the second group having the maximum diversity next to Noctuidae, of which only *P. ricini* was present during the entire study period. One species each were identified from all the other families. Only *O. exvinacea* (Pyralidae) had a significant number in the total collection after noctuids. About 61% of total collection were formed of Noctuidae and the next largest contributor is the Pyralidae (12%) followed by Arctiidae (11%), Lymantriidae (7%) and others (9%) (Fig. 1).

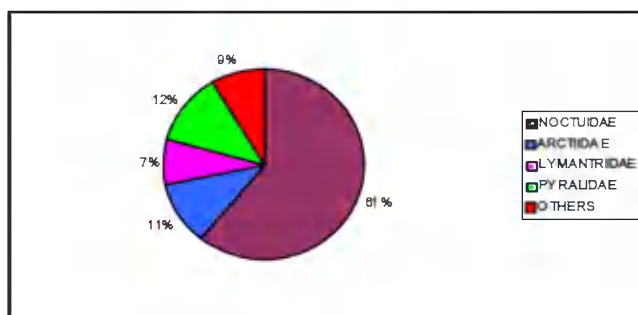


Fig. 1. The percentage of total number of individuals from different families

Spodoptera mauritia (Noctuidae) formed the dominant species in the entire collection. This is a polyphagous pest whose primary host is paddy. Hence the vast stretches of paddy in Kakkani area have supported their growth. The major factors that must have favored their population build up are:

- Prolonged dry condition followed by heavy rainfall favors its outbreak.
- Wind and rainstorm helps in migration of moths to long distance.
- Pest occurs throughout the year on alternate hosts and move to paddy during season.
- Heavy rainfall leads to high mortality of larval population. Hence, their number decreases during June in the collection.
- Pests migrate from older rice to grassy areas for off-season survival.

Table 1. Specimens collected during the entire period of study

SPECIES	FEB	MAR	APR	MAY	JUN
<i>Spodoptera litura</i>	1	5	4	2	0
<i>Spodoptera mauritia</i>	2	12	8	6	8
<i>Earias vitella</i>	1	1	0	0	0
<i>Helicoverpa armigera</i>	1	2	5	4	2
<i>Othreis fullonia</i>	2	3	3	1	1
<i>Othreis materna</i>	0	1	0	0	0
<i>Pericallia ricini</i>	2	1	1	4	1
<i>Syniomis sp.</i>	1	3	1	0	0
<i>Euproctis fraterna</i>	5	3	0	1	0
<i>Thalassodes sp.</i>	0	0	0	1	0
<i>Hippotion rosetta</i>	0	2	1	0	0
<i>Hyblaea puera</i>	2	0	0	1	1
<i>Asoto sp.</i>	0	0	0	2	1
<i>Orithaga exvinacea</i>	0	6	5	3	1

E. vitella, *O. fullonia*, *O. materna*, *P. ricini*, *H. armigera*, *O. exvinacea*, *E. fraterna*, *H. rosetta* etc. were the fruit boring pests identified and their presence can be attributed to the vast mango and banana plantations in the study region. *H. puera* did not show a significant contribution in the entire collection, as only a few isolated teak trees are present in the collection site, as against the high intensity outbreaks reported earlier

from several places in Kerala. They did not have a continuous representation in the samples (Table 1). Presence of large monoculture plantations in the region justifies the observation that majority of the heterocerans obtained were pests.

There is a significant relationship between the occurrence and abundance of moth community with the climatic factors such as temperature and rainfall of that region⁷. In the present study too, the diversity in collection may be due to the unique geographical features of the study area. The long Western Ghats is perhaps the most influential factor for the unique characteristics of the district. The presence of Malampuzha dam is also a major factor affecting the climatic conditions of the Kakkani region.

Many species of moths are strictly seasonal, preferring only a particular set of habitats. From the table given below it is clear that the onset of monsoon in June decreases the number of specimens in the collection, hence their occurrence can be considered as good indicator of climatic conditions as well as ecological changes⁸. Lepidopterans offer good opportunities for studies on population and community ecology⁹. Close monitoring on heterocerans will provide much valuable information regarding climatic changes on diversity.

Acknowledgements

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Major pathological conditions and illnesses of Falcons (*Falcon sp.*) in United Arab Emirates

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Abstract

The healthcare practice of Falcons (Peregrine Falcon *Falco peregrinus*, Saker Falcon *Falco cherrug* and Gyr Falcon *Falco rusticolus*) in UAE was studied from 1999 to 2003. The study was conducted in falcon hospitals, clinics and breeding centres in Abu Dhabi, Dubai, Sharjah and Al Ain in the United Arab Emirates. A critical examination of the current healthcare practices was carried out and attempts were made to develop a strategy to pay attention to the shortcomings. A variety of viral, bacterial and fungal diseases were recorded in Falcons. However, most of the diseases are not fatal if precautions and treatment are given in time. Bacterial infections were less serious than the viral diseases and they are transmitted to Falcons through prey. The main prophylactic actions involved optimizing the conditions in captivity to meet all the natural requirement of birds. Proper management, better hygiene, balanced diet and routine check up prevented most of the diseases. Falcons constantly forced to land on cold muddy floors are likely to have health problems and hypothermia. Bark or wood chips are not suitable substrate in falcon enclosures as they harbour spores of *Aspergillus sp.* In summer climates the falcons are completely cared in these enclosures.

Key words: *Aspergillus sp.*, new castle, chlamydiosis, salmonellosis

Introduction

A variety of infectious diseases are prevalent in falcons (Peregrine falcon *Falco peregrinus*, Saker falcon *Falco cherrug* and Gyr falcon *Falco rusticolus*). The majority of these infections are transmitted through natural prey. Wild falcons are resistant to the infectious diseases due to their acquired immunity¹. The primary role of disease prevention in falcons involves optimizing the conditions in captivity to meet all the natural requirements of the birds. As a routine management procedure, this includes cage rest, providing nourishment or simple rehydration techniques². Maintaining of these birds, which occupy special status in the trophic chain and are highly sensitive to local environment, need better understanding and experience. As no detailed information on the health care practices of falcons in UAE is available an attempt was made to study the pathological conditions and illness of falcons³. In this study attempts were made document the viral, bacterial and fungal diseases, the disease caused by internal and external parasites and other diseases by non-biological agents in the falcon species of UAE⁴.

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Materials and Methods

The data was collected from falcon hospitals, clinics and breeding centers in Abu Dhabi, Dubai, Sharjah and Al Ain in the United Arab Emirates. Hospitals and clinics were visited to study the Falcons and document the health status. Diseased and inactive birds were examined and the medical histories of selected birds were collected. The management strategy adopted by the clinics and hospitals in each case was studied and documented. Experts who handled the birds were interviewed and the technical details collected. Detailed examination of the birds was carried out after they were anesthetized, using isoflurane gas. The fecal samples, pharyngeal swabs and blood samples were tested for the presence of microbes in laboratories under detailed microscopic examination. In certain cases X-ray, biopsies and endoscopies were also done for the detailed examination of the feces. The research study was conducted during 1999 to 2003.

Results

The major infectious diseases recorded in Falcons were viral, bacterial, mycoplasmal, chlamidial and fungal diseases. The common internal parasites were protozoans and helminths such as trematodes, cestodes and nematodes. The external parasites included arachnids such as ticks, fowl mites, red mites, quill mites and epidermatid mites and insects such as feather lice, feather flies and blowflies. Some unidentified endoparasites were also recorded during the study. The symptoms of major diseases and effects are given in Table 1. It was found that, the common diseases are affecting both the wild and captive populations (Fig. I). However it may be noted that the wild-bred individuals were under captivity for some period and hence it is likely that the observed disease prevalence is due to captivity.

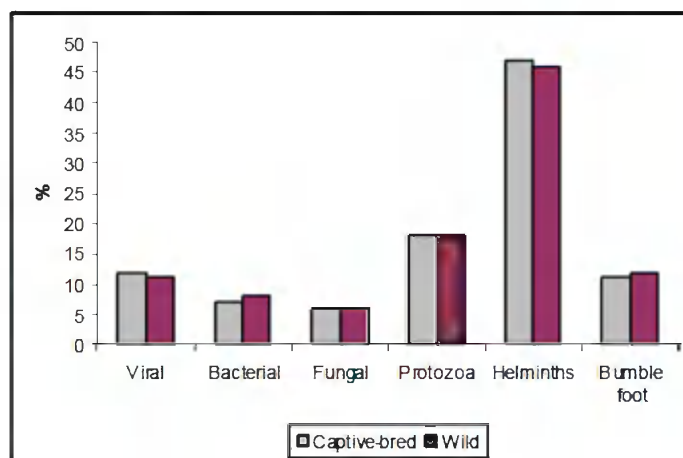


Fig. 1. Percentage of various diseases of the pure-bred and captive Falcons during 1999 to 2003

Table 1. Major diseases affecting Falcons, their symptoms, pathogens and treatment

	Diseases	Pathogen	Symptoms	Medicines	Remarks
Viral diseases	Viral Pox	<i>Avipox falconi</i>	Pinhead sized papules on the unfeathered areas of the skin, feet and eyelids.	No specific treatment. Supportive treatment (broad spectrum, antibacterial and anti fungal therapy).	Vaccination, protection from mosquito bites
	Newcastle disease	<i>Paramyxovirus</i>	Mild neurological signs, gentle tremors, severe ataxia.	No specific treatment, but supported treatment	Prevention by vaccination.
Bacterial diseases	Chlamydiosis	<i>Chlamydia psittaci</i>	Discharge from eyes and nose, diarrhea, weight loss etc.	Antibiotics like Doxycycline, Enrofloxacin effective	Avoid feeding with ducks, turkeys and pigeons affected with the disease.
	Salmonellosis	<i>Salmonella sp.</i>	The Falcons were depressed, dehydrated and had greenish urates.	Enrofloxacin, Tetracycline etc.	Avoid feeding pigeons affected with the disease.
	Mycoplasma	<i>Mycoplasma sp.</i>	Respiratory dysfunction, air sacculitis, pneumonia and tracheitis.	Enrofloxacin, tylosin.	Avoid feeding pigeons affected with the disease.
Fungal diseases	Aspergillosis	<i>Aspergillus fumigatus</i>	Weakness, exercise intolerance and dyspnea with open mouth and abdominal breathing.	Treatment successful if given earlier. Flucytosine, Amphotericin effective.	Optimal nutrition, good management, maintenance of body condition
	Candidiasis	<i>Candidia albicans</i>	Reduced food intake, yellowish white plaque on the oral mucosa.	Broad-spectrum antifungal drugs.	As above
Protozoan	Trichomoniasis	<i>Trichomonas gallinae</i>	Yellowish caseous lesions develop in the oral cavity, and on tongue. And foul necrotic odor.	Carnidazole successful supplementation vitamins.	is and of Maintaining healthy disease resistant birds
	Coccidiosis	<i>Caryospora sp.</i>	Weight loss, lethargic, depressed and changes in fecal consistency.	Clazuril, toltrazuryl are very effective.	Hygienic measures important
Helminthes	Trematodes	<i>Fibres</i>	Severe infection, diarrhea and weakness	Fenbendazole, praziquantel effective	are Rare in captivity
	Cestodes	<i>Tape worms</i>	Diarrhea and weakness.	Praziquantel effective	Rare in captivity
	Nematodes	<i>Capillaria, Serratospeculum sp., Ascarids etc.</i>	Weight loss, depression, yellowish deposits on the mucus of the pharynx etc.	Fenbendazole is very effective.	Serratospeculum may be removed endoscopically.
Bacteria	Bumble foot	Secondary bacterial infection	Inflammation and swelling on foot, advanced case may lead to death.	Therapy dependant on stage of disease	Commonly seen in captive birds

Nutrition deficiencies and metabolic disorders occur in Falcons when they are not provided with balanced diet. Morbidity is also widely seen with sub-optimal environment. Inadequate levels of certain vitamins in the diet can result in metabolic disorders (Table 2). It is believed that falcons have a higher requirement for vitamin A than mammals and are prone to deficiency complications in captivity, which can be managed by supplements. Inadequate levels of calcium and phosphorous caused rickets in growing falcons or osteomalacia in adults.

Providing constant supply of chopped rats prevented rickets in growing individuals in 15 out of 20 cases observed. Boned meat consumption also controlled the development of osteomalacia in adults. Whole animal diet can prevent the deficiency diseases successfully. In the case of administering artificial food supplements such as tablets, they need to be placed in the digestive tract carefully to guide to the stomach. In few occasions during the present study falcons had breathing problems due to choking and required immediate medical attention. Newcastle disease, raptor pox, falcon herpes virus and influenza A virus are common viral diseases.

Table 2. Diseases due to vitamin deficiency in Falcons kept in captivity

Vitamin	Effect	Deficiency
Vit. A	Protection of mucous tissue Resistance against infection	Lesions on beak and talons, Eye problems, Poor hatching, high chick mortality, predisposing factor for visceral gout of respiratory and alimentary tract
Vit. B, B1, B2	Important for nerve system	Biotin; necessary for good skin, feather and skin problems
Vit. C	Wound healing	Level reduced in times of stress like training, transport and Prolonged wound healing
Vit. D, D3	Balance of calcium and phosphorous	Weak bones, rickets
Vit. E, K	Immunity, protective effect on stress, stored in liver, blood-clotting factor.	Low immune system, easily contracts by diseases and bleeding.

New Castle disease caused by virus was fatal to the birds⁵. Raptor pox affects Falcon's activities, but was not fatal. Herpes virus and influenza A virus were also recorded. Chronic superficial keratitis is a viral infection of cornea, rare among falcons, but a case has been reported in Saker falcon for which grid keratectomy was performed. Chlamydiosis, Salmonellosis, Mycoplasma and Avian tuberculosis was

common bacterial disease in falcons⁶. Because mycoplasma is low in virulence and close contact is necessary for horizontal transmission. Avian Tuberculosis was common in captive falcons. Consuming prey especially pigeons infected with bacteria caused these type of diseases. Falcons tested in UAE had Salmonella infection, which was originated from prey animals like mouse, pigeon etc. The common fungal diseases were Aspergillosis and Candidiasis⁷. These were widely recorded in Gyr and Merlin falcons of far northern climates⁸. This is an infection, influenced by environmental hygienic factors and the animal's resistance to infection. Though Aspergillosis can be cured by surgery, success in advanced stage is difficult. Candidiasis affect esophagus and can be easily treated with antifungal drugs.

Falcons are carriers of parasites and diseases caused by them were more or less dangerous and common. Endoparasites include protozoans and helminths. Better hygiene and good management were effective in preventing these diseases⁹. The protozoan diseases occurred in falcons was Trichomoniasis, Coccidiosis and Babesiosis. Trichomoniasis affects Falcons through the infected prey especially pigeons¹⁰. Appropriate quality assurance and ensuring that the prey species were not infected reduced the possibility of infected pigeons transferring trichomoniasis to Falcons. Removing head, neck and internal organs of prey for the falcons was an effective step to control infections. Furthermore pigeon flocks should be medicated with antiprotozoal drugs to reduce the number of protozoan carriers¹¹. Freezing the prey at least for 24 hrs was likely to inactivate the trichomonads and reduce the risk of infection. It was found that more than 50 % of pigeons in UAE are infected with this protozoan.

Coccidiosis is the commonly reported disease all over the world and also in the Gulf¹². Completion of the parasite's life cycle results in destruction of the intestinal cells. It was recorded that the Falcons showing weight loss, lethargy and diarrhoea, were mostly affected by *Coccidia*¹³. Ascarids, *Serratospeculum*, *Capillaria* and Filarial worms were Nematodes seen in falcons. *Serratospeculum seurati* was a common parasite especially during hunting season and widespread in Middle East¹⁶. These species infects the respiratory system. Saker Falcons, which were caught from wild in their countries of origin, were infected with these parasites. Such falcons already have lungworms in their air sacs when they were brought to UAE or other GCC countries. Coccidian eggs were common in falcons caught from wild, while they were less in captive bred individuals indicating that the infection is possible in the field if they consumed the vector beetle, the intermediate host of the parasite¹⁴. Various species of worms belonging to *Filaridae* occur in falcons from tropical climates. Many wild caught birds used for falconry in Arab country carry the parasites. The Falcons were treated for this disease by endoscopic removal of the parasites. Trematodes like flukes residing in small intestine of falcons were causing severe infections.

As mentioned earlier, in captivity, the nutrient deficiency causes metabolic disorders in the Falcons. Vitamin A deficiency and dehydration may lead to gout in Falcons. Organophosphates, carbonates, polychlorinated biphenyles, chlorinated hydrocarbons, which are present in a variety of fungicides; herbicides and insecticides were badly affecting falcons. Lead-induced mortality appears to have been a major factor in the decline of the California Condor¹². There was a significant relationship between lead shot ingestion in falcons and consumption of waterfowl during the hunting season⁵, indicating that waterfowl can be an important cause of lead toxicity in Falcons.

The pressure and temperature of the feet increased at the time of moulting and may cause the bumble foot¹¹. Sometimes the talons may grow and pierce the foot resulting in injury. In captivity the falcons were tied in '*waqr*' and it increased the chance for bumble foot. Poor perches in aviaries are known to cause over 80 % of the bumble foot disease. In 1990s, bumblefoot posed a major health problem to the falcons in the United Arab Emirates¹⁵. Traumatic injury also caused the death of falcons. Raptors with traumatic injuries need emergency stabilization¹⁶. It was reported that 15% incidents of eye diseases in falcons were by accidents and 50% of that was due to traumatic injury, primarily from automobiles and gun shot wounds.

A variety of viral, bacterial and fungal diseases were reported in Falcons from UAE. Many of the diseases were not critical, if precautions and treatment were taken at appropriate time. The common viral diseases, reported in falcons were Newcastle disease, raptor pox, falcon herpesvirus and influenza A virus. The common bacterial diseases recorded were Chlamydiosis, Salmonellosis, Mycoplasma and Avian tuberculosis. Bacterial infections were not serious as viral diseases. Most of the bacterial and viral diseases were transmitted to falcons through their prey. The common fungal diseases were Aspergillosis and Candidiasis. The protozoan diseases occurring in falcons were Trichomoniasis, Coccidiosis and Babesiosis. Ticks, mites, lice, louse flies, blowflies, and feather flies were common Ectoparasites. In captive falcons nutrition deficiencies and metabolic disorders were directly related to the quality of the food and the environment provided¹⁷. Proper management, better hygiene, balanced diet and routine check up will prevent most of the diseases to a certain extent.

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Taxonomic studies on *Hygroplasta meyrick* (Lecithoceridae) with new records to Western Ghats

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Abstract

Two species namely, *Hygroplasta spoliatella* Walker and *Hygroplasta lygaea* Meyrick are reported from the first time from Western Ghats. Diagnosis, nomenclature aspects and distribution are presented in this paper.

Key words: Lecithoceridae, *Hygroplasta*, Western Ghats

Introduction

The family Lecithoceridae has a rectangular forewing compared to that of Gelechiidae, which has oval forewings, the antennae may be longer than the forewing and the abdomen is often spined dorsally. Lecithoceridae usually lack an antennal pecten. They rest with the wings folded flat over the body and parallel to the substrate, often with the antennae held forward.

Materials and Methods

The adults were collected during the night time with the help of portable light traps. Besides this, some specimens were collected at night to an illuminated vertical white sheet. The light source we used was an 18-watt CFL (Compact Fluorescent Lamp) powered by a 12-watt car battery¹. The methodology discussed by workers such as Mikkola², as well as Landry and Landry³, was followed for the pinning, stretching and preservation of specimens. The standard techniques given by Zimmerman⁴, and Robinson *et al.*⁵, have been followed for wings and genitalia respectively. With regard to systematic arrangement of families⁶ classification for Lepidoptera were followed.

Results

In this study, genitalial morphology of two species of the subfamily *Torodorinae* was studied. Keys and descriptions of which are given below:

Super Family Gelechioidea

Vertex and frons decorated with smooth scales; labial palpus 3 segmented and upturned, forewing with veins $R_4 + R_5$ stalked; scaled haustella.

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Key to the genera of the subfamily *Torodorinae*

Forewing upper surface with two black spots, vein R3 absent; male genitalia with vinculum produced anteriorly into a well developed saccus..... *Hygroplasta* Meyrick

Key to the species of the genus *Hygroplasta* Meyrick

1. Alar expanse 23 mm; forewing with black spot rounded, and a small spot present in discal cell; male genitalia with valvae broad, saccus somewhat long, aedeagus straight and long, bent at apex***spoliatella* Walker**
- Alar expanse 17-22 mm; dorsal surface of forewing with discocellular spot more prominent, discal cell with spot rather prominent; male genitalia with valvae small, aedeagus small **2**
2. Male genitalia with saccus small, broad distally, saccus margin concave medially, aedeagus short; Female genitalia with ductus bursae open near middle of the corpus bursae, signum spindle shaped***lygaea* Meyrick**

***Hygroplasta spoliatella* (Walker)**

Hygroplasta spoliatella (Walker), *Gelechia spoliatella* Walker, 1864, *List Specimens lepid. Insects Colln Br. Mus.*, 29: 659.

Collection data: Amarambalam; October, 2001 (2 ex.).

Distribution: Himachal Pradesh from India.

Host: Unknown.

Alar expanse: 23 mm.

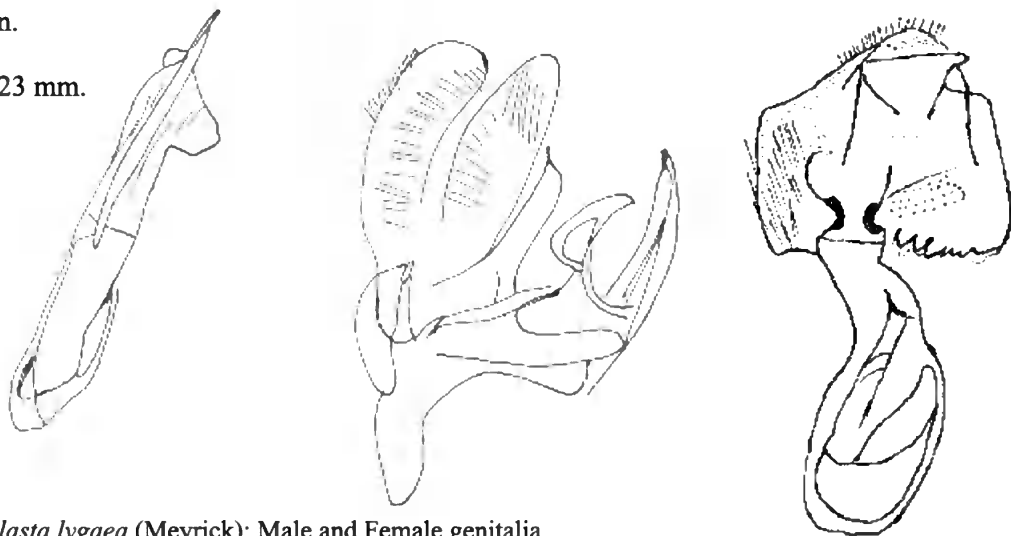


Fig. 1. *Hygroplasta lygaea* (Meyrick): Male and Female genitalia

Male genitalia: Uncus broader basally, pointed at the apex, sparsely setose dorsally; socii absent; gnathos small, somewhat hook-like, weakly sclerotized; tegumen short, weakly sclerotized; vinculum V-shaped, poorly sclerotized; saccus moderately long and broad, apex rounded; juxta large and broad, moderately sclerotized; valvae symmetrical, small, broad in the middle, costal margin narrowed at the base, strongly convex medially; sacculus convex at the base, narrowed, almost straight medially and apically, cucullus concave dorso-distally, pointed apically, weakly sclerotized and densely setose; aedeagus long, broad at the base, apex slightly curved and pointed, relatively less sclerotized; vesica without cornutus (Fig. 1).

Remarks: *Hygroplasta spoliatella* (Walker) is being reported for the first time from South India in view of its earlier distribution mentioned above⁷.

***Hygroplasta lygaea* (Meyrick)**

Hygroplasta lygaea (Meyrick), *Pachnistis lygaea* Meyrick, 1911, *Journ. Bombay. Nat. Hist. Soc.* 20: 707.

Collection data: Peechi; April, 2002 (2 ex.).

Distribution: Dalhousi, Kashmir, Himachal Pradesh, Uttaranchal.

Host: Unknown.

Alar expanse: 24 mm.

Male genitalia: Uncus moderately long; broad at the base, narrowed distally, apex rounded, moderately sclerotized; gnathos large; tegumen small, hood-like; vinculum V-shaped; juxta slightly broader; saccus small; valvae symmetrical, moderately sclerotized, sparsely setosed, costal margin broad at the base, strongly concave medially; aedeagus small (Fig. 2).

Female genitalia: Ovipositor small and rounded; posterior apophyses longer than the anterior apophyses; ostium bursae large, ductus bursae long, slightly curved and narrowed at the middle; signum large and spindle-shaped.

Remarks: Meyrick⁸ has reported the species *lygaea* under the genus *Pachnistis* Meyrick and the localities reported by him for the collection of this lone specimen include Dalhousie and Kashmir in North-West India, which



Fig. 2. *Hygroplasta spoliatella* (Walker): Male genitalia

seems to be apparently wrong. The present survey shows that this species being reported for the first time from South India in view of its earlier distribution mentioned above⁷.

Discussion

According to Park⁹, the taxonomic status of the family Lecithoceridae has not been well defined, due to various differing opinions as to its rank. Marchand¹⁰ proposed the subfamily Lecithocerinae with *Lecithocera* Herrich-Schäffer as the type-genus, which was placed under the family Gelechiidae by Janse¹¹. In a recent publication, Park⁹ has stated, there is no doubt that it should be considered as a family rank by such autapomorphic characters, as antenna being longer than forewing length (except Oditinae established by Lovovsky, 1996) and gnathos always bent downwardly. Meyrick¹² proposed the genus *Hygroplasta* with *Gelechia spoliatella* Walker as its type-species kept the same in the family Gelechiidae. However, Clarke¹³ transferred the genus from the latter family to the family Lecithoceridae, under which it is being dealt with presently. It has been observed that different individuals of this genus complex may have one or two black spots on the wings, which may be conspicuous, faint or even obscure.

Acknowledgements

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Evidence of Oceania-native frigate bird *Frigata minor* as a straggler at the Calicut University campus

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Abstract

Frigate birds are ocean birds. The Frigate bird belongs to the *Frigata minor* species found in the Pacific and Indian Ocean. The Frigate bird, weighing nearly 2kg, found trapped in a bush might have been affected by the strong winds that lashed the region. It is supposed that the bird landed on the campus having lost its way during a migratory flight.

Keywords: Ocean bird, gular pouches, *Frigata minor*

This is to report the above Frigate Bird *Frigata minor* as an accidental straggler from its original location on the Oceanic (West Indies area) at the Calicut University campus. The Frigate bird, weighing nearly 2 kg¹, found trapped in a bush might have been affected by the strong winds that lashed the region. It is supposed that the bird landed on the campus having lost its way during a migratory flight. The Great Frigatebird (*Fregata minor*) is a large dispersive seabird in the frigate bird family.

Frigate birds are ocean birds. The Frigate bird belongs to the *Frigata minor* species found in the Pacific and Indian Ocean. Frigate birds are also found in the South Atlantic. They attack other sea birds, and hence the name frigate. Related to the pelicans, frigate birds are also called man of war birds or pirate birds. Also called Iwa (meaning thief in Hawaiian language), these birds are infamous for stealing the food of other sea birds. They depend on the sea for their survival, eating a diet of mostly fish, squid and sea turtle hatchlings.

Frigate birds found over tropical surface, do not swim or walk, and cannot take off from a flat surface. Having the largest wingspan to body weight ratio among birds, they are essentially aerial, able to stay aloft for more than a week, landing only to roost or breed on trees or cliffs. They also hold the flight speed record, diving at up to 400 kmph. They lay one or two white eggs. The bird could rotate its head by more than 180m degrees. "It is a wonderful bird capable of turning your imagination on".

As members of Pelecaniformes, frigate birds have the key characteristics of all four toes being connected by the web, a gular sac (also called gular skin), and a furcula that is fused to the breastbone. Although there is definitely a web on the frigate bird foot, the webbing is reduced and part of each toe is free. Frigate birds produce very little oil and therefore do not land in the ocean. The gular sac is used as part of a courtship display and is, perhaps, the most striking frigate bird feature.

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Both parents take turns feeding for the first three months but then only the mother feeds the young for another eight months. It takes so long to rear a chick that frigate birds cannot breed every year. It is typical to see juveniles as big as their parents waiting to be fed. When they sit waiting for endless hours in the hot sun, they assume an energy-efficient posture in which their head hangs down, and they sit so still that they seem dead. But when the parent returns, they will wake up, bob their head, and scream until the parent opens its mouth. The hungry juvenile plunges its head down the parent's throat and feeds at last. Frigate birds' feeding habits are pelagic. Lacking the ability to take off from water, they snatch prey from the ocean surface or beach using their long, hooked bills. Frigate birds will rob other seabirds such as boobies, tropicbirds, and shearwaters of their catch, using their speed and maneuverability to outrun and harass their victims until they regurgitate their stomach contents.



Fig.1. *Frigate Bird, Frigata minor*

Frigate birds are large², with iridescent black feathers (the females have a white underbelly), with long wings (male wingspan can reach 2.3 metres) and deeply-forked tails. The males have inflatable red-coloured throat pouches called "gular pouches", which they inflate to attract females during the mating season. Frigate birds are found over tropical oceans and ride warm updrafts. Therefore, they can often be spotted riding weather fronts and can signal changing weather patterns. The bird, the stretched wing of which measures two metres from tip to tip, was found trapped near a house. The bird had no injuries. We started examining the bird by feeding it within different kinds of fish and made a gut analysis by examining its faeces. It was the first time a frigate bird had appeared in the region.

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