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Biochemical Parameters  
Analysis

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Chronic Psychological Stress  
Mediated

Natural Distribution of Pistius  
Algae



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IMAGE: OBSERVATORY WITH STAR TRAILS ON MOUNTAINS FOR CLEAR SKY

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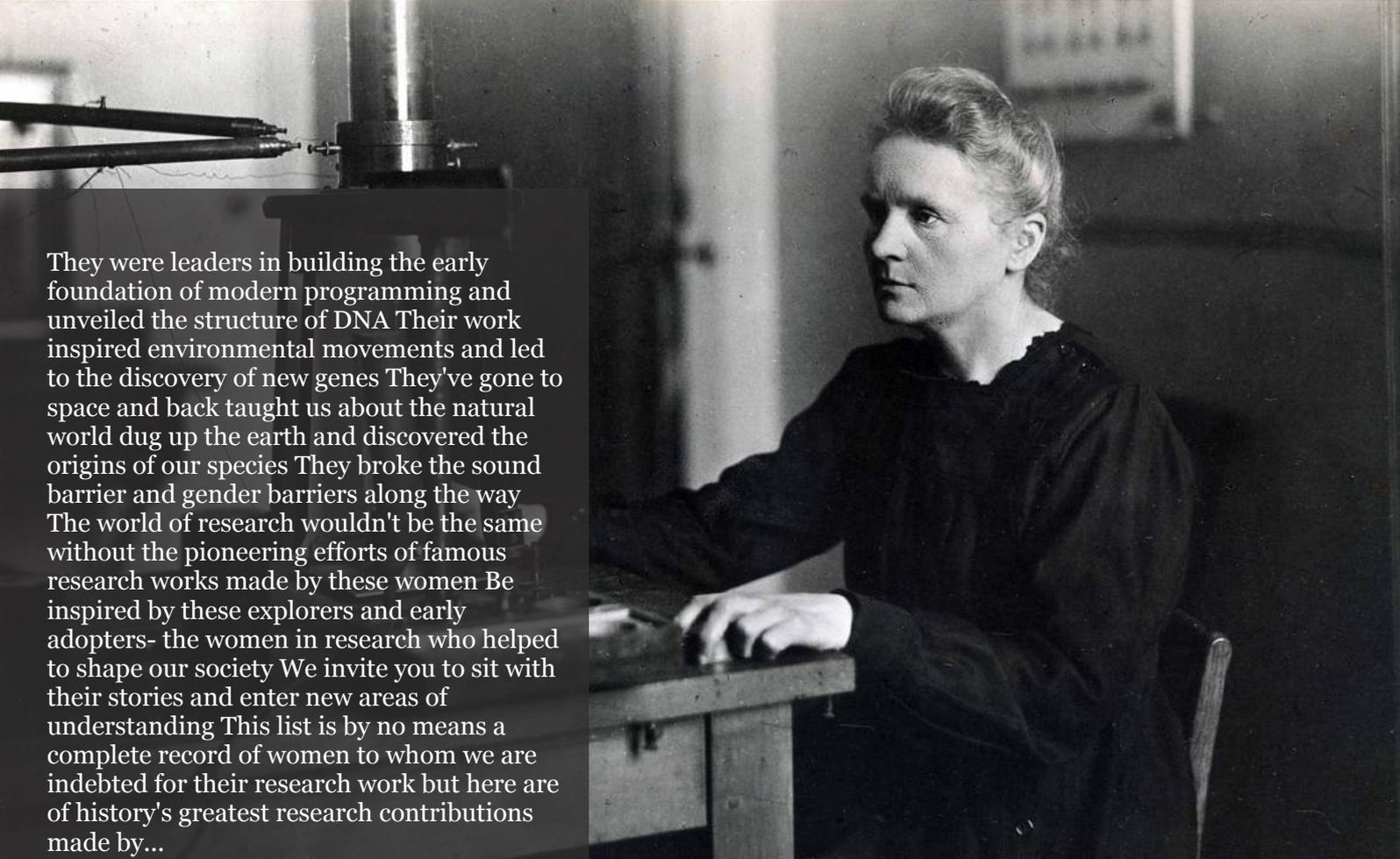
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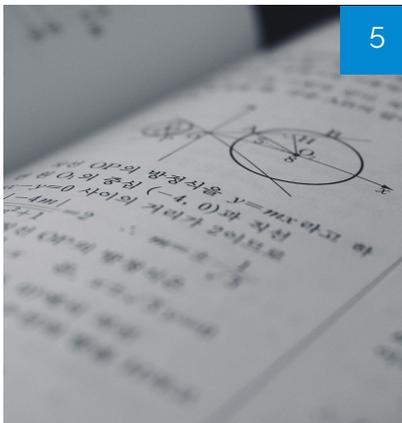


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# The Underlying Vectors and Surfaces with the Same Timescales as Causes of Order Emergence in the Turbulent Flows

*A. Aptsiauri & G. Aptsiauri*

*Georgian Aviation University*

## ABSTRACT

The paper shows that vectors of the integrated mass and energy flow set the surfaces, on which the oscillatory processes have the same timescales or frequencies. Consequently, there is introduced the concept of synchronous surfaces. The correlation vector of density and velocity lies on the same surface, which indicates that chaotic motions are conceived and relatively steadily exist on such surfaces. The partially ordered (coherent) structures may also be conceived on the similar surfaces.

*Keywords:* turbulence, synchronous surfaces, frequency preservation, time scale preservation.

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# The Underlying Vectors and Surfaces with the Same Timescales as Causes of Order Emergence in the Turbulent Flows

A. Aptsiauri<sup>α</sup> & G. Aptsiauri<sup>σ</sup>

## ABSTRACT

*The paper shows that vectors of the integrated mass and energy flow set the surfaces, on which the oscillatory processes have the same timescales or frequencies. Consequently, there is introduced the concept of synchronous surfaces. The correlation vector of density and velocity lies on the same surface, which indicates that chaotic motions are conceived and relatively steadily exist on such surfaces. The partially ordered (coherent) structures may also be conceived on the similar surfaces.*

**Keywords:** turbulence, synchronous surfaces, frequency preservation, time scale preservation.

**Author α:** Professor, Kutaisi technological academy, Gaponov str., 11, Kutaisi, 4600.

**σ:** PhD, Georgian Aviation University, K. Tsamebuli str., 16, Tbilisi, 0144, Georgia.

## I. OBTAINING THE DIFFERENTIAL EQUATIONS OF CONSERVATION BY INTRODUCING THE INTEGRATED FLOWS.

With the known values of the flow thermodynamic parameters (pressure, temperature, density) and rate ( $W$ ), at an arbitrary point in space, the instantaneous values of the mass and energy flow vectors shall be set:

$$g = \rho W, \quad (1.1)$$

$$e_{\Sigma} = \rho W [c_v T + P / \rho] + \rho W W^2 / 2 - W \sigma(W) - \lambda \text{grad} T = e_i + e_k + e_{\mu} + q \quad (1.2)$$

where

$e_i$  - the instantaneous enthalpy flow;

$e_k$  - the instantaneous kinetic energy flow;

$e_{\mu}$  - the instantaneous flow of energy of viscose surface force;

$q$  - the instantaneous heat flow.

$\sigma(W)$  - viscous stress tensor.

By integrating (1.1-1.2) during a time interval  $\tau_0$ , it is possible to determine the values of the integrated flows or the averaged vectors of mass and energy:

$$\overline{g}_{\tau} = \frac{1}{\tau_0} \int_0^{\tau_0} \rho W dt, \quad (1.3)$$

$$\overline{e}_{\Sigma\tau} = \frac{1}{\tau_0} \int_0^{\tau_0} e_{\Sigma} dt. \quad (1.4)$$

If we have stationary turbulence, then, after the completion of one cycle, all flow parameters should take their initial value and, accordingly, the condition of conservation of the integrated (or averaged) fluxes must be met, which has the following form:

$$\overline{\text{div} g}_{\tau} = 0, \quad (1.5)$$

$$\overline{\text{div} e}_{\Sigma\tau} = 0. \quad (1.6)$$

Thus, on the basis of the condition of conservation of the integrated flows, we obtain the averaged differential conservation equations in the form of 1.5–1.6. At the same time, the averaged conservation equations are also obtained by direct

integration of differential equations of a continuous medium.

## II. OBTAINING THE AVERAGED DIFFERENTIAL EQUATIONS OF CONSERVATION BY INTEGRATING THE DIFFERENTIAL EQUATIONS OF MASS AND ENERGY CONSERVATION.

Consider the differential equations of mass and energy conservation:

$$\partial\rho/\partial t + \text{div}\rho W = 0, \quad (2.1)$$

$$\partial[\rho c_v T + \rho W^2 / 2] / \partial t + \text{div}[e_\Sigma] = 0. \quad (2.2)$$

If we integrate these equations within a cyclic process, under conditions of variables over the space of timescales

$$(A = -\text{grad} \ln \tau_0 = \text{grad} \ln f \neq 0), \quad \text{we}$$

obtain the different equations:

$$\text{div} \bar{g}_\tau = \bar{g}_\tau A, \quad (2.3)$$

$$\text{div} \bar{e}_{\Sigma\tau} = \bar{e}_{\Sigma\tau} A.$$

A comparison of these equations with equations 1.5-1.6. leads to the conclusion:

$$\bar{g}_\tau A = 0, \quad (2.5)$$

$$\bar{e}_{\Sigma\tau} A = 0 \quad (2.6)$$

As will be seen later, the equations obtained can play an important role in the search for a closed equation system for solving the problem of turbulence.

If we determine the instantaneous values of density and velocity as the sum of the average value and pulsation ( $\rho = \bar{\rho} + \rho', W = V + w$ ), then the averaged value of the mass flux vector will be as follows:

$$\bar{g}_\tau = \bar{\rho} V + \frac{1}{\tau_0} \int_0^{\tau_0} \rho' w dt, \quad (2.7)$$

Or:

$$\bar{g}_\tau = \bar{\rho} V + \bar{\rho} A_\rho, \quad (2.8)$$

where  $A_\rho$  expresses some vectoral value, which reflects mass propagation due to the velocity pulsation - or the velocity of the turbulent mass propagation.

$$A_\rho = \frac{1}{\rho \tau_0} \int_0^{\tau_0} \rho' w = \frac{\overline{\rho' w}}{\rho}, \quad (2.9)$$

As we can see, if there is a correlation between the velocity and density pulsations, then the mass flux depends not only on the average velocity vector -  $V$ , but also on the velocity vector of the turbulent mass diffusion -  $A_\rho$ . In the incompressible fluid flows, this term is understandably eliminated, but, as will be seen later, in the turbulent compressible fluid flows, this quantity has a clearly defined value.

The orthogonality of the velocity vector and timescale gradient ( $AV = 0$ )

Consider a differential equation of continuity in another form:

$$\rho \text{div} W + W \text{grad} \rho = -\partial \rho / \partial t, \quad (3.1)$$

Imagine the velocity and density in the form of the sum of the average value and pulsation and implement the time integration within a cycle:

$$\overline{\rho \text{div}(V+w) + \rho' \text{div} w + \rho' \text{div} V + V \text{grad} \rho + w \text{grad} \rho} = 0, \quad (3.2)$$

We shall take into account that, according to the rules of integration, at  $A \neq 0$ , the following conditions are correct:

$$\overline{\text{div}(V+w)} = \text{div} V - AV, \quad (3.3)$$

$$\overline{\rho' \text{div} V} = \overline{\rho' \text{div} V} = 0, \quad (3.4)$$

$$\overline{w \text{grad} \rho} = \overline{w \text{grad}(\bar{\rho} + \rho')} = \overline{w \text{grad} \rho'}. \quad (3.5)$$

Respectively, (3.2) will be as follows:

$$\overline{\rho \text{div} V} - \overline{\rho} AV + \overline{V \text{grad} \rho} + \overline{\rho' \text{div} w} + \overline{w \text{grad} \rho'} = 0 \quad (3.6)$$

or:

$$\overline{\rho} \operatorname{div} V + V \operatorname{grad} \overline{\rho} - \overline{\rho} AV + \operatorname{div} \overline{\rho'w} = 0 . \quad (3.7) \quad VA = 0 , \quad (4.1)$$

We shall take into account that:

$$A_\rho A = 0 , \quad (4.2)$$

$$V \operatorname{grad} \overline{\rho} = V \operatorname{grad} \overline{\rho} - \overline{\rho} AV , \quad (3.8) \quad \overline{e}_{\Sigma\tau} A = 0 , \quad (4.3)$$

$$\operatorname{div} \overline{\rho'w} = \operatorname{div} \overline{\rho'w} - \overline{\rho'w} A . \quad (3.9)$$

Respectively, (3.7) yields:

$$\operatorname{div} \overline{\rho} V + \operatorname{div} \overline{\rho'w} - \overline{\rho'w} A - 2 \overline{\rho} AV = 0 , \quad (3.10)$$

Applying the definition of the averaged mass flow vector 2.8, the latter expression yields:

$$\operatorname{div} \overline{g}_\tau - \overline{g}_\tau A - \overline{\rho} AV = 0 , \quad (3.11)$$

And with provision for 2.3 - 2.5, we obtain:

$$AV = 0 , \quad (3.12)$$

or

$$V \operatorname{grad} \tau_0 = \frac{d\tau_0}{dt} = 0 , \quad (3.13)$$

Thus, on the basis of a fundamentally different approach, a more general result was obtained, than it was shown when analyzing one-dimensional pulsating flows [2], which indicates that the timescale (or main frequency) along the jet remains unchanged.

Taking into account the condition (2.3), we obtain that the correlation vector  $A_\rho$  is also oriented perpendicular to the frequency gradient:

$$\overline{\rho'w} A = 0 , \quad (3.14)$$

### III. THE SURFACES OF THE SAME TIMESCALES OR THE SYNCHRONOUS SURFACES, SET BY THE DIRECTIONS OF PROPAGATING THE PRINCIPLE SUBSTANCES - PRIORITY AREAS OF TURBULENT MOTIONS

Thus, we have obtained the equations indicating that the timescale gradients are orthogonal to three vectors simultaneously.

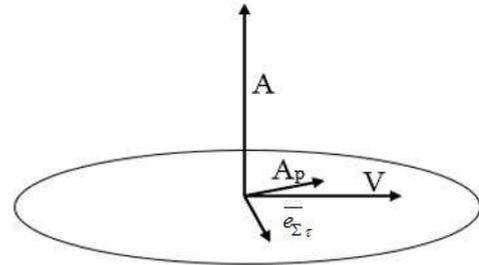


Fig. 1: Average velocity  $V$ , vector of the cumulative flow of energy  $\overline{e}_{\Sigma\tau}$  and correlation  $A_\rho$  on a plane orthogonal to the timescale gradients  $A$ .

The conditions (4.1-4.3) can be met in the following different cases:

A.  $A = 0$  - no timescale gradients. The presence of this condition in a compressible medium can be considered to be the unlikely special case.

B. The vectors  $V$ ,  $A_\rho$ ,  $\overline{e}_{\Sigma\tau}$  are arranged on the same plane (Fig. 1). Naturally, the vector can be perpendicular to three vectors, simultaneously, only if these vectors are arranged on the same plane. This situation can be considered to be the most common characteristic of the compressible media.

As we can see, vectors of the mass and energy flows set such surfaces, on which the timescales (or the frequencies) are the same. Therefore, they can be called the synchronous surfaces. Obviously, on the surfaces of synchronous oscillations, we should expect synchronization of processes. Consequently, more ordered motions or the turbulent structures with the relatively large scales (and, accordingly, the large timescales) may be conceived on such surfaces. In the theory of turbulence, such

structures are called coherent, and the turbulence itself is considered to be a mixture of chaos and order, in which, against the background of regular disordered motions, periodically, spontaneously or regularly, there are appeared the ordered structures, the development of which, to some extent, has an autonomous character.

Consequently, vectors of the mass and energy flows set the special surfaces, which determine the priority directions of motion with identical frequencies, which, in turn, contributes to the appearance of order in the depth of chaotic motion.

The fact that the correlation vector  $A_\rho$  lies also on this plane, once again indicates that turbulent oscillations occur mostly in this plane. Moreover, this feature allows for determining the dependence of  $A_\rho$  on  $V$  and  $\overline{e_{\Sigma\tau}}$ , what will be shown later.

Analysis of stress tensors, which depend on 6 scalar values, shows that for their mathematical description it is enough to introduce two base vectors (or major axes), which, in the stationary turbulent flows, also set some stable reference surfaces. As is known, in the system of such axes, some components of tensor take a zero value, and this fact also indicates that the structure of turbulence is always associated with anisotropy due to the presence of the priority directions and elements of order in a chaotic process.

Based on the above, it can be said that not only the ordered coherent structures, but also the smallest turbulent vortices, are conceived on the certain surfaces in a partially ordered form, and the deviation from such a pattern can be explained only by the fact that the shape and orientation of the synchronous surfaces can vary not only in space, but also in time, since the stationary turbulent processes, do not exist in their pure

form, and, due to variation of  $V$  and  $\overline{e_{\Sigma\tau}}$  not only in space, but also in time (in the strict sense), a stable process of conceiving of the vortex structures, with the same spatial orientation, at a fixed point of space is obviously difficult to be observed.

The issues of representation of the turbulent stress tensor based on the underlying vectors will be considered in a separate section.

#### IV. CONCLUSION

The vectors of the integrated mass and energy flow set the surfaces directed orthogonally to the timescale gradients, on which the oscillatory processes have the same timescales or frequencies - the synchronous surfaces. The correlation vector of density and velocity lies on the same surface, which indicates that chaotic motions are conceived and relatively steadily exist on such surfaces. The partially ordered (coherent) structures may also be conceived on the similar surfaces. Similarly to one-dimensional pulsating streams, in more complex flows timescale (or main frequency) along the jet remains unchanged.

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# Об одной задаче изгиба пластинки в случае прямоугольника, ослабленного отверстием и вырезками в вершинах

*Г. А. Капанадзе & Л. А. Гоголаури*

## ABSTRACT

Рассматривается задача отыскания равнопрочного контура теории изгиба пластинки для прямоугольника, ослабленного отверстием и вырезками в вершинах (неизвестная часть границы) при условии, что на каждом линейном отрезке границы прикреплена жесткая планка и пластинка изгибается нормальными моментами, приложенными к планкам таким образом, что углы поворота средней поверхности пластинки принимают кусочно-постоянные значения, а неизвестная часть границы свободна от внешних усилий. Условие равнопрочности искомого контура (совокупность границ отверстий и вырезов) заключается в том, что действующий на него тангенциальный нормальный момент принимает постоянное значение.

**Ключевые слова:** Задача изгиба пластинки; Конформное отображение; Задачи Римана-Гильберта и Келдыша Седова; Эллиптические интегралы.

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Г. А. Капанадзе<sup>α</sup> & Л. А. Гоголаури<sup>σ</sup>

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Рассматривается задача отыскания равнопрочного контура теории изгиба пластинки для прямоугольника, ослабленного отверстием и вырезками в вершинах (неизвестная часть границы) при условии, что на каждом линейном отрезке границы прикреплена жесткая планка и пластинка изгибается нормальными моментами, приложенными к планкам таким образом, что углы поворота средней поверхности пластинки принимают кусочно-постоянные значения, а неизвестная часть границы свободна от внешних усилий. Условие равнопрочности искомого контура (совокупность границ отверстий и вырезов) заключается в том, что действующий на него тангенциальный нормальный момент принимает постоянное значение.

Методами комплексного анализа, комплексные потенциалы выражающие прогиб средней поверхности пластинки и уравнение искомого равнопрочного контура построены эффективно (в аналитическом виде). Приведен анализ упомянутых результатов в случае квадрата.

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Ключевые слова: Задача изгиба пластинки; Конформное отображение; Задачи Римана-Гильберта и Келдыша-Седова; Эллиптические интегралы.

*Author α:* A. Razmadze Mathematical Institute of Iv. Javakhishvili, Tbilisi State University 6, Tamarashvili Str. Tbilisi 0186 Georgia I.Vekua Institute of Applied Mathematics of Iv. Javakhishvili Tbilisi State University, 2, University str. Tbilisi 0186, Georgia.

*σ:* A. Razmadze Mathematical Institute of Iv. Javakhishvili, Tbilisi State University 6, Tamarashvili Str. Tbilisi 0186 Georgia.

Введение. Задачи отыскания равнопрочного контура плоской теории упругости и изгиба пластинки можно отнести к обширному классу задач оптимизации форм упругих тел (см. [1]) и они всегда были в центре внимания многих ученых. Среди методов, разработанных для изучения этих задач одно из важных мест занимают методы комплексного анализа (методы теории граничных задач аналитических функций и конформного отображения). Этим подходом упомянутые задачи рассмотрены в работах [2-10].

*Постановка задачи.* Пусть срединная поверхность упругой изотропной пластинки на плоскости  $Z$  комплексной переменной занимает двухсвязную область  $S_0$ , внешняя граница которой представляет прямоугольник с вырезками в вершинах, а внутренняя граница – гладкий замкнутый контур (фиг. 1). Предположим, что на каждом линейном участке внешней границы прикреплена жесткая планка и пластинка изгибается нормальными моментами приложенными к планкам таким образом, что углы поворота средней поверхности пластинки принимают кусочно постоянные значения, перерезывающая сила равна нулю, а неизвестная часть границы (искомый равнопрочный контур) свободна от внешних усилий. Рассмотрим симметричный случай и будем считать, что на каждом отрезке внешней границы заданы значения главного нормального изгибающего момента.

Рассмотрим задачу: найти прогиб  $w(x, y)$  средней поверхности пластинки и аналитическую форму неизвестной части границы при условии, что действующий на неё тангенциальный нормальный момент принимает постоянное значение  $M_s(t) = K_0 = const$ .

**Решение задачи.** В силу симметрии, мы можем рассмотреть упругое равновесие заштрихованной части  $S$  области  $S_0$  (фиг. 1), граница которой состоит из прямолинейных отрезков  $L_1 = \cup L_1^{(j)} (L_1^{(j)} = A_j A_{j+1}, j = 1, 2, 4, 5)$  и из дуги  $L_0 = L_0^{(1)} \cup L_0^{(2)} (L_0^{(1)} = \overset{\cup}{A_3 A_4}; L_0^{(2)} = \overset{\cup}{A_6 A_1})$ .

Согласно приближенной теории изгиба пластинки прогиб  $w(x, y)$  средней поверхности в рассатриваемом случае удовлетворяет уравнению

$$\Delta^2 w(x; y) = 0, \quad z = x + iy \in S \tag{1}$$

и граничным условиям

$$\begin{aligned} M_n(t) &= f(t) \left( N t \frac{\partial w}{\partial n} = t(\chi) \right), \quad (\chi) = 0, \quad \in L_1, \\ M_n(t) &= 0; \quad M_{ns}(t) = 0, \quad M_s(t) = K_0, \quad N(t) = 0, \quad t \in L_0, \end{aligned} \tag{2}$$

где  $f(t) = f_k = const (k = 1, 2, 4, 5)$  - главные нормально изгибающие моменты;  $d(t) = d_k = tg \gamma_k$  ( $\gamma_k$  - углы поворота),  $t \in L_1$ ;  $N(t)$  - перерезывающая сила;  $M_{ns}(t)$  - крутящий момент;  $M_s(t)$  - тангенциальный нормальный момент.

Отметим, что в классических постановках задач изгиба пластинки, на свободной части границы

$$M_n(t) = 0; \quad N(t) + \frac{\partial H_{ns}(t)}{\partial s} = 0$$

обычно имеем два условия: , но в случае равнопрочного контура, который характеризуется условием  $M_s(t) = K_0 = const$ , как это увидим ниже, условие  $N(t) = 0$  выполняется автоматически, и таким образом, на  $L_0$  остаются два условия  $M_n(t) = H_{ns}(t) = 0$ , а  $M_s(t)$  - принадлежит к внутренним силовым факторам.

На основании известных формул (см. [12]-[14]) имеем

$$\begin{aligned} \frac{\partial w}{\partial n} + i \frac{\partial w}{\partial s} &= e^{-i\alpha(t)} \left[ \varphi(t) + t \overline{\varphi'(t)} + \overline{\psi(t)} \right], \\ (\sigma - 1) d \left[ \chi \varphi(t) - t \overline{\varphi'(t)} - \overline{\psi(t)} \right] &= \left[ M_n(t) + i \int_0^s N(t) ds \right] dt, \\ M_n + M_s &= -8D_0(1 + \sigma) \operatorname{Re}[\varphi'(t)], \end{aligned} \tag{3}$$

где  $\alpha(t)$  - угол между осью  $ox$  и внешней нормалью границы в точке  $t \in L_1$ .  $D_0$  - цилиндрическая жесткость пластинки ( $D_0 = Eh^3[12(1 - \sigma^2)]^{-1}$ ),  $\sigma$  - коэффициент Пуассона,  $\chi = (\sigma + 3)(1 - \sigma)^{-1}$ .

На основании (2) и (3), относительно искомым функций  $\varphi(z)$  и  $\psi(z)$  получим граничные условия

$$M \quad \operatorname{Re} \left[ e^{-i\alpha(t)} (\varphi(t) + t \overline{\varphi'(t)} + \overline{\psi(t)}) \right] = d(t), \quad t \in L_1; \tag{4}$$

$$N \quad \operatorname{Re} \left[ e^{-i\alpha(t)} (\chi \varphi(t) - t \overline{\varphi'(t)} - \overline{\psi(t)}) \right] = c(t), \quad t \in L_1; \tag{5}$$

$$\chi\varphi(t) - t\overline{\varphi'(t)} - \overline{\psi(t)} = 0, \quad t \in L_0; \quad (6)$$

$$\operatorname{Re}[\varphi'(t)] = \frac{K}{4}, \quad t \in L_0, \quad (7)$$

где

$$c(t) = c_k = \sum_{j=1}^k M_j \sin(\alpha_k - \alpha_j), \quad t \in L_1^{(k)}, \quad M_j = [2D_0(\sigma - 1)]^{-1} \int_{L_1^{(j)}} M_n(t) ds,$$

$$K = -K_0 [8D_0(1 + \sigma)]^{-1}, \quad (k, j = 1, 2, 4, 5).$$

В дальнейшем от искомым функций  $\varphi(z)$  и  $\psi(z)$  потребуем, чтобы  $\varphi(z)$  была непрерывна в замкнутой области  $S + L$ , а  $\varphi'(z)$  и  $\psi(z)$  были непрерывно продолжимы на границе области  $S$  всюду, за исключением быть может вершин  $A_3$  и  $A_4$ , в окрестности которых выполняется условие

$$|\varphi'(z)|, |\psi(z)| < N |z - A_k|^{-\delta_k}, \quad N = \text{const}, \quad 0 \leq \delta_k \leq \frac{1}{2}, \quad k = 3, 4. \quad (8)$$

Сложением равенств (4) и (5) и затем дифференцированием по дуговой абсциссе  $s$ , учитывая, что функции  $d(t)$  и  $c(t)$  кусочно постоянные, получим

$$\operatorname{Im}\varphi'(t) = 0, \quad t \in L_1. \quad (9)$$

Отображением области  $S$  на единичный круг  $|\eta| < 1$  и введением функции  $\Phi_0(\eta) = \varphi[\omega_0(\eta)] - \frac{K}{4} (z_0 = \omega_0(\eta))$  - конформно-отображающая функция), граничные условия (7) и (9) запишутся в виде однородной задачи Римана-Гильберта (см. [12], [15]).

$$\operatorname{Re}[\Phi_0(\sigma)] = 0, \quad \sigma \in l_0^{(0)}; \quad \operatorname{Im}[\Phi_0(\sigma)] = 0, \quad \sigma \in l_0^{(1)}, \quad (10)$$

где  $l_0^{(0)}$  и  $l_0^{(1)}$  - части окружности  $l_0 = \{|\eta| = 1\}$  соответствующие контурам  $L_0$  и  $L_1$  соответственно. Задача (10) при условии (8) имеет только тривиальное решение, и, таким образом, имеем

$$\varphi(z) = \frac{K}{4} z. \quad (11)$$

Отметим, что линейность комплексного потенциала  $\varphi(z)$  является необходимым условием существования равнопрочного контура. Кроме того, в силу линейности функции  $\varphi(z)$ , заключаем, что перерезывающая сила  $N(z) = 0$  во всей области, и, таким образом, условие  $N(t) = 0, t \in L_0$  выполняется автоматически (см. сказанное выше).

Пусть функция  $z = \omega(\zeta)$  конформно отображает верхнюю полуплоскость ( $\operatorname{Im}\zeta > 0$ ) на область  $S$ . Обозначим через  $a_k$  прообразы точек  $A_k (k = \overline{1, 6})$  и будем считать, что  $a_1 = 1; a_6 = -1; \zeta_0 = \infty$  ( $A_0 = \omega(\zeta_0)$  - серединная точка дуги  $A_3 A_4$  (см. фиг. 1)).

Введением функций

$$\Phi_1(\zeta) = p\omega(\zeta) + \psi(\zeta) - iM_1; \quad \Phi_2(\zeta) = i[p\omega(\zeta) - \psi(\zeta) - M_2], \quad \left( p = \frac{K}{4}(\chi - 1) \right), \quad (12)$$

граничные условия (5) и (6) с учетом (11) приводятся к граничным задачам Келдыша-Седова для полуплоскости  $\text{Im } \zeta > 0$  (см. [12], [15])

$$\begin{aligned} \text{Im } \Phi_1(t) &= 0, \quad t \in (-\infty; a_5] \cup [a_3; \infty); \\ \text{Re } \Phi_1(t) &= 0, \quad t \in [a_5; -1]; \\ \text{Im } \Phi_1(t) &= -M_1, \quad t \in [-1; a_2]; \\ \text{Re } \Phi_1(t) &= -M_2 + 2pa, \quad t \in [a_2; a_3]. \end{aligned} \tag{13}$$

$$\begin{aligned} \text{Im } \Phi_2(t) &= 0, \quad t \in (-\infty; a_4] \cup [a_2; \infty); \\ \text{Re } \Phi_2(t) &= M_1 - 2pb, \quad t \in [a_4; a_5]; \\ \text{Im } \Phi_2(t) &= -M_2, \quad t \in [a_5; 1]; \\ \text{Re } \Phi_2(t) &= 0, \quad t \in [1; a_2], \end{aligned} \tag{14}$$

и

где  $2a$  и  $2b$  - длины сторон прямоугольника (см. фиг. 1).

Будем искать ограниченные на бесконечности решения задачи (13) и (14) класса  $h(a_4; a_5; -1; 1; a_2; a_3)$  (об этом классе см. [12]) удовлетворяющие условию

$$\Phi_j(\zeta) = \overline{\Phi_j(\bar{\zeta})}, \quad (j=1, 2). \tag{15}$$

Индексы упомянутых задач данного класса равны  $(-2)$ .

Необходимые и достаточные условия существования ограниченных на бесконечности решений задачи (13) и (14) упомянутого класса имеют соответственно вид (см. [11], [12])

$$-iM_1 \int_{-1}^{a_2} \frac{d\tau}{\chi_1(\tau)} + (-M_2 + 2pa) \int_{a_2}^{a_3} \frac{d\tau}{\chi_1(\tau)} = 0, \tag{16}$$

$$(M_1 - 2pb) \int_{a_4}^{a_5} \frac{d\tau}{\chi_2(\tau)} - iM_2 \int_{a_5}^1 \frac{d\tau}{\chi_2(\tau)} = 0, \tag{17}$$

а само решение дается формулами

$$\Phi_1(\zeta) = \frac{\chi_1(\zeta)}{\pi i} \left[ -iM_1 \int_{-1}^{a_2} \frac{d\tau}{\chi_1(\tau)(\tau - \zeta)} + (-M_2 + 2pa) \int_{a_2}^{a_3} \frac{d\tau}{\chi_1(\tau)(\tau - \zeta)} \right], \tag{18}$$

$$\Phi_2(\zeta) = \frac{\chi_2(\zeta)}{\pi i} \left[ (M_1 - 2pb) \int_{a_4}^{a_5} \frac{d\tau}{\chi_2(\tau)(\tau - \zeta)} - iM_2 \int_{a_5}^1 \frac{d\tau}{\chi_2(\tau)(\tau - \zeta)} \right], \tag{19}$$

где

$$\chi_1(\zeta) = \sqrt{(\zeta - a_5)(\zeta + 1)(\zeta - a_2)(\zeta - a_3)}; \quad \chi_2(\zeta) = \sqrt{(\zeta - a_4)(\zeta - a_5)(\zeta - 1)(\zeta - a_2)}. \tag{20}$$

(под радикалами подразумевается ветвь, разложение которой в окрестности бесконечно удаленной точки имеет вид  $\zeta^2 + \delta_1 \zeta + \delta_0 + \dots$ ).

Легко показать, что функции  $\Phi_j(\zeta)$  ( $j=1, 2$ ) удовлетворяют условию (15).

После нахождения функции  $\Phi_j(\zeta)$  ( $j=1, 2$ ), на основании (12), для функций  $\omega(\zeta)$  и  $\psi(\zeta) = \psi[\omega(\zeta)]$  получаем формулы

$$\omega(\zeta) = \frac{1}{2p} [\Phi_1(\zeta) - i\Phi_2(\zeta) + M_2 + iM_1], \quad (21)$$

$$\psi(\zeta) = \frac{1}{2} [\Phi_1(\zeta) + i\Phi_2(\zeta) - M_2 + iM_1]. \quad (22)$$

Для определения аналитической формы искомого равнопрочного контура, воспользуемся формулами (18), (19), (21). Очевидно, что уравнения для частей (дуг)  $\overset{\cup}{A_3A_4}$  и  $\overset{\cup}{A_6A_1}$  упомянутого контура получаются из образа функции  $\omega(\zeta)$ , при  $\zeta = \xi \in (-\infty; a_4] \cup [a_3; \infty)$ . и  $\zeta = \xi \in [-1; 1]$  соответственно.

Если в формуле (21) перейдем к пределу при  $\zeta \rightarrow \xi \in [-1; 1]$ , на основании формулы Сохоцкого-Племеля, получим

$$\omega(\xi) = \frac{1}{2p} [\Phi_1(\xi) - i\Phi_2(\xi)], \quad \xi \in [-1; 1], \quad (23)$$

причем интегралы, входящие в этой формуле понимаются в смысле главного значения по Коши. Аналогично, для контура  $\overset{\cup}{A_3A_4}$  (дуги) имеем при  $\zeta \rightarrow \xi \in (-\infty; a_4] \cup [a_3; \infty)$

$$\omega(\xi) = \frac{1}{2p} [\Phi_1(\xi) - i\Phi_2(\xi) + M_2 + iM_1]. \quad (24)$$

Вернемся теперь к формулам (16)-(19) и (23), (24). Интегралы, участвующие в этих формулах выражаются через эллиптические интегралы первого и третьего рода. А именно (см. [16])

$$\begin{aligned} \int_{-1}^{a_2} \frac{d\tau}{\chi_1(\tau)} &= -\frac{2}{\Delta_1} F\left[\frac{\pi}{2}; k_1^{(1)}\right]; & \int_{a_2}^{a_3} \frac{d\tau}{\chi_1(\tau)} &= -\frac{2i}{\Delta_1} F\left[\frac{\pi}{2}; k_1^{(2)}\right]; \\ \int_{a_4}^{a_5} \frac{d\tau}{\chi_2(\tau)} &= \frac{2i}{\Delta_2} F\left[\frac{\pi}{2}; k_2^{(1)}\right]; & \int_{a_5}^1 \frac{d\tau}{\chi_2(\tau)} &= -\frac{2}{\Delta_2} F\left[\frac{\pi}{2}; k_2^{(2)}\right]; \end{aligned} \quad (25)$$

$$\begin{aligned} \int_{-1}^{a_2} \frac{d\tau}{\chi_1(\tau)(\tau - \zeta)} &= \frac{2}{(\zeta - a_3)(\zeta - a_2)\Delta_1} \left\{ (a_2 - a_3) \Pi\left(\frac{\pi}{2}; n_1^{(1)}; k_1^{(1)}\right) + (\zeta - a_2) F\left[\frac{\pi}{2}; k_1^{(1)}\right] \right\}; \\ \int_{a_2}^{a_3} \frac{d\tau}{\chi_1(\tau)(\tau - \zeta)} &= -\frac{2i}{(a_2 - \zeta)(\zeta + 1)\Delta_1} \left\{ (a_2 + 1) \Pi\left(\frac{\pi}{2}; n_1^{(2)}; k_1^{(2)}\right) + (\zeta - a_2) F\left[\frac{\pi}{2}; k_1^{(2)}\right] \right\}; \\ \int_{a_4}^{a_5} \frac{d\tau}{\chi_2(\tau)(\tau - \zeta)} &= -\frac{2i}{(\zeta - 1)(\zeta - a_5)\Delta_2} \left\{ (a_5 - 1) \Pi\left(\frac{\pi}{2}; n_2^{(1)}; k_2^{(1)}\right) + (\zeta - a_5) F\left[\frac{\pi}{2}; k_2^{(1)}\right] \right\}; \\ \int_{a_5}^1 \frac{d\tau}{\chi_2(\tau)(\tau - \zeta)} &= \frac{2}{(\zeta - a_5)(\zeta - a_4)\Delta_2} \left\{ (a_5 - a_4) \Pi\left(\frac{\pi}{2}; n_2^{(2)}; k_2^{(2)}\right) + (\zeta - a_5) F\left[\frac{\pi}{2}; k_2^{(2)}\right] \right\}, \end{aligned} \quad (26)$$

где

$$\begin{aligned} \Delta_1 &= \sqrt{(a_3+1)(a_2-a_5)}; \quad k_1^{(1)} = \frac{1}{\Delta_1} \sqrt{(a_2+1)(a_3-a_5)}; \quad k_1^{(2)} = \frac{1}{\Delta_1} \sqrt{(a_3-a_2)(-1-a_5)}; \\ \Delta_2 &= \sqrt{(1-a_4)(a_2-a_5)}; \quad k_2^{(1)} = \frac{1}{\Delta_2} \sqrt{(a_2-1)(a_5-a_4)}; \quad k_2^{(2)} = \frac{1}{\Delta_2} \sqrt{(1-a_5)(a_2-a_4)}; \\ n_1^{(1)} &= \frac{(a_2+1)(\zeta-a_3)}{(a_3+1)(\zeta-a_2)}; \quad n_1^{(2)} = \frac{(a_3-a_2)(\zeta+1)}{(a_3+1)(\zeta-a_2)}; \\ n_2^{(1)} &= \frac{(a_5-a_4)(\zeta-1)}{(1-a_4)(\zeta-a_5)}; \quad n_2^{(2)} = \frac{(1-a_5)(\zeta-a_4)}{(1-a_4)(\zeta-a_5)}; \end{aligned} \tag{27}$$

$$F\left[\frac{\pi}{2}; k\right] = \int_0^{\pi/2} \frac{d\varphi}{\sqrt{(1-k^2 \sin^2 \varphi)}} - \text{полный эллиптический интеграл первого рода};$$

$$\Pi\left[\frac{\pi}{2}; n; k\right] = \int_0^{\pi/2} \frac{d\varphi}{(1-n \sin^2 \varphi)\sqrt{1-k^2 \sin^2 \varphi}} - \text{полный эллиптический интеграл третьего рода}.$$

Если удовлетворимся приближениями

$$F\left[\frac{\pi}{2}; k\right] \approx \frac{\pi}{2} \left(1 + \frac{k^2}{4}\right); \quad \Pi\left[\frac{\pi}{2}; n; k\right] \approx \frac{\pi}{2} \left(1 + \frac{k^2}{4} + \frac{n}{2}\right),$$

условия (16) и (17) запишутся в виде

$$M_1 \left(1 + \frac{k_1^{2(1)}}{4}\right) + (M_2 - 2pa) \left(1 + \frac{k_1^{2(2)}}{4}\right) = 0, \tag{28}$$

$$(M_1 - 2pb) \left(1 + \frac{k_2^{2(1)}}{4}\right) + M_2 \left(1 + \frac{k_2^{2(2)}}{4}\right) = 0, \tag{29}$$

а для функций  $\Phi_1(\xi)$  и  $\Phi_2(\xi)$  на основании (18)), (19), (26)-(29) получаем формулы

$$\begin{aligned} \Phi_1(\xi) &= \frac{\chi_1(\xi)}{2\Delta_1} \cdot \frac{(a_3-a_2)(a_2+1)}{(a_3+1)(\xi-a_2)^2} (2pa + M_1 - M_2); \\ \Phi_2(\xi) &= \frac{\chi_2(\xi)}{2\Delta_2} \cdot \frac{(a_5-a_4)(1-a_5)}{(1-a_4)(\xi-a_5)^2} (-2pb + M_1 - M_2), \end{aligned} \tag{30}$$

Причем  $\xi \in [-1; 1] \cup (-\infty; a_4] \cup [a_3; \infty)$ .

Таким образом для определения параметров  $p, a_2, a_3, a_4, a_5$  имеем два условия (28) и (29).

Рассмотрим случай циклической симметрии, т. е. примем, что  $a = b; M_1 = M_2 = M; a_2 = \delta; a_5 = -\delta; a_3 = \gamma; a_4 = -\gamma$ .

Как легко заметить, в этом случае

$$k_1^{(1)} = k_2^{(2)} = \sqrt{\frac{(\delta+1)(\gamma+\delta)}{2\delta(\gamma+1)}}; \quad k_1^{(2)} = k_2^{(1)} = \sqrt{\frac{(\delta-1)(\gamma-\delta)}{2\delta(\gamma+1)}} \tag{31}$$

и из условий (28) и (29) остается одно условие

$$M \left(1 + \frac{k_1^{2(1)}}{4}\right) + (M - 2pa) \left(1 + \frac{k_1^{2(2)}}{4}\right) = 0. \tag{32}$$

(здесь, как и в дальнейшем, подразумеваем  $k_1^{2(1)} \equiv [k_1^{(1)}]^2, \dots$ ).

Учитывая, что

$$k_1^{2(1)} + k_1^{2(2)} = 1, \tag{33}$$

из (32) следует

$$p = \frac{9M}{8a \left( 1 + \frac{k_1^{2(2)}}{4} \right)}. \tag{34}$$

Так, как  $0 < k_1^{2(2)} < k_1^{2(1)} < 1$ , из (33) имеем  $k_1^{2(2)} < \frac{1}{2}$ , и, таким образом, из (34) получаем область изменения параметра  $p$  (считая  $2a = 1$ )

$$p \in \left( 2M; \frac{9}{4}M \right). \tag{35}$$

После соответствующих вычислений, из (34), с учетом (31), получим равенство

$$\frac{\delta^2 + \gamma}{\delta(\gamma + 1)} = \frac{9(p - 2M)}{p}. \tag{36}$$

Введем обозначение

$$\alpha = \frac{9(p - 2M)}{p}, \quad (\alpha < 1), \tag{37}$$

из (36), относительно  $\delta$  получим уравнение

$$\delta^2 - \alpha(\gamma + 1)\delta + \gamma = 0. \tag{38}$$

Потребуем, чтобы  $\gamma > \frac{2}{\alpha} - 1$ , тогда будем иметь  $\frac{\alpha(\gamma + 1)}{2} > 1$ , и для однозначного определения  $\delta$  (учитывая, что для функции  $f(\delta) = \delta^2 - \alpha(\gamma + 1)\delta + \gamma$  имеем  $f(1) = (\gamma + 1)(1 - \alpha)$ ) необходимо, чтобы дискриминант уравнения (38) равнялся нулю, т. е.  $D = \alpha^2(\gamma + 1)^2 - 4\gamma = 0$ , из которого, относительно  $\gamma$  получим уравнение

$$\gamma^2 + \frac{2\alpha^2 - 4}{\alpha^2}\gamma + 1 = 0. \tag{39}$$

Так, как для трехчлена  $g(\gamma) = \gamma^2 + \frac{2\alpha^2 - 4}{\alpha^2}\gamma + 1$  имеем  $g(0) = 1 > 0$ ;  $g(1) = -4 \cdot \frac{1 - \alpha^2}{\alpha^2} < 0$ ,

дискриминант  $D_1 = \frac{16(1 - \alpha^2)}{\alpha^4} > 0$ , для  $\gamma_1$ , которая удовлетворяет условию  $\gamma > 1$ , получим формулу

$$\gamma = \frac{\left( \sqrt{1 - \alpha^2} + 1 \right)^2}{\alpha^2}. \tag{40}$$

После нахождения  $\gamma$ , для определения  $\delta$  из (38) имеем формулу

$$\delta = \frac{\alpha(\gamma + 1)}{2}. \tag{41}$$

На основании приведенных результатов заключаем:

При заданных внешних усилиях, для конкретного значения  $P$  из интервала (35) (и тем самым значения параметра  $K_0$ , так как при введенных обозначениях  $K_0 = 16pD_0(1-\sigma)$ ), определяя  $\alpha$  из (37), а параметры  $\gamma$  и  $\delta$  из (40) и (41), уравнения искомого равнопрочного контура на основании (23) и (24) (с учетом формулы (30) и равенства  $\Phi_2(\xi) = -\Phi_1(-\xi)$ ) даются формулами

$$\omega(\xi) = \frac{1}{2p} [A(\xi) + iA(-\xi)], \quad \xi \in [-1; 1], \tag{42}$$

$$\omega(\xi) = \frac{1}{2p} [A(\xi) + M + i(A(-\xi) + M)], \quad \xi \in (-\infty; a_4] \cup [a_3; \infty), \tag{43}$$

где

$$A(\xi) = \frac{p(\gamma - \delta)(\delta + 1)}{\sqrt{\delta} [2(\gamma + 1)]^{3/2}} \cdot \frac{\sqrt{(\xi + 1)(\xi^2 - \delta^2)(\xi - \gamma)}}{(\xi - \delta)^2}. \tag{44}$$

Так, как из (44) имеем

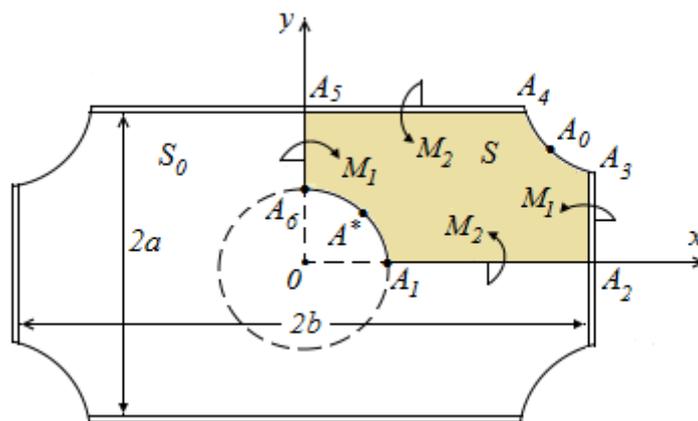
$$A(\infty) = \frac{p(\gamma - \delta)(\delta + 1)}{\sqrt{\delta} [2(\gamma + 1)]^{3/2}},$$

то для точки  $A_0$  на основании (43) получаем

$$A_0 = \frac{1}{2} \left[ \frac{(\gamma - \delta)(\delta + 1)}{\sqrt{\delta} [2(\gamma + 1)]^{3/2}} \right] (1 + i),$$

а для серединной точки  $A^*$  дуги  $\overset{\cup}{A_6 A_1}$  (см. фиг. 1) имеем:

$$A^* = \frac{(\gamma - \delta)(\delta + 1)\sqrt{\gamma}}{2[2\delta(\gamma + 1)]^{3/2}} (1 + i).$$



Фиг. 1

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# Diclorvos-Mediated Heavy Metal Uptake in Leafy Vegetables and Potential Health Risk on Consumption

*I.B watanglang*

*Adamawa State University Mubi*

## ABSTRACT

In this study, the mediating role of 2, 2-Dichlorovinyl Dimethyl Phosphate (diclorvos) in the uptake of metal ions in vegetables is investigated and the health risk assessment conducted toward establishing a health-based approach in vegetable farming for human consumption. The concentrations of Cd and Pb in the vegetables analyzed using Atomic Absorption Spectroscopy (AAS) were observed to be higher in the pesticides treated vegetables compared to the untreated (control). The consumption of the vegetables treated with the pesticides were observed to increase the estimated daily intake (EDI) of the metal ions in humans compared to the EDI level in the untreated groups. The EDI levels were observed to fall in this order Spinach>Sorrel and Pb>Cd. The consumption of the vegetables treated with the pesticides were observed to expose children to a highest EDI of  $1.50E-02$  mg/kg/day and  $2.46E-02$  mg/kg/day for Cd and Pb compared to the  $1.19E-02$  mg/kg/day and  $1.55E-02$  mg/kg/day in the adults. The susceptibility of the children to non-carcinogenic risk from Cd and Pb exposure through the consumption of the pesticides treated Spinach were observed to be higher. For the metals, a target hazard quotient (THQ) of  $1.76E-01$  and  $8.26E-01$  were observed in the treated groups compared to the untreated ( $1.40E-01$  and  $5.21E-01$ ). Based on species, the THQ established in the Spinach specie is higher than in Sorrel.

**Keywords:** pesticides; sorrel; spinach; heavy metals; dichlorvos; trace elements; risk assessment; estimated daily intake; target hazard quotient.

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## ABSTRACT

In this study, the mediating role of 2, 2-Dichlorovinyl Dimethyl Phosphate (diclorvos) in the uptake of metal ions in vegetables is investigated and the health risk assessment conducted toward establishing a health-based approach in vegetable farming for human consumption. The concentrations of cadmium (Cd) and lead (Pb) in the vegetables analyzed using Atomic Absorption Spectroscopy (AAS) were observed to be higher in the pesticides treated vegetables compared to the untreated (control). The consumption of the vegetables treated with the pesticides were observed to increase the estimated daily intake (EDI) of the metal ions in humans compared to the EDI level in the untreated groups. The EDI levels were observed to fall in this order Spinach>Sorrel and Pb>Cd. The consumption of the vegetables treated with the pesticides were observed to expose children to a highest EDI of 1.50E-02 mg/kg/day and 2.46E-02 mg/kg/day for Cd and Pb compared to the 1.19E-02 mg/kg/day and 1.55E-02 mg/kg/day in the adults. The susceptibility of the children to non-carcinogenic risk from Cd and Pb exposure through the consumption of the pesticides treated Spinach were observed to be higher. For the metals, a target hazard quotient (THQ) of 1.76E-01 and 8.26E-01 were observed in the treated groups compared to the untreated (1.40E-01 and 5.21E-01). Based on species, the THQ established in the Spinach specie is higher than in Sorrel. All the THQs values recorded in the study are observed to be <1. The health index (HI) for the non-carcinogenic risk were also observed to follow similar trend with the THQ values. Though, the THQ and HI values were observed to

be <1 and safe for human consumption, the continual application of the pesticides will increase the concentrations of Cd and Pb and hence, the non-carcinogenic (THQ and HI) risk to a level of concern. Furthermore, the application of pesticides were observed to increase the carcinogenic risk for Cd and Pb exposure through the consumption of the vegetables. The CRI for Cd were higher than the unacceptable range ( $>10^{-4}$ ). The combine effect (TCRI) further show high carcinogenic risk for children compared to the adults. From the results it will suffice to say that the application of pesticides in vegetable cultivation for human consumption should be regulated or discouraged where possible.

**Keywords:** pesticides; sorrel; spinach; heavy metals; dichlorvos; trace elements; risk assessment; estimated daily intake; target hazard quotient.

**Author:** Department of Pure and Applied Chemistry, Faculty of Science, Adamawa State University Mubi, Nigeria.

## I. INTRODUCTION

Food in a more practical sense are source of nutrition that can modulate toxicity, inhibits diseases, reduce risk from environmental stressors, and improves life expectancy<sup>[1]</sup>. Besides been a reservoir of nutrient and an antidote for quenching hunger, in contaminated form can also increase a person's vulnerability to additional environmental stressors. In food profiling, vegetables are ranked among the widely accessible nutrient-dense foods, providing the essential nutrients, dietary fibres and other bioactive components necessary for human health and vitality<sup>[2]</sup>. Dietary daily consumption of vegetables

have significant health effect, such as lowering the risk of cardiovascular diseases, regulating body weight and increasing the immune system to fight carcinogens [3,4]. These growing popularity of vegetables as a source of healthy, affordable and nutrient-rich food has attracted considerable array of research to improve output and make it readily available.

In Nigeria, community garden are fast becoming a household practice, virtually every available space is being cultivated with locally grown vegetables. This is presumably expected to meets the demands for fresh vegetables owed largely to the health-improving awareness as highlighted above. However, gardening on polluted soils were observed to contaminate the whole food chain. Soil as a repository of contaminants can readily accumulate heavy metals and transfer same into the plants. The buildup of contaminants in soils is multidimensional, emanating from human activities such as waste incineration, mining, oil combustion, use of leaded gasoline, paints and from galvanized metal parts [5]. Gardening on soils contaminated from such activities can increase the risk of exposure to both adults and children through either incidental soil ingestion, inhalation, dermal contacts or from the consumptions of plants grown on such a soil [6]. Several research relates the association of heavy metals in contaminated soils to the levels in edible plants [7-9]. Voutsas *et al*, [8], and De Nicola *et al*, [9] reported high accumulation of Pb, Cr and Cd in leafy vegetables. The level of preponderance were observed to be predominant in the leafy or root portion of the plants [10-13]. Other studies shows a direct relationship between population/vehicular density and other anthropogenic activities with heavy metals concentration on leafy vegetables [14-22].

Excessive applications of plant protection products to bust the cultivation of vegetables were observed to influence the chemistry of the soil and the health-status of the plants. Plant protection products were observed to modulate metal bioavailability in soil and streaming rate in plants [23]. This were observed to be influenced by the complexation chemistry between the pesticides and the metal ions in the soil [23, 24]. This complexation reaction helps in facilitating the mobility of the pesticides-metal complex in the soil and translocation in plants [23-25]. Pesticides

residues were reported to facilitate the mopping of heavy metals in soil by chelating the metals ions and stimulates the uptake and translocation in plants [23, 26]. In our previous study, 2, 2-dichlorovinyl dimethyl (diclorvos) were observed to influence heavy metal uptake and bioavailability in the roots, stem and leaves of spinach (*Spinacia oleracea*) and sorrel (*Rumex acetosa*), compared with the same vegetable cultivated without the pesticides [23]. Related study were also reported by Chiroma *et al*, [18]. Metals ions like Cd, Pb, Zn and Fe readily chelates with the phospheryl oxygen groups on dichlorvos and by so doing enhances the uptake by plants [24, 27].

This study is intended as a community project and conducted to assess the level of exposure to heavy metals through the consumption vegetables grown on experimental garden. The study further examine the potential association between pesticides application in the garden and heavy metal concentrations and their potential health risk on consumption. Dietary intake of lead (Pb) and cadmium (CD) through the consumption of spinach and sorrel were used to determine the level of concern for both non-carcinogenic and carcinogenic risks.

## II. MATERIALS AND METHODS

### 2.1 Sample collection and treatment

A representative standard plot of garden were evenly demarcated into four beds (B1, B2, B3, and B4). *Spinacia oleracea* (Spinach) were planted in plot B1 and B2 while sorrel (*Rumex acetosa*) in B3 and B4. Three weeks after planting, B2, B4 beds (treated plants) were sprayed with 2, 2-dichlorovinyl dimethyl phosphate, while B1, B3 beds were used as the control beds (untreated plants) following the same procedure described by Garba *et al* [23]. After harvesting, the powdered samples taken from each beds were digested and analysed for Cd and Pb using atomic absorption spectroscopy (VGP 210, Buck Scientific) [23].

### 2.2 Health Risk Characterization

The estimated daily intake of Cd and Pb based on the average consumption of the vegetable by adults and children in Nigeria were determined

using the USEPA recommended procedure described in equation 1 [28, 29].

$$EDI = C \frac{F_{IR}}{BW} \quad (1)$$

Where EDI is the average daily intake (mg/kg body weight/day); C is the concentration of the elements in the vegetable plant, and  $F_{IR}$  is the average daily consumption of vegetables (0.2 kg). The BW is the body weight (kg); set at 60 for an average adult and 15 for children.

Furthermore, target hazard quotient (THQ) as described in equation 2 was used to determine the human health risk posed by the long-time exposure to the metals following the consumption of the vegetables.

$$THQ = \frac{C \times F_{IR} \times EF \times ED}{BW \times AT \times RfD} \quad (2)$$

Where, EF is the exposure frequency (365 days/year); ED is the exposure duration (70 years); BW is the body weight in kg; and AT is the average time for non-carcinogens (365 days/year  $\times$  ED). The oral reference dose (RfDs) of 0.001, and 0.00035 mg/kg/day were adopted for Cd and Pb respectively. The parameters, C, and  $F_{IR}$  are already described in Eq.1 [30-33]. The description hypothesis a  $THQ < 1$  to signify no associated risk, meaning the exposed population is unlikely to experience any adverse health hazard. However, if the  $THQ \geq 1$ , then there is a potential health risk [33].

The health index (HI), expressed as the sum of THQ as described in equation 3 is used in this study to describe the cumulative effect pose by the combination of the individual metal ions presents in the vegetable plants. Thus, the greater the value of HI, the greater the level of concern. [28, 29].

$$HI = \sum THQs \quad (3)$$

The description in equation (4) is use to estimate the carcinogenic risk (CRI), which is the lifetime probability of an individual developing any type of cancer following the consumption of the vegetables contaminated with the metal ions.

$$CRI = EDI \times CSF_{ing} \quad (4)$$

Where, EDI is the estimated daily intake of each heavy metal (mg/kg/day),  $CSF_{ing}$  is ingestion

cancer slope factor which is used to evaluates the probability of an individual developing cancer from oral exposure to the heavy metals over a period of a lifetime. The CSF for Cd and Pb are 0.38, and 0.0085 mg/kg. A CRI values of  $10^{-6}$  (1 in 1,000,000) to  $10^{-4}$  (1 in 10,000) represent a range of permissible predicted lifetime risks for carcinogens [30].

### III. RESULTS

#### 3.1 Concentration of Cd and Pb in the vegetables

The average mean concentrations of Cd and Pb analyzed in the Spinach and Sorrels plants are presented in Fig. 1. The results show a significant ( $p < 0.05$ ) increase in the concentrations of Cd ( $13.20 \pm 0.87 \mu\text{g/g}$ ) and Pb ( $21.67 \pm 1.47 \mu\text{g/g}$ ) in the spinach treated with the pesticides when compared to the untreated (control) groups ( $10.47 \pm 1.22 \mu\text{g/g}$  and  $13.67 \pm 1.21 \mu\text{g/g}$ ). Furthermore, an insignificant ( $p > 0.05$ ) increase in Cd ( $10.23 \pm 0.92 \mu\text{g/g}$ ) and Pb ( $12.53 \pm 2.26 \mu\text{g/g}$ ) were observed in the pesticide treated Sorrels compared to the untreated groups ( $10.00 \pm 0.57 \mu\text{g/g}$  and  $10.17 \pm 2.18 \mu\text{g/g}$ ). For both species, the metal accumulation were observed to follow the trend,  $Pb > Cd$ . The concentration of both Cd and Pb were observed to be significantly ( $p < 0.05$ ) higher in the Spinach than in the sorrel.

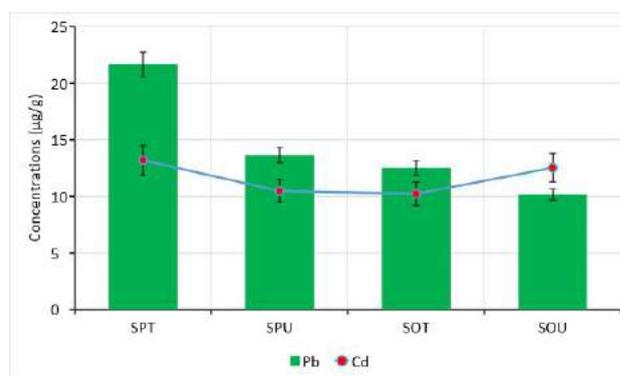


Fig. 1: Concentration of heavy metals (Cd and Pb) in spinach cultivated with (SPT) and without (SPU) pesticides treatment. The SOT and SOU stands for Sorrel cultivated with and without the pesticides treatment. Results are presented as Mean  $\pm$  SD of triplicate analysis..

### 3.3 Estimated daily intake of metals for adults and children

As shown in table 1, the consumption of the vegetables treated with the pesticides were observed to increase the EDI for Cd and Pb in the adults and children respectively. The EDI of Cd and Pb in the Spinach treated with the pesticides are 3.74E-03 mg/kg/day and 6.14E-03 mg/kg/day compared to the control groups (2.97E-03 mg/kg/day and 3.87E-03 mg/kg/day). The EDI for Cd and Pb in the children were 1.50E-02 mg/kg/day and 2.46E-02 mg/kg/day following the consumption of Spinach treated pesticides compared to the EDI of 1.19E-02 mg/kg/day and 1.55E-02 mg/kg/day in the control groups. Similar trend were also observed

for Sorrel. An EDI of 2.90E-03 mg/kg/day and 3.55E-03 mg/kg/day were observed in the treatment groups while, 2.83E-03 mg/kg/day and 2.88E-03 mg/kg/day were observed in the control groups for the adults. In children, an EDI of 1.16E-02 and 1.42E-02 were observed in the treatment groups compared to the 1.13E-02 and 1.15E-02 in groups cultivated without pesticides treatment. Overall, the EDI were observed to be higher for children than the adults and the activity were equally higher in Spinach compared to Sorrel. The EDI for both Spinach and Sorrel and for each weight category were observed to be lower than the RfD values of 1.00E-03 and 3.50E-04 for Cd and Pb respectively

**Table 1:** Estimated Daily Intake (EDI) in mg/kg/d for Cd and Pb in the Vegetables

Elements	Spinach				Sorrel			
	Adults		Children		Adults		Children	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cd	3.74E-03	2.97E-03	1.50E-02	1.19E-02	2.90E-03	2.83E-03	1.16E-02	1.13E-02
Pb	6.14E-03	3.87E-03	2.46E-02	1.55E-02	3.55E-03	2.88E-03	1.42E-02	1.15E-02

### 3.3 Target hazard quotient (THQ) and Health index (HI) in adults and children for Cd and Pb in the vegetables:

The non-carcinogenic risk of the two heavy metals in the Spinach and Sorrel are presented in table 2. The THQ value of the heavy metals in the Spinach treated with the pesticides were observed to be higher (4.40E-02 and 2.06E-1) than the untreated (3.49E-03 and 1.30E-01) for Cd and Pb in the adults. The values for the adults were observed to be much lower to the THQ values in children. Showing a THQ of 1.76E-01 and 8.26E-01 for the treated and 1.40E-01 and 5.21E-01 for the control groups for Cd and Pb respectively. Target hazard quotient of 3.41E-02 and 1.19E-01 were observed in Sorrel treated with the pesticides compared with the 3.33E-02 and 9.68E-02 in the untreated for the adults. For the same species in children, a THQ of 1.36E-01 and 4.77E-01 were observed in the treated groups, while THQ of 1.33E-01 and

3.87E-01 were observed in the untreated. In all, the non-carcinogenic risk were observed to be higher in children than in adults and higher for Pb than Cd. Based on species, THQ of Spinach is higher than in Sorrel. All the THQs values recorded in the study are observed to be <1. The health index (HI) for the non-carcinogenic risk were also observed to follow similar trend with the THQ values except in children whose HI were observed to be 1.00E+00 through the consumption of pesticides treated Spinach

**Table 2:** Target hazard quotient (THQ) and Health index (HI) for Cd and Pb in the Vegetables

Elements	Spinach				Sorrel			
	Adults		Children		Adults		Children	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cd	4.40E-02	3.49E-02	1.76E-01	1.40E-01	3.41E-02	3.33E-02	1.36E-01	1.33E-01
Pb	2.06E-01	1.30E-01	8.26E-01	5.21E-01	1.19E-01	9.68E-02	4.77E-01	3.87E-01
HI	2.50E-01	1.65E-01	1.00E+00	6.60E-01	1.53E-01	1.30E-01	6.14E-01	5.21E-01

### 3.4 Cancer risk index (CRI) in adults and children for Cd and Pb in the vegetables

The CRI for Cd and Pb following the consumption of the vegetables treated with and without pesticides are presented in table 3. Highest CRI of 5.68E-03 were observed in children exposed to Cd through the consumption of treated spinach when compared to the untreated (4.57E-03). A CRI of 1.42E-03 and 1.13E-03 were observed in the adults exposed to the metals following the consumption of the treated and untreated spinach respectively. From the result,  $CRI \leq 10^{-4}$  were observed in both age categories exposed to Pb through the consumption of spinach treated with and without pesticides. For the treated groups, a CRI of 5.22E-05 and 2.09E-04 were observed in adults and children exposed to the metal ion compared to the CRI of 3.25E-05 and 1.32E-04 for the untreated. The cumulative cancer risk of the metal ions (TCRI) following the consumption

of Spinach were also observed to be above the unacceptable limits ( $> 10^{-4}$ ). For Cd levels in the pesticides treated Sorrel, a CRI of 1.10E-03 and 4.41E-03 were observed in the adults and children, compared to the 1.08E-03 and 4.31E-03 observed in the control groups. The CRI for Pb following the consumption of the vegetable by children were 3.02E-05 and 1.24E-04 for Sorrel treated with the pesticides while, 2.45E-05 and 9.79E-05 were observed for the untreated groups respectively. The TCRI following the consumption of the vegetables containing both Cd and Pb are 1.13E-03 and 4.53E-03 in the pesticides treated sorrel for both the adults and children. The untreated groups show a TCRI of 1.10E-03 and 4.40E-03 for the same age categories. The highest TCRI were observed in children. Overall, the results show a higher CRI and TCRI in children exposed to the heavy metals through the consumption of the vegetables than adults.

**Table 3:** Cancer risk index (CRI) in adults and children for Cd and Pb in the vegetables

Elements	Spinach				Sorrel			
	Adults		Children		Adults		Children	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cd	1.42E-03	1.13E-03	5.68E-03	4.51E-03	1.10E-03	1.08E-03	4.41E-03	4.31E-03
Pb	5.22E-05	3.29E-05	2.09E-04	1.32E-04	3.02E-05	2.45E-05	1.21E-04	9.79E-05
TCRI	1.47E-03	1.16E-03	5.89E-03	4.64E-03	1.13E-03	1.10E-03	4.53E-03	4.40E-03

## V. DISCUSSIONS

Pesticides application were reported to mediate metal ions bioavailability in soils and plants [23, 34-40]. As observed in Fig.1, the concentrations of Cd and Pb in the vegetables were higher in the pesticides treated groups compared to the

untreated. Study shows that the phospheryl oxygen groups in organophosphate pesticides has strong affinity to chelates metal ions such as Cd, Pb, Zn and Fe in soil/water medium [24, 26, 27]. The application of the dichlorvos might have initiated a change in the soil pH, increasing the solubility of

the sparingly soluble metals ions and chelating potential with the biological components of the plants [24]. Based on this chemistry increases the absorption capacity and translocation of the metal-ions by the plants [41, 42]. The concentrations of the metals ions in the vegetables were observed to be higher than the maximum permissible (PL) limits of 0.2 mg/kg and 0.3 mg/kg set by the FAO/WHO for Cd and Pb in edible plants [43]. The concentration was observed to fall in this order Cd<Pb. Lead ions compared to other heavy metals were reported to have the ability to preferential chelate with the uronic acids expressed on the root surface of plant [44, 45] mostly in the form of extracellular precipitates [46]. Besides this pathway, atmospheric deposition of the Pb-pesticides complexes on the leaves is another possible explanations for the observed higher concentration of the ions in the vegetables [47, 48]. The high concentration of the metal ions in the Spinach compared to Sorrel could be due to the difference in their cell biology response to physiological stress. Schaidler *et al*, [49] observed that Pb or Cd complexes in the soil can physiologically increase stress in plant, initiating both transient and pronounced changes in the cells biology, facilitating the absorption of metal ions in dissociation with the diffusive boundary layer [50]. On the contrary, the poor absorption of the metal ions by Sorrel could be connected to the barrier limiting properties of the roots applying exclusion mechanism on sensing toxic-induce stress to inhibit absorption [51-53].

As discussed earlier, the metal ion absorption potentials were enhanced following the application of pesticides in the cultivation of the vegetables [23]. The increase in the EDI values influence by the pesticides were observed to be more pronounce in the Spinach species than in the Sorrel. Even though, the EDI for both the Spinach and Sorrel were observed to be lower than their RfD values of 1.00E-03 mg/kg/day and 3.50E-04 mg/kg/day for Cd and Pb, the results however draw attention on the need to apply caution on the indiscriminate applications of pesticides in food cultivation. The RfD often used for the assessment of non-carcinogenic health risk is a reference oral dose values set as an estimate for the tolerable daily intake of metals that will

pose no health risk during a lifetime [54]. The cultivation of the vegetables using pesticides poses potential health risk to the consumers and more so to the populace that eat more of spinach. On the same note, body weight and age variations were also observed to influence the vulnerability to Cd or Pb toxicity. The EDI were observed to be higher for children than the adults and the activity were equally higher in Spinach compared to Sorrel. Which further suggest that children could be more susceptible to possible non-carcinogenic or carcinogenic risk from Cd and Pb exposure than the adults through the consumption of the vegetables especially the consumption of Spinach.

The target hazard quotient for non-carcinogenic risk were conducted to further share light into the level of concerns to Cd or Pb exposure following the consumption of these vegetables cultivated with or without pesticides. The risk characterization processes based on THQ analysis is a health-based statistical probability expressed as a function of the quantified level of concern; a process developed to estimate the potential health risk associated with long-term exposure to environmental pollutants [30, 32, 55]. The THQ values of <1 observed in the study suggest no health risk associated with the level of Cd or Pb in the vegetables, thus the population consuming these vegetable are in no immediate danger for non-carcinogenic risk. The HI for Cd and Pb in the vegetables were observed to be <1. The result therefore means that the consumption of the vegetables by the population posed no immediate health risk. However, The HI of 1.00E+00 observed for Spinach suggest possible health concerns for children eating the vegetable if the exposure to the metals persisted. Polluted vegetable increases the likelihood of heavy metal induced toxicity. An HI values >1 were observed in some vegetables in Kpanshia and Swali markets in Bayelsa state, Nigeria [13]. Similar finding are reported in leafy vegetables collected in Lagos state, Nigeria by Adedokun *et al*, [56]. Higher THQ for Cd, Pb, and Ni were also reported by Singh *et al*, [57] in vegetables from wastewater irrigated area. Higher THQ for Cd and Pb in an area near a lead (Pb) and antimony (Sb) were also reported by Cui *et al*. [58] and Zhou *et al*. [59] in vegetable species planted in contaminated soils.

Though the risk characterization conducted in this study might have suggest no immediate danger for non-carcinogenic risk, the continual application of pesticides in the cultivation of vegetables will increase the likelihood of heavy metal buildup in the plants and consequently transmit potential non-carcinogenic risk to a level of concern.

The lifetime cancer risk (CRI) for the adults and children as presented in table 3 were analyzed for the ingestion exposure pathways only. For regulatory purposes, a cancer risk in the range of  $10^{-6}$  to  $10^{-4}$  are considered acceptable [55, 59]. From the results presented in the table, the CRI were found to be in this order Cd>Pb and Children>Adults. The CRI for Cd were higher than the unacceptable range ( $>10^{-4}$ ). The level were found to be higher in the pesticides treated groups compared to the untreated suggesting that the consumption of vegetables cultivated with pesticides will significantly increase the likelihood of heavy metal exposure. From the results it will suffice to say that children are more susceptible to carcinogenic risk than the adults. The combine effect (TCRI) further show high carcinogenic risk for children. The order for exposure was observed to be in the decreasing order, Sorrel<Spinach. According to the results, the carcinogenic risk is higher in the pesticides treated vegetables and largely due to the presence of Cd, which was observed to exceed the limit for acceptable risk of developing cancer indicating some concern about the application of pesticides in vegetable garden.

#### IV. CONCLUSION

Cadmium and Pb ions concentrations were observed to be influence by the pesticides, with significantly ( $p < 0.05$ ) higher activities recorded in leads levels in both vegetables. The 2, 2-dichlorovinyl dimethyl phosphate was observed to facilitate significant ( $p < 0.05$ ) metal uptake in the spinach compared to the sorrel plants. The EDI of the metal ions were observed to be higher in children and significantly higher through the consumption of pesticides treated Spinach compared to the adults and Sorrel species. Though, the THQ and HI values were observed to be  $<1$  and safe for human consumption, the consumptions of the vegetables treated with the pesticides were observed to increase THQ and HI

values closer to a point where continual application of the pesticides will increase the likelihood for non-carcinogenic risk to a level of concern. The application of pesticides were observed to increase the carcinogenic risk from Cd and Pb exposure through the consumption of the vegetables. The CRI for Cd were higher than the unacceptable range ( $>10^{-4}$ ). The combine effect (TCRI) further show high carcinogenic risk for children compared to the adults.

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# Natural Distribution of Pistius Algae Plant and its Systematic Place and Biological Features

*Ch. H. Kuchkarova & B. Sh. Ismailhodjaev*

## ABSTRACT

This article presents the materials of household purification methods - waste water on the example of a plant pistil, highlights effective methods of household purification - wastewater by analyzing in laboratory conditions the physical properties and chemical composition of the plant pistil.

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# Natural Distribution of Pistia Algae Plant and its Systematic Place and Biological Features

Ch. H. Kuchkarova<sup>α</sup> & B. Sh. Ismailhodjaev<sup>σ</sup>

## ABSTRACT

*This article presents the materials of household purification methods - waste water on the example of a plant pistil, highlights effective methods of household purification - wastewater by analyzing in laboratory conditions the physical properties and chemical composition of the plant pistil.*

**Keywords:** algae pest plants, azolla, biological purification, domestic runoff, eichornia.

**Author α:** Andijan Machine Building Institute (Andijan, Uzbekistan).

## I. INTRODUCTION

Pistia belonging to the family of water cabbage and street flowers (*Pistia stratiotes* L) is a perennial aquatic plant that melts on the surface of the water and forms a watery vineyard. Pistia is considered the oldest plant. Its remains are found in the south of France and in North America (Angler, 1924). Currently, the range of Pistias is gaining in Asia, Africa, Australia and Europe (Wolf, Maleeva, 1966; Grudzinskaya, 1982; Gillet, 1989). Pistia fruit is dry, in the form of a single-celled box and has several seeds. The crust of the fruit is thick and green. The crust of the ripe fruit is thin and picks up a light brown color. As a result of the ripening of the fruit, the fruit bursts and the seeds come out of the cradle. Some of them enter the lower part of the water, and some are glued to the roots of the plant. Fully matured seeds are brown in color, not matured — green in color; seeds are long-cylindrical in shape, fineness is 1.5–3.0 mm. The mass of 1000 pieces of seeds is 2.2 g. The quiet period of Pistia seeds under conditions of

introduction is very short under natural conditions. In convenient conditions (water temperature is 25-26 ° C and it is necessary in sufficient amount of light) the seeds start to grow after 14 days out of the seed bag. With the growth of pistia seeds, the decisive factor is considered to be light, because although with sufficient temperature, the seeds will not grow in a dark place. Pistia seeds are also resistant to long-term (up to 60 days) cold temperatures (3-5°), continue to grow after 14-16 days. Under laboratory conditions (distilled water, temperature 26-28° C and especially light is needed) seed yield was 72 percent. In the botanical gardens, pistias are sown as ornamental (Paramonova, 1961; Minokhina, 1984) and in an aquarium (Zhdanov, 1973; Cyriling, 1991). In addition, Pistia biomass is used as food for pigs (Woolf, Maleeva, 1969). Manure of various animals (ram, cow, pig, horse), sewage water of flax processing plants, enterprises producing mineral fertilizers, biochemical plants, alkali factories, meat producing factories, urban public services enterprises are good for the cultivation of pistias (R.Sh.Shoyakubov et al., 1993). According to scientists, the growth of algae plants depends not only on the composition of the food environment and the type of plants, but also on the density of seedlings planted. The density of the primary sowing planted on the surface is 1 m<sup>2</sup> of water, depending on the food environment and the concentration of sewage water, 1-3 kg, in some cases 5 kg / m<sup>2</sup> of wet biomass. Pistia is well grown in the sewage waters of pork complexes and poultry farms. Its yield per day for 1m<sup>2</sup> of the water surface is up to 1,400 grams of wet biomass (R.Sh.Shoyakubov et al., 1993, 1997). Below is a view of the algae plant *Pistia telerizoid* (Fig. 1).



*Fig. 1:* Pistia telerizoid

In the conditions of Uzbekistan, the methods of growing a pistia plant are given in the works of R.Sh.Shoyakubova (1993). In our country, as an ornamental plant, it grows in the Botanical Gardens and from the amateur ribodov in aquariums. Pistia algae plants in winter conditions can be grown in greenhouses in glass, plastic vessels and in aquariums, concrete reservoirs, and in summer conditions in ferro-concrete trays and cemented reservoirs in the open air. When the pistol is increased in the laboratory and in the open air, the growth of its individual fully grown parts is 20-40 cm. The root system consists of shaggy, long, browed, small roots. The root is pale, transparent light, 0.5-0.6 m and may be longer from it. Her body is short, the leaves have a triangular shape. The upper part of the leaves is velvety green, and the lower part of silver green, consists of 9 to 12 pieces prominent vessel. Due to the porosity of the structure of the structure of the leaves, that they are filled with air, the pistol grows on the surface of the water.

Pistia can bloom in the open air from the second half of April to November, and in greenhouse conditions throughout the year. Pistia has a feature of rapid reproduction through vegetation as well as from seeds. Vegetative reproduction mainly occurs through the process (stolon) of leaves that grow from the plant. New pistias are developed on the final processes. This circumstance continues throughout the summer and until late autumn can be repeated several times.

With the arrival of a comfortable climate in the seating arrangement, the pistols are transferred to

the discovery of reservoirs. By the end of April and during the month of May, the pistia is rapidly developing, and from the point of development, looking at the center in a circular shape, organs of multiplication are formed. During vegetation, up to 4-5 circles can form on each plant. Thus, the formation of leaves occurs from the center to the outer side, and the formation of the organs of reproduction, flowering and ripening of seeds occurs from the outer side to the center.

The method of multiplication of the seed piston has several advantages from the method of multiplication through vegetation. In this, in the autumn-winter period, in not very large vessels, it is possible to grow seedlings sufficient for sowing in spring. To prepare the seeds for the pistia, in the months from September to October, vibrating the picty's undergrowth over some material can be collected. The collected picty's seeds are placed in a glass jar and filled with tap water or, if possible, distilled water. Tanks should not be large and not deep, for lighting you can use fluorescent lamps. In order for the ripe seeds to come to the surface of the water there must be a necessary condition. Seeds come out well at a temperature of 250, with a decrease in temperature, the yield of seeds decreases. Pista emerging from the seeds should be illuminated within 7-8 hours. In this case, you can use the day lamp. After the appearance of a few roots at the exit of the pistia comes to the surface of the water. They are not only from the endosperm, but are transferred to a sovereign formation and therefore, it is necessary to replace distilled or tap water with sewage. Thus, young seedlings in early spring, with warming days, do not leave seeds in greenhouse conditions in small reservoirs built in the open air for Kriba and after preparing in sufficient quantity of seedlings, in April and May, sow livestock farms or industrial plants in biological reservoirs. For use in a large number of plants Pistia in the conditions of Uzbekistan is of great importance to study flowering seeds. Mass flowering begins in 45-55 days from the flowering of the initial seating and lasts 60-65 days. With the growth of the pistols, the formations of 2 types of seeding were

determined, that is, colorless and colored seed color. Both types of color are defined as seed flowers (Tursunov, 1989). In conclusion, it can be said that in recent years, municipal and industrial enterprises have been increasing in the republic, they are developing and they are working intensively. With the development of production, the amount of waste and sewage water leaving the plant increase. Biological purification of sewage water leaving municipal and industrial enterprises, that is, accelerated biotechnology of purification through the use of various algae plants and high aquatic plants, is considered one of the advantages of biological discipline. Given this, it is necessary to prevent the outflow of blue-green aquatic plants from under water, to develop measures of protection against anthropogenic influences. Because, with the help of hydrobionts living in biological reservoirs, which they develop in their vital activity special chemical things (antibiotics), as a result of which pathogenic bacteria spreading waterborne diseases are destroyed.

The purpose of our research work in the purification of municipal sewage waters of the city of Andijan is to increase the high (surface) water plant of the piston in laboratory conditions and to develop a biotechnology for the purification of urban municipal sewage water from various organic-mineral substances.

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# Oxidative and Biochemical Parameters Analysis of Alloxan-Induced Diabetic Rats Administered Methanol Leaf and Fruit Extracts of *Kigelia Africana*

Uhuo, E. N, Ezeanyika, L.U.S & Ogugua, V.N

University of Nigeria Nsukka

## ABSTRACT

Free radicals have been implicated in the pathogenesis of diabetes mellitus leading to various complications including atherosclerosis. *Kigelia africana* is highly used for ethnomedical purposes although there is paucity of scientific information on its uses. This study is designed to investigate the possible anti diabetic and anti oxidative effects of methanol extract of leaf and fruit of *k. africana*. Diabetes was induced with alloxan in a dose of 160mg b.wt. i. P. After 4 weeks of treatment, diabetic rats untreated (positive control) showed an apparent reduction in the body weight, significant increase in the blood glucose level, triacylglycerol (TG) total cholesterol and low density lipoprotein cholesterol (LDLC) with corresponding decrease in serum high density lipoprotein-cholesterol (HDLC) as compared to the normal control. In addition, there was significant deviation of lipid peroxide measured as malondialdehyde (MDA), with masked reduction in serum Glutathione Peroxidase activity, SOD, CAT and Vitamin C concentration. On the other hand, oral daily treatment of animal with *K. africana* in a dose of 200mg/kg bwt for the period of 4 weeks ameliorated alloxan-induced alterations in the animal body weight as well as blood glucose, MDA, lipid profile, activities of SOD, GPx and CAT. In conclusion, *K. africana* extract offers promising results mainly could be attributed to its potent antioxidant potential. Further studies will be required in future to determine which one (or more) of its active constituent has the main antidiabetic and antioxidative effects.

**Keywords:** diabetes, alloxan, antioxidant, *kigelia africana*.

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Uhwo, E. N<sup>a</sup>, Ezeanyika, L.U.S<sup>o</sup> & Ogugua, V.N<sup>p</sup>

## ABSTRACT

*Free radicals have been implicated in the pathogenesis of diabetes mellitus leading to various complications including atherosclerosis. Kigelia africana is highly used for ethnomedical purposes although there is paucity of scientific information on its uses. This study is designed to investigate the possible anti diabetic and anti oxidative effects of methanol extract of leaf and fruit of k. africana. Diabetes was induced with alloxan in a dose of 160mg b.wt. i. P. After 4 weeks of treatment, diabetic rats untreated (positive control) showed an apparent reduction in the body weight, significant increase in the blood glucose level, triacylglycerol (TG) total cholesterol and low density lipoprotein cholesterol (LDLC) with corresponding decrease in serum high density lipoprotein-cholesterol (HDLC) as compared to the normal control. In addition, there was significant deviation of lipid peroxide measured as malondialdehyde (MDA), with masked reduction in serum Glutathione Peroxidase activity, SOD, CAT and Vitamin C concentration. On the other hand, oral daily treatment of animal with K. africana in a dose of 200 mg/kg bwt for the period of 4 weeks ameliorated alloxan-induced alterations in the animal body weight as well as blood glucose, MDA, lipid profile, activities of SOD, GPx and CAT. In conclusion, K. africana extract offers promising results mainly could be attributed to its potent antioxidant potential. Further studies will be required in future to determine which one*

*(or more) of its active constituent has the main antidiabetic and antioxidative effects.*

**Keywords:** diabetes, alloxan, antioxidant, kigelia africana.

**Author a:** Department of Biochemistry, Michael Okpara University of Agriculture, Abia State.

**o:** Professor of Biochemistry, University of Nigeria Nsukka.

**p:** Professor of Biochemistry, Department of Biochemistry, University of Nigeria Nsukka.

## I. INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism [1]. During diabetes or insulin resistance, failure of insulin-stimulated glucose uptake by fat and muscle cause glucose concentration in the blood to remain high, consequently glucose uptake by insulin-independent tissue increases. Increased glucose flux both enhances oxidant production and impairs antioxidant defenses by multiple interacting non-enzymatic, enzymatic and mitochondrial pathways [2, 3]. These include activation of protein kinase C isoforms [4], increased hexosamine pathway [5], glucose autoxidation [6], increased methylglyoxal and advanced glycation end-product (AGEs) formation [7] and increased polyol pathway flux [8]. These seemingly different mechanisms are the results of a single process-that is, overproduction of

superoxide by the mitochondrial electron transport system [9]. This hyperglycemic-induced oxidative stress ultimately results in modification of intracellular proteins resulting in an altered function, DNA damage, activation of the cellular transcription (NF KB), causing abnormal changes in gene expression, decreased production of nitric oxide, and increased expression of cytokines, growth factors and procoagulant and proinflammatory molecules [10, 11, 2, 12].

Oxidative stress is responsible for molecular and cellular tissue damage in a wide spectrum of human diseases [13], amongst is diabetes mellitus. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation [14], which is responsible for increased incidence of atherosclerosis [15], a major complication of diabetes mellitus. An enhanced oxidative stress has been observed in these patients as indicated by increased free radical production, lipid peroxidation and diminished antioxidant status [16].

Globally, the estimated incidence of diabetes and projection for year 2030, as given by International Diabetes Federation is 350 million [17]. Currently available pharmacotherapies for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these drugs do not restore normal glucose homeostasis and they are not free from side effects [18]. In view of the adverse effects associated with the synthetic drugs and as plants are safer, affordable and effective, conventional antidiabetic plants can be explored [19]). Over 400 traditional plants have been reported for the treatment of diabetes [20]. Furthermore, following World Health Organization recommendations, investigation of hypoglycemic agents from medicinal plants has become more important [19].

*Kigelia africana* (Lam.) Benth (Family: Bignoniaceae) plant has many medicinal properties due to the presence of numerous secondary metabolites. Crude extracts of herbs and spices and other materials rich in phenolics

are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Flavonoids are groups of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [21].

Due to paucity of scientific documentation on anti-diabetic properties of the plant, it is therefore the aim of the study to explore anti-diabetic and antioxidative potential of Methanol extracts the plant.

## II. MATERIALS AND METHOD

**Plant Materials:** The leave and fruit of *Kigelia africana* were collected from Omor, Ahamelu Local Government Area, Anambra State, Nigeria. The plant was authenticated by the Department of plant Science and Biotechnology University of Nigeria Nsukka.

**Chemicals:** All the used chemicals were of the highest analytical grades commercially available.

**Extraction of the Plant Material:** The leaves and fruits of *K. africana* were air-dried at room temperature for after which it was grounded into powders. A quantity of 500mg each of the powdered leaves and fruits of *K. fricana* macerated in 2 litres of Methanol for 72h. The solution was filtered with whatman no 4 and concentrated using rotary evaporator.

**Animals:** Male Wistar Albino rats between 12 to 14 weeks of age, with average weight of 108±5 g were obtained from the Department of Zoology, University of Nigeria Nsukka. They were housed in the animal facilities of Department of Home Science and dietetics, University of Nigeria, Nsukka for one week before starting the experiment. The animals were allowed free access to standard diet, water and maintained under optimum conditions of temperature, relative humidity and light period. (12h light/12h dark).

**Induction Of Diabetics:** The rats were fasted (12h) prior to injection of alloxan dissolved in cold citrate buffer (pH. 4.5) in a dose of 160 mg/kg intraperitoneally. The baseline blood glucose level was determined before the induction. On the fourth day blood samples were taken from the tail vein to measure the blood glucose level using Accu-check glucose meter. Rat with blood glucose level of 200 mg/dl and above were considered diabetic and used for the study.

The treatment was for a period of 4 weeks in which the bloods obtained were used for parameters analysis.

**Experimental Design:** Thirty (30) male Wistar albino rats with average weight of 108±5g were classified into 6 groups (5 rats per group) and subjected to treatment as follows.

**Group i:** Normal control rats.

**Group ii :** Diabetics untreated rats.

**Group iii:** Diabetic rats treated 2.5mg/kg bwt glibenclamide

**Group iv:** Diabetics rats treated with 500 mg/kg btw methanol leaf extracts.

**Group v:** Diabetic rats treated with 500 mg/kg btw methanol fruit extracted.

**Group vi:** Diabetic rats treated with equal ratio of methanol leaf and fruit extracts.

At the end of the experiment, rats were starved for 12h and blood glucose levels were determined.

**Table 1:** Qualitative phytochemical composition of methanol leaf and fruit extracts of *Kigelia africana*

Extract	Soluble carbohydrate	Tannin	Alkaloid	Hydrogen cyanide	Saponin	Flavonoid	Reducing sugar	Steroid	Glycoside	Terpenoid
Methanol fruit	++	+++	++	+	+	+++	+++	+	++	+
Methanol leaf	++	+++	++	++	+	++	++	++	++	+

**NB**

- + Present in trace concentration
- ++ Present in moderately high concentration
- +++ Present in very high concentration

Blood samples were received into clean dry centrifuge tubes and use for the analysis of the parameters.

**Estimation of the Chosen Biochemical Parameters:** All the chosen biochemical and oxidative parameters were estimated using bio-diagnostic kits and the procedures were strictly followed as outlined in the manual guide.

**Statistical Analysis:** Results were reported as mean± SEM, where appropriate. Both one-and two-way analysis of variance (ANOVA) were used to analyse the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurement. Difference were considered significant where  $p < 0.05$ .

**IV. RESULTS**

**4.1 Qualitative Phytochemical Composition of Methanol Leaf and Fruit Extracts of *K. africana***

**Table1.** Shows relative trace presence of saponin and terpenoids in all the extract samples. In the same vein, hydrogen cyanide and steroid were found to be present in trace concentrations. Relative moderate amount of soluble carbohydrates was found in all the extracts. Interestingly, flavonoid was found in high concentration in the extracts.

#### 4.2 Quantitative Phytochemical Composition of Methanol Leaf and Fruit Extracts of *Kigelia africana*

Table 2 shows the quantitative composition of bioactive compounds present at various concentrations. Significant increase of flavonoid methanol compared with the leaf extracts. Trace concentration of hydrogen cyanides was found in the extracts. All the extracts contained moderate

concentration of alkaloid. High tannin level was equally recorded.

Table 2: Quantitative Phytochemical Composition of Methanol Leaf and Fruit Extracts of *K. africana*.

Table 2: Quantitative Phytochemical Composition of Methanol Leaf and Fruit Extracts of *K. africana*.

Extract	Soluble carbohydrate (mg/100g)	Tannin (mg/100g)	Alkaloid mg/100g	Hydrogen cyanide mg/100g	Saponin mg/100g	Flavonoid mg/100g	Reducing sugar (mg/100g)
Methanol leaf	1.44±0.05	10.31±0.42	3.12±0.11	0.29±0.04	0.52±0.01	2.84±0.01	26.67±1.02
Methanol fruit	1.92±0.25	10.87±0.22	3.21±0.13	0.91±0.01	0.53±0.01	3.36±0.02	20.35±1.01

#### 4.3 Percentage Proximate Composition of Leaf and Fruit Extracts of *K. africana*

The percentage proximate composition of *K. africana* was shown on the Table 3. *K. africana* leaf demonstrated high percentage protein

concentration compared with the fruit. Relative percentage of fibre content was found. Carbohydrates concentration was high in fruit as against leaf

Table 3: Percentage Proximate Composition of Leaf and Fruit Extracts of *K. africana*

	Moisture (%)	Ash (%)	Fats (%)	Fibre (%)	Protein (%)	Carbohydrate (%)
<b>Leaf</b>	5.5	2.7	11.4	2.2	13.9	63.5
<b>Fruit</b>	5.1	1.8	3.7	1.3	10.4	77.5

#### 4.4 Effects of Methanol Extracts of Leaf and Fruit of *K. africana* on Sugar Level of Diabetic Rats

The sugar levels of rats before the experiment in all groups were found to be non-significant ( $p > 0.05$ ) compared with the sugar level of group 2 rats (diabetic untreated) as shown in Table 4. At day 21, a significant increase ( $p < 0.05$ ) was also observed in the sugar level of rats in all groups compared with the sugar level of rats in group 2

(diabetic untreated). There was no significant ( $p > 0.05$ ) variation in the sugar level of rats in group 1 (normal control) at 72 hours after induction and day 21 after treatment compared with the sugar level before the induction. On the other hand, the sugar level of rats in group 4 significantly increased ( $p < 0.05$ ) at 72 hours after induction.

**Table 4:** Effect of Methanol Extracts of Leaf and Fruit of *K. africana* on sugar level of Diabetic Rats

Treatment Groups	Sugar Level (mg/dl)		
	Before Induction	72 Hours After Induction	After 21 Days Treatment
Group 1 (Normal Control)	76.20±5.02 <sup>ab</sup>	78.80±2.71 <sup>ab</sup>	75.40±4.22 <sup>ab</sup>
Group 2 (Diabetic Untreated)	67.40±3.50 <sup>ab</sup>	558.40±14.01 <sup>ac*</sup>	405.40±15.96 <sup>ac*</sup>
Group 3 (Standard Control)	66.40±3.91 <sup>ab</sup>	321.00±115.16 <sup>ab*</sup>	241.20±116.79 <sup>ab*</sup>
Group 4 (Diabetic + Methanol Leaf Extract)	69.80±10.37 <sup>ab</sup>	393.00±150.16 <sup>ab*</sup>	163.80±68.81 <sup>ab</sup>
Group 5 (Diabetic + Methanol Fruit Extract)	64.00±5.33 <sup>ab</sup>	467.60±122.21 <sup>ac*</sup>	185.80±53.60 <sup>ab*</sup>
Group 6 (Diabetic + Methanol Leaf and Fruit Extract)	66.60±12.73 <sup>ab</sup>	342.40±121.43 <sup>ab*</sup>	219.80±131.40 <sup>ab*</sup>

*Results are expressed in mean ± SD; n = 5*

Mean values with different letters as superscripts across the column compared with group 2 (diabetic untreated) are considered significant ( $p < 0.05$ ) while mean values with asterisk (\*) as superscripts across the row compared with the sugar level before the experiment are considered significant ( $p > 0.05$ )

after experiment compared with that obtained before the experiment. Conversely, non-significant ( $p > 0.05$ ) decrease was observed in the body weights of the animals in other groups after the experiment compared with the body weights of the animals before the experiment (Table 5).

#### 4.5 Body Weights of Diabetic Rats Treated with Methanol Extracts of Leaf and Fruit of *K. africana* before and after Experiment

Significant ( $p < 0.05$ ) increases in the body weight of group 1 rats (normal control) and diabetic rat in groups 4 and 5 treated with methanol leaf and methanol fruit extracts of *K. africana* respectively

**Table 5:** Body weights of Diabetic Rats treated with Methanol Extracts of leaf and fruit of *K. africana* before and after experiment

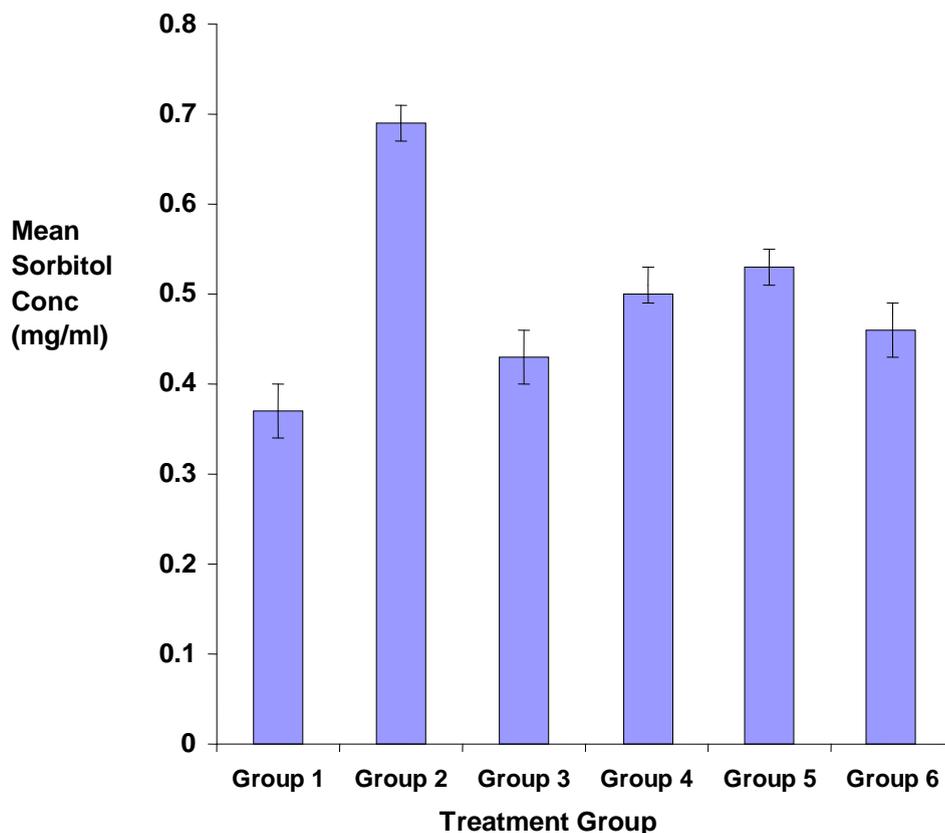
Treatment Groups	Body Weight (g)	
	Before Experiment	After Experiment
Group 1 (Normal Control)	92.59±5.87 <sup>ab</sup>	130.36±17.83 <sup>ac</sup>
Group 2 (Diabetic Untreated)	173.66±12.24 <sup>aa</sup>	156.16±13.14 <sup>aa</sup>
Group 3 (Standard Control)	94.58±5.80 <sup>aa</sup>	107.34±18.41 <sup>aa</sup>
Group 4 (Diabetic + Methanol Leaf Extract)	81.40±4.45 <sup>ab</sup>	112.44±13.83 <sup>ac</sup>
Group 5 (Diabetic + Methanol Fruit Extract)	75.38±6.05 <sup>ab</sup>	101.38±17.57 <sup>ac</sup>
Group 6 (Diabetic + Methanol Leaf and Fruit Extract)	70.29±4.42 <sup>ab</sup>	90.06±18.75 <sup>aa</sup>

*Results are expressed in mean ± SD; n = 5*

Mean values with different letters as superscripts across the row are considered significant ( $p < 0.05$ )

#### 4.6 Effects of Methanol Leaf and Fruit Extracts of *K. africana* on Sorbitol Concentration in Alloxan-Induced Diabetic Rats

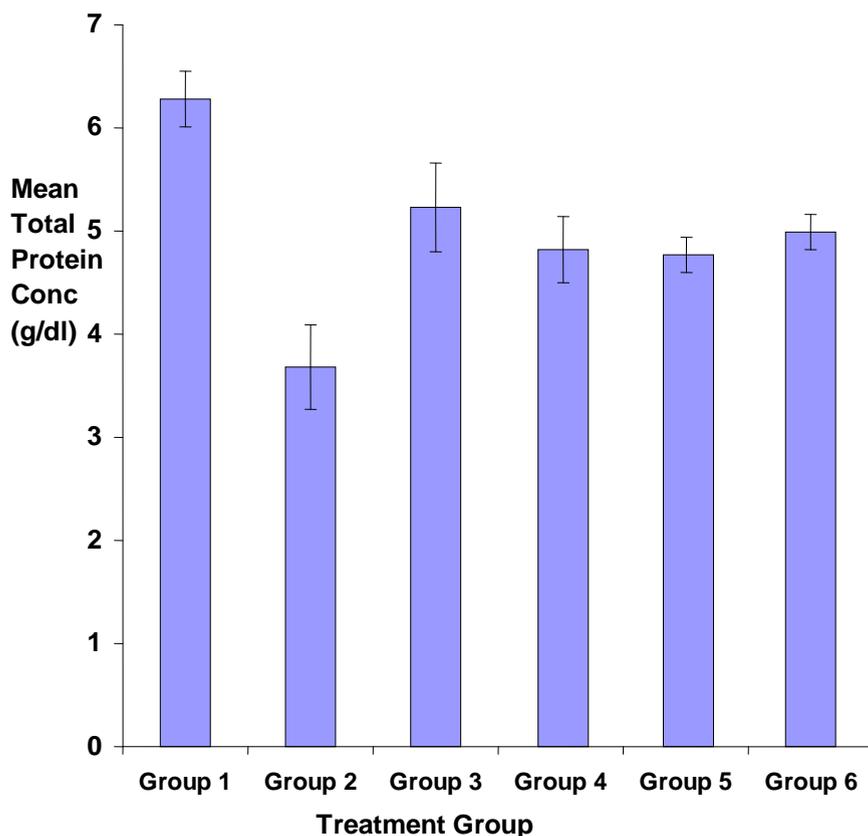
The sorbitol concentration in all the test groups decreased significantly ( $p < 0.05$ ) compared with the untreated diabetic animals (Group 2). A significant ( $p < 0.05$ ) reduction of sorbitol concentration was recorded in groups 6 treated with a combination of the leaf and fruit extracts of *K. africana* compared with the diabetic rats not treated. There was non-significant increase ( $p > 0.05$ ) of sorbitol concentration in all the test rats compared with the normal control rats (group 1) as depicted in Fig. 1 Similarly, non significant increase ( $p > 0.05$ ) of sorbitol concentration was recorded in group 6 (diabetic + methanol leaf and fruit extract) in comparison with group 3 treated with the reference drug, glibenclamide



*Fig.1: Effects of methanol extracts of leaf and fruit of Kigelia africana on Sorbitol concentration in alloxan-induced diabetic rats*

#### *4.7 Effects of Methanol Leaf and Fruit Extract of K. africana on Total Protein Concentration in Alloxan-Induced Diabetic Rats*

Fig. 2 reveals observable significant increased ( $p > 0.05$ ) of total protein was recorded in all test groups compared with the positive control rats (group 2). Total protein concentrations in group 6 orally fed with a combination of the leaf and fruit extracts showed significant increase ( $p < 0.05$ ) compared with test groups 4 and 5 administered methanol leaf and fruit extracts only. A non-significant increase ( $p > 0.05$ ) of total protein was noted across all test groups (groups 4-6) compared with the total protein concentration of group 3 rats fed with the standard drug.

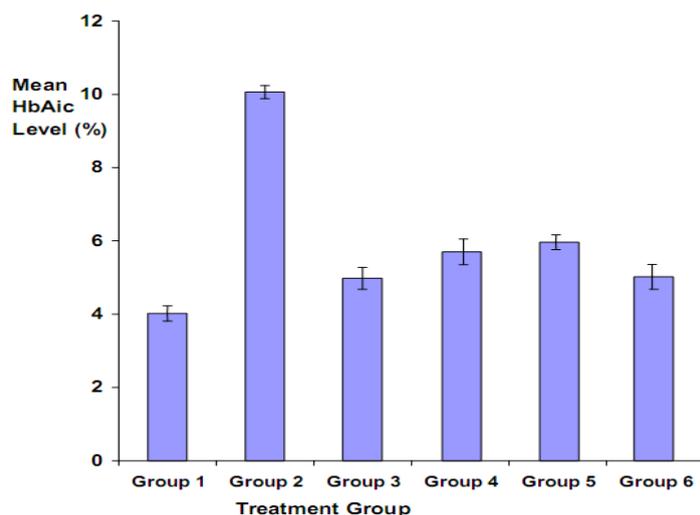


*Fig. 2:* Effects of methanol extracts of leaf and fruit of *K. africana* on Total protein concentration in alloxan-induced diabetic rats

*4.8 Effects of Methanol Leaf and Fruit Extract of K. africana on Glycosylated Haemoglobin Concentration in Alloxan- Induced Diabetic Rats*

The mean HbA1c level decreased significantly ( $p < 0.05$ ) in all the test groups compared with the HbA1c level of untreated diabetics rats (group 2).

Changes in HbA1c level was observed in group 6 rats treated with a combination of methanol leaf and fruit extracts in ratio of 1:1 compared with group 3 rats treated with the standard drug. A significant increase ( $p < 0.05$ ) HbA1c level was recorded in all the test groups against the normal control rats (negative control) Fig. 3.



*Fig.3:* Effects of methanol extracts of leaf and fruit of *K. africana* on Glycated Haemoglobin level in alloxan-induced diabetic rats

#### 4.9 Effects of Methanol Extracts of Leaf and Fruit of *K. africana* on Malondialdehyde (MDA) Concentration in Alloxan-Induced Diabetic Rats

Lipid peroxidation measured as malondialdehyde (MDA) observed significantly increase ( $p < 0.5$ ) in all the test groups compared with untreated control as shown in Fig. 4. A significant decrease

of MDA concentration was recorded in groups 6 treated with the combination of the plant extract compared with the groups administered with single extract (groups 4 & 5). Similarly, concentration of MDA in diabetic rat treated with methanol leaf and fruit extracts (group 6) significantly reduced ( $p < 0.05$ ) as against group 4 & 5.

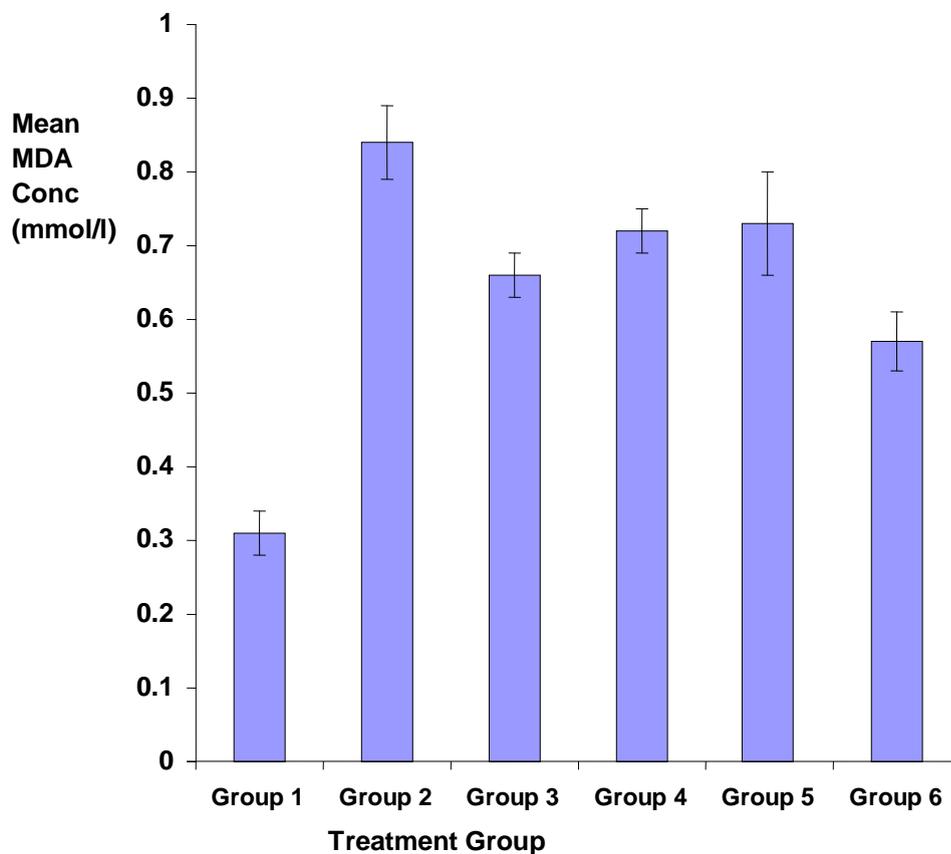
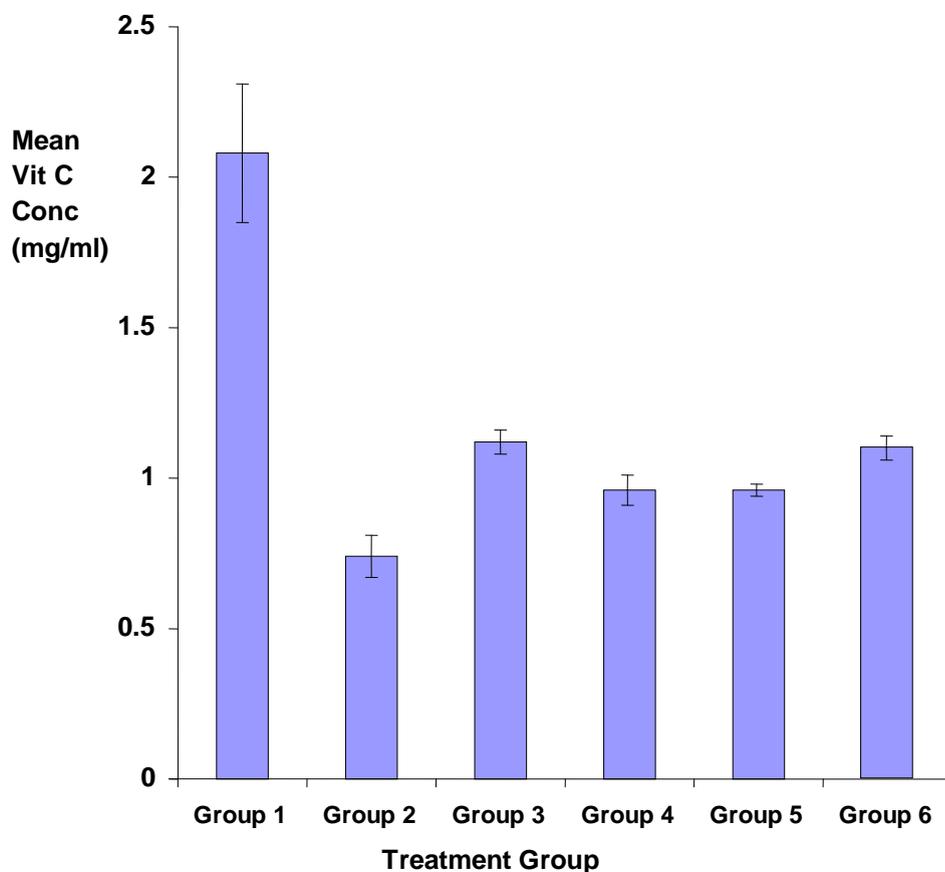


Fig.4: Effects of methanol extracts of leaf and fruit of *K. africana* on Malondialdehyde concentration in alloxan-induced diabetic rats

#### 4.10 Effects of Methanol Leaf and Fruit Extracts of *K. africana* on Vitamin C Concentration in Alloxan-Induced Diabetic Rats

There was a general decrease in vitamin C concentration in all the test groups and the untreated diabetic group compared with the vitamin concentration of normal control rats (group 1). There was statistically significant increase ( $p < 0.05$ ) of vitamin C level in group 6 rats treated with a combination of methanol leaf and fruit extracts compared with other test groups

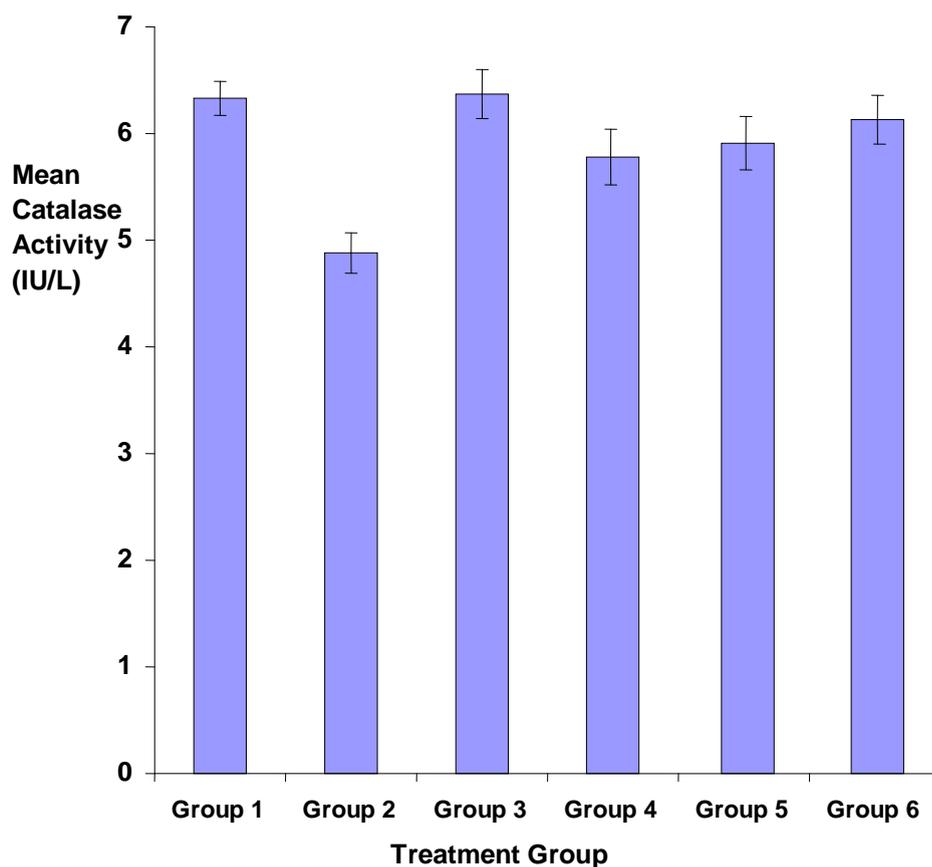
4 & 5. The diabetic rats administered 2.5 mg/kg of glibenclamide demonstrated an increased ( $p < 0.05$ ) vitamin c level compared with the vitamin C concentration of rats in group 2 (diabetic untreated rates), see Fig. 5.



*Fig. 5:* Effects of methanol extracts of leaf and fruit of *Kigelia africana* on Vitamin C concentration in alloxan-induced diabetic rats

#### 4.11 Effects of Methanol Leaf and Fruit Extracts of *K. africana* on Catalase Activity in Alloxan-Induced Diabetic Rats

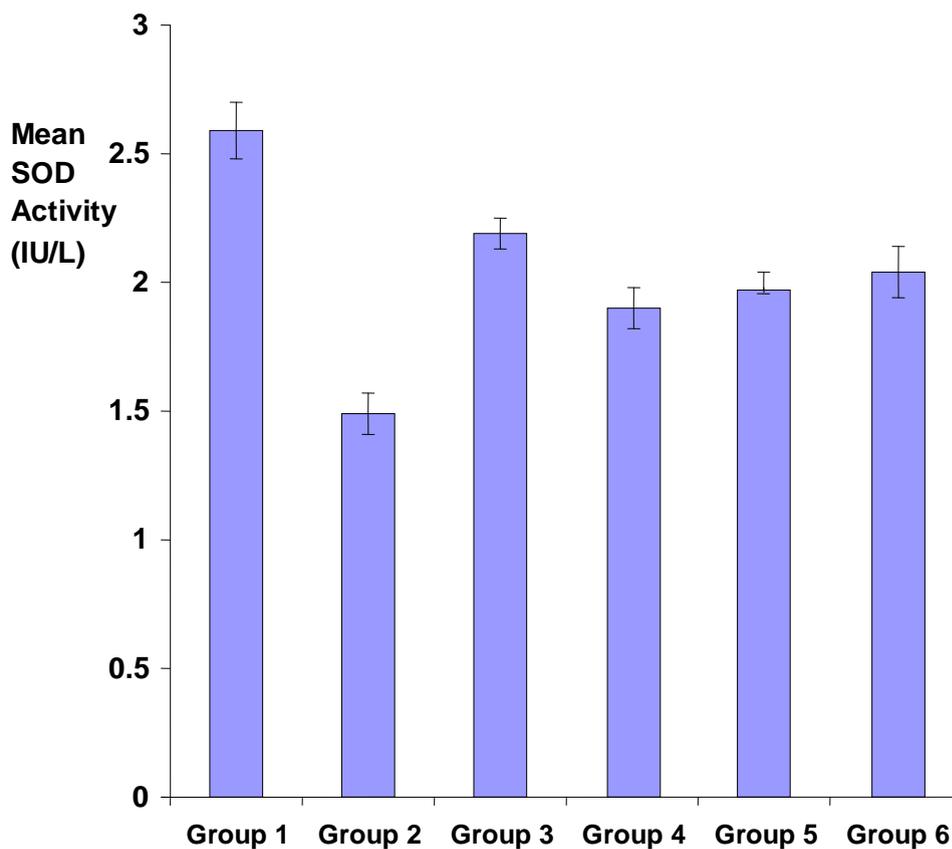
Across the test groups was recorded a statistically significant increase ( $p < 0.05$ ) of serum catalase activity (Fig. 6) compared with the untreated diabetic rats (positive control; group 2). Similarly, a significant increase ( $p < 0.05$ ) of catalase activity was observed in the diabetic rats treated with reference drug (glibenclamide) in comparison with the catalase activity of all the test groups. In the same pattern, groups 6 treated with equal ratio of methanol leaf & fruit extracts demonstrated a non significant increase ( $p > 0.05$ ) of catalase activity compared with other test groups (groups 4 to 5) administered with a single plant extract.



*Fig. 6:* Effects of methanol extracts of leaf and fruit of *K. africana* On Catalase activities in alloxan-induced diabetic rats

#### 4.12 Effect of Methanol Leaf and Fruit Extracts of *K. africana* on Superoxide Dismutase (SOD) Activity in Alloxan-Induced Diabetic Rats

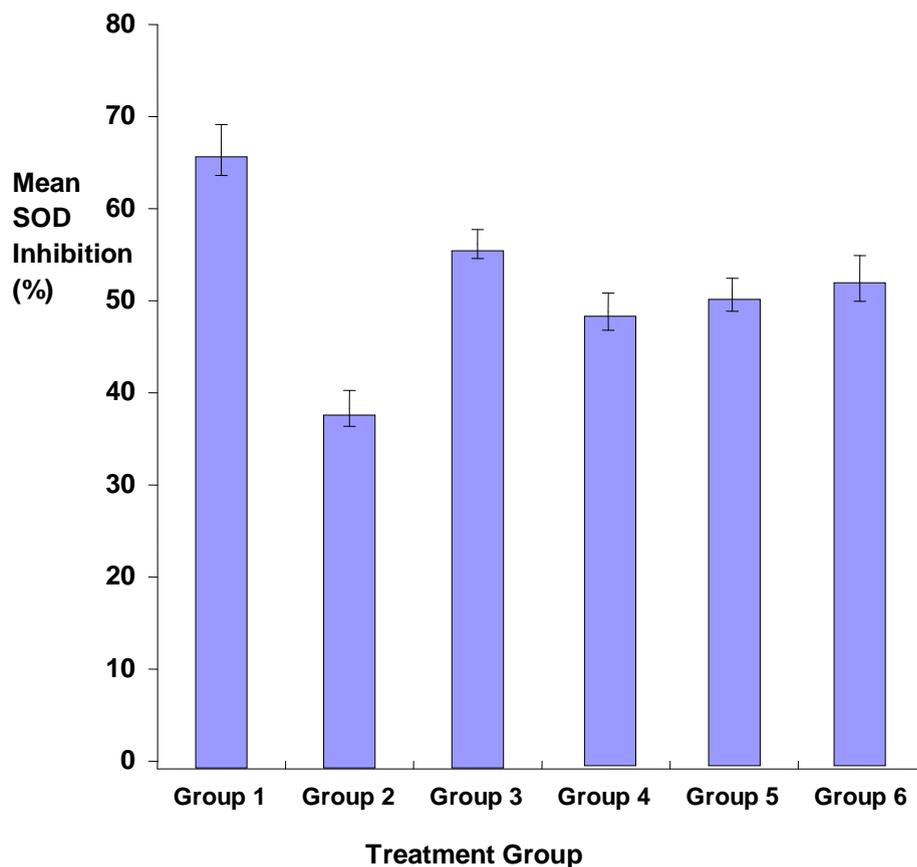
The activities of superoxide dismutase (SOD) reduced significantly ( $p < 0.05$ ) in all the test groups compared with the normal control (group 1). There were statistically significant ( $p < 0.05$ ) decreases in SOD activities of all test groups compared with the untreated diabetic rats (group 2) as shown in Fig. 7. Superoxide dismutase activities of the test group 6 administered with the combination of the extracts was significantly increased ( $p < 0.05$ ) compared with other test groups treated with the single extracts (groups 4 & 5). In the same vein, the activities of SOD in the diabetic rat administered with 2.5 mg /kg body weight of glibenclamide increased significantly ( $p < 0.05$ ) as against test groups.



*Fig.7:* Effects of methanol extracts of leaf and fruit of *K. Africana* on Superoxide dismutase activity in alloxan-induced diabetic rats

#### 4.13 Effects of Methanol Leaf and Fruit Extracts of *K. africana* on Percentage Inhibition of SOD Activity in Alloxan-Induced Diabetic Rats

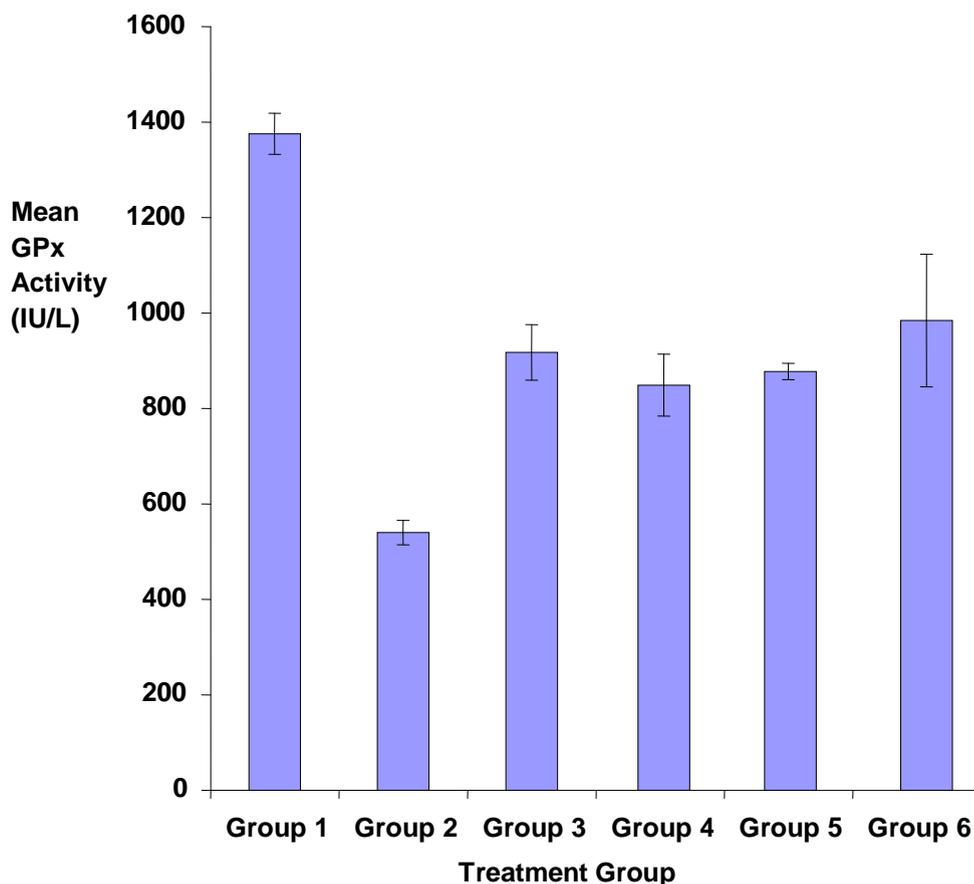
Fig.8 demonstrates statistically significant decrease ( $p < 0.05$ ) of percentage inhibition of SOD activity in the test groups compared with the normal control group. A significant reduction ( $p < 0.05$ ) of percentage inhibition of SOD activity occurred in the diabetic untreated rats (group 2) compared with the percentage inhibition of SOD activity in normal control. Diabetic rats in group 6 treated with a combination of leaf and fruit extracts recorded a non-significantly ( $p > 0.05$ ) increase of percentage inhibition of SOD activity compared with groups 4 & 5 administered monotherapeutically with leaf and fruit extracts of *K. africana*. Furthermore, non-significant reduction ( $p > 0.05$ ) of percentage inhibition was observed in groups 4 & 5 compared with the diabetic rats treated with 2.5 mg/kg body weight of glibenclamide (group 3)



*Fig. 8:* Effects of methanol extracts of leaf and fruit of *K. Africana* on Superoxide dismutase percentage inhibition in alloxan-induced diabetic rats

*4.14 Effects of Methanol Leaf and Fruit Extract of K. africana on Glutathione Peroxidase Activity in Alloxan-Induced Diabetic Rats*

Fig. 9 represents activity of glutathione peroxidase (GPx) which increased significantly ( $p < 0.05$ ) in all the test groups treated with both single and combination of the leaf and fruit of *K. africana* extract in comparison with the GPx activity of the rats in group 1 (normal control rats). The combination therapy in groups 6 demonstrated significant increase ( $p < 0.05$ ) of GPx activity compared with groups 4 & 5 of the test groups treated with a single plant extract (monotherapy). The test group 6 of diabetic rats treated with combined leaf and fruit extracts increased in GPx activity significantly relative to group 3 treated with the standard drug.



*Fig. 9: Effects of methanol extracts of leaf and fruit of K. Africana on Glutathione peroxidase activities in alloxan-induced diabetic rats*

#### *4.15 Effects of Methanol Leaf and Fruit Extract of K. africana on Total Cholesterol Concentration in Alloxan-Induced Diabetic Rats*

Fig. 10 shows relative increase in the total cholesterol concentration in diabetic rats treated in groups 5 & 6 compared with the total cholesterol concentration of normal control in group 1 however such increase was found to be non significant ( $p > 0.05$ ). A significant ( $p < 0.05$ ) decrease was noted in the diabetic rats administered with the standard drug compared with the untreated diabetic rats (group 2). Similar trend of result was observed in total cholesterol concentration of groups 6 treated with a combination of the extracts compared with the total cholesterol concentration in diabetic untreated rats. Furthermore, significant decrease ( $p < 0.05$ ) of total cholesterol concentration in group 3 was observed in comparison with diabetic untreated rats.

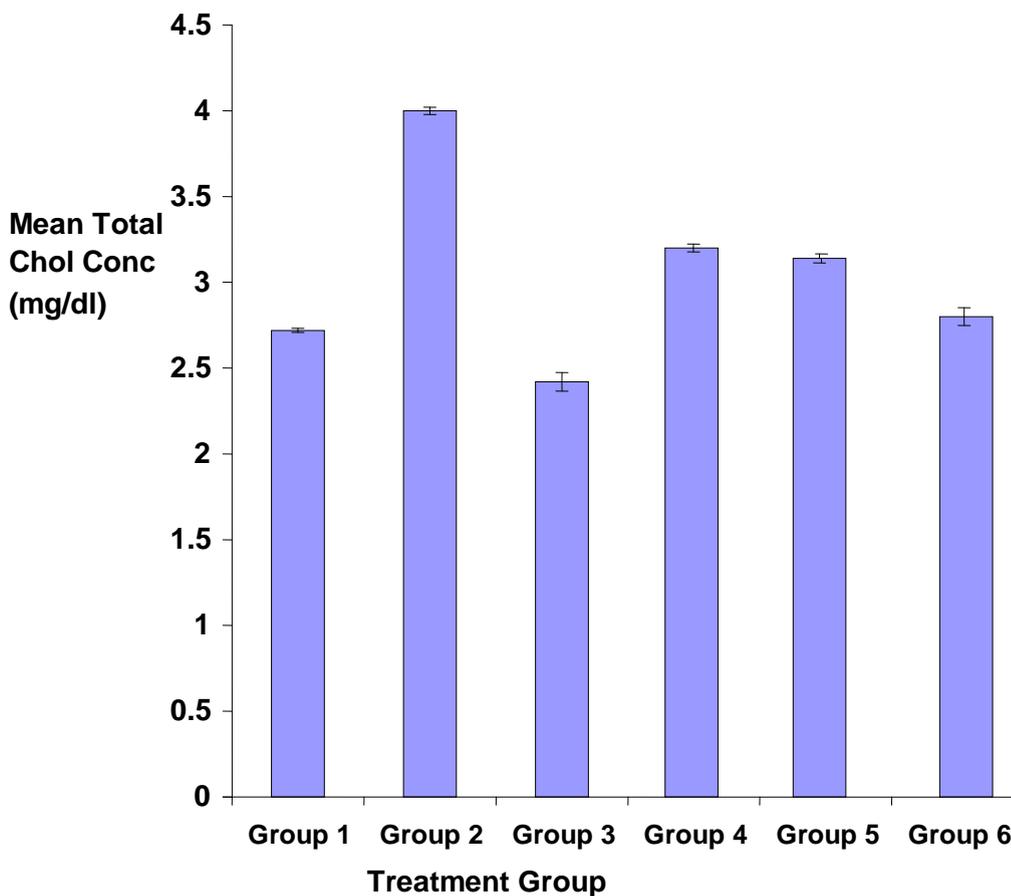


Fig. 10: Effects of methanol extracts of leaf and fruit *K. Africana* on Total Cholesterol concentration in alloxan-induced diabetic rats

## V. DISCUSSION

In animals, diabetes induced experimentally has provided considerable approach on the physiologic and biochemical derangement of the diabetic state. Many of the derangement have been characterized in hyperglycemic animals. Significant changes in lipid metabolism and structure also occur in diabetes.

This study evaluated the antidiabetic and antioxidative properties of *K. africana* in alloxan-induced diabetic rats. From the results obtained; diabetic rats had much higher blood glucose level than that of the normal control. Changes in blood glucose levels reflect abnormalities in  $\beta$ - cells structure and function. In this study, rats with blood sugar level of 500 mg/dl and greater were considered diabetic. Administration of *K. africana* leaf and fruit extracts restored glucose level in alloxan- induced diabetic rats near the normal level. Glibenclamide was used as a standard drug to compare the activity of *K. africana* extract in reference to blood glucose reduction. The results revealed that the extracts in a dose of 500 mg/kg

body weight showed significant effect at 21st day indicating that the extracts possess extra pancreatic hypoglycemic activities. The comparable effect of the extract (500 mg/kg) with glibenclamide (2.5 mg/kg) may suggest similar mode of action, since the main mechanism of the action of glibenclamide is the stimulation of insulin release and the inhibition of glucagon secretion. The possible mechanism by which the plant extract brings about its hypoglycemic action may be by potentiating the insulin effect by increasing pancreatic secretion of insulin from  $\beta$ -cells [22]. The findings also suggest that *K. africana* leaf and fruit extracts may generate  $\beta$ -cells and have protective effect on  $\beta$ - cells from glucose toxicity. Some plants have also been observed to exert hypoglycemic activity through insulin release stimulatory effect [23]. In general, there is little biological knowledge on the specific modes of action of plants in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, and flavonoid that are frequently implicated as having antidiabetic effect [24]. This

was also buttressed by the results of the phytochemistry of *K. africana* which revealed high percentage of flavonoid, glycoside, alkaloid, terpenoid among other constituents. These plant constituents can lower blood glucose level.

The alloxan-induced diabetic rats had a marked loss in body weight. Free radical generated under hyperglycemic condition could attack major biomolecules such as proteins, DNA and lipids and which could lead to the weight loss recorded in this work. Increased synthesis of ketone bodies coupled with increased lipolysis seen in diabetes leads to a severe body weight loss. However, the diabetic rats orally fed with *Kigelia* plant extracts had a remarkable gain in body weight compared with diabetic untreated rats. Significant increase ( $p < 0.05$ ) in body weight was recorded in the group administered a combination of the plant extracts in comparison with the group treated with a single extract. In addition, the observed decrease in body weight of diabetic animals agreed with Torres *et al.*, 1999, who also noticed a marked reduction in the body weight of animal with significant increase in serum triacylglycerol in STZ- induced diabetic rats

Sorbitol concentration significantly decreased ( $P < 0.05$ ) across all the test animals in reference to diabetic untreated rats. This reduction is probably due to the antioxidant contents of the plant extracts. Sorbitol is a product of polyol pathway and is a feature of diabetic complications. It could be suggested that some of the active constituents of *K. africana* extracts inhibit the activity of aldose reductase; the major enzyme in the polyol pathway. An increased flux of glucose via the polyol pathway leads to intracellular accumulation of sorbitol. Accumulation of this non-permeable sugar in cells especially the lenses and nerves results in osmotic stress, cellular edema, redox imbalance, depletion of water soluble antioxidants and susceptibility to oxidative insult [25]. This is implicated in the pathogenesis of long term complication in diabetes mellitus.

This study further revealed significant reduction ( $P < 0.05$ ) of sorbitol concentration in group 6 rats treated with the combination of leaf and fruit extracts relative to animals treated with the

reference drug (2.5 mg of glibenclamide). This is in line with the fact that synthetic drugs do not restore normal glucose homeostasis and are not free from side effect [18].

A significant ( $p < 0.05$ ) increase in glycosylated haemoglobin level in the diabetic rats untreated with reference to the normal control animals (group 1) was recorded in this study. The increase is in accord with the report of several other researchers [26, 27, 28]. The increased glycosylation may be as a result of diabetic complications caused by oxidative stress. Generally, decreased in glycosylated haemoglobin level was observed in diabetic rats treated with *K. africana* extracts as against diabetic rats not treated. Decrease in glycohemoglobin level could be attributed to the extracts' ability to reduce glucose level in the blood stream with corresponding decrease in glycated haemoglobin level.

A Significant increase ( $p < 0.05$ ) in serum total protein was recorded in all the test groups treated with the plant extracts in comparison with the diabetic untreated rats. Decrease in serum total protein was observed in untreated diabetic rats with reference to test groups administered both single and combination of the plant extracts. This is in tandem with the proximate composition of the plant that revealed approximate 13% protein.

High concentration of MDA in diabetic untreated established oxidative stress status in the animals. In hyperglycemic condition, glucose is one of the major sources of free radicals. Malondialdehyde (MDA) significantly ( $p < 0.05$ ) decreased in all the test groups compared with diabetic rats untreated (group 2). Group 6 treated with a combination of leaf and fruit extracts showed significant ( $p < 0.05$ ) decrease in MDA concentration as against groups 4&5 treated with single extract. Reduction in the lipid peroxidation index in treatment groups indicates the ability of the extracts to stem down the oxidative stress by mopping up free radical that lead to lipid breakdown. The bioactive constituents of the extracts such as flavonoids, alkaloids could be implicated in free radical scavenging properties of the extracts.

This study revealed marked increase in serum total cholesterol level. Diabetes is associated with altered lipid levels. The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [29, 30] and these contribute to coronary artery disease. This lends credence to the significant ( $P < 0.05$ ) increase of total cholesterol in the diabetic rats used in this study. *K. africana* treated rats, showed a reduction in total cholesterol which buttressed the hypolipidemic effect of the plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis [1]. It could also be attributed to the increase in the reverse cholesterol transport pathway and decreased cholesterol concentration from the intestine due to  $\alpha$ -glucosidase inhibition. In normal metabolism insulin activates the enzymes lipoprotein lipase and hydrolyses triacylglycerol. A deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia [31, 32]. Administration of a combination of leaf and fruit extracts of *K. africana* resulted in a significant ( $p < 0.05$ ) decrease in lipid parameter compared with the diabetic control animals (group-2). It can be further stated that *K. africana* plant extracts have the potential to correct the lipid abnormalities, thus delaying lipid peroxidation in diabetic condition.

In this study, significant ( $P < 0.05$ ) decreases in the activities of SOD, CAT and GPx were recorded in diabetic rats not treated compared with the normal control group. An observed significant ( $p < 0.05$ ) increases of these antioxidant enzymes were recorded in group 6 treated with a combination of two parts of *K. africana* extracts as against groups 4&5 with mono therapeutic administration of leaf and fruit extracts of the same. Reduction of the antioxidant enzymes was observed in diabetic rats not treated with reference to test rats treated with the standard drug. This is in line with the report that products of membrane lipid peroxidation and other oxidants like  $H_2O_2$  may react with superoxide dismutase resulting in oxidative modification thereby causing loss of enzyme activity in diabetic condition [33]. The result, also concurs with the

reports that the relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, pancreatic  $\beta$ -cells may be vulnerable to reactive oxygen species (ROS) attack when the system is under oxidative stress situation [34,35]. Similarly, elevated levels of free radicals, due to insufficiency of the antioxidant defense system, may lead to disruption of cellular functions, oxidative damages to protein, DNA, membranes and enhance their susceptibility to lipid peroxidation [16] under uncontrolled diabetic condition. Also hyperglycemia leads to glycation and inactivation of superoxide dismutase thus attributing to its decrease. In the study, the animals treated with *K. africana* extracts showed increase in the activity of antioxidant enzymes as against untreated diabetic rats (group 2) and this unveiled the extracts' potential in mopping up or scavenging free radicals generated under oxidative stress mediated diabetes. The bioactive compound, flavonoid may be implicated in the scavenging activity of the plant extracts in oxidative condition.

The fact that normal cells are protected against peroxidative damage *in vivo* can be attributed to efficient antioxidant mechanisms. This antioxidant protection is in part a function of the integrity of each cellular constituent, and in part a reflection of antioxidant system within the cell. In disease conditions, where oxidative stress plays causative and/or exacerbating roles, this mechanism is impaired. Antioxidant vitamins, such as vitamin C and E may be then low in such system. From the above premise, the low plasma vitamin C concentration obtained in group 2 of untreated diabetic rats compared with the control group 1 and the test rats (group 6) is a manifestation of oxidative stress. The fact that vitamin C protects against oxidative stress is now generally accepted [36,37]. Thus; decrease in vitamin C concentration in the present study of untreated diabetic rats is probably a consequence of its protective roles.

## VI. CONCLUSION

From the results, it can be concluded that 500 mg of *K. africana* extracts possess antihyperglycemic

effect via  $\alpha$ -glucosidase inhibition with corresponding increase in body weight of diabetic rats treated with the extracts. Significant reduction of glycohemoglobin level and sorbitol concentration was obtained in the all the diabetic treated groups in reference to positive control and therefore supporting sugar reduction ability of the extracts. The extracts were found to have lipid lowering effects through reduction of total cholesterol probably by increasing reverse cholesterol transport pathway. *K. africana* extracts exhibit antioxidant scavenging properties by reducing malondialdehyde concentration; hence retard lipid peroxidation. Antioxidative potential of the extracts was ascertained through increase in activities of antioxidant enzymes; CAT, SOD, GPx and antioxidant vitamin (Vitamin C) in test animals as against positive group. The increase in the antioxidant activities was due to the ability of the extracts to mop-up free radical generated under stress conditions. Total protein was improved in all the treatment groups. In general, the possible mechanisms by which *K. africana* brings about antidiabetic activities include: glycosidase (glucosidase) inhibitor mechanism,  $\alpha$ -amylase inhibitor mechanism, inhibition of hepatic glucose metabolizing enzyme mechanism, antioxidant mechanism, inhibition of glycosylation of haemoglobin mechanism and modulation of glucose absorption from the gut. Therefore, management and prevention of diabetes complications can be achieved by use of *K. africana* extracts.

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# Rejuvenation of Bio-Fertilizer: An Alternative Source

*Digambar Shelke*

*Savitribai Phule Pune University*

## ABSTRACT

Indian scenario shows the infertility of chemical fertilizer is increasing day by day as compare to natural or bio-fertilizer so we are facing the symptoms of illness like birth defect, neurological condition like attention deficit, hyperactivity disorder (ADHD), chlorine illnesses like diabetes and degenerative diseases like cancer also, the effect of chemical fertilizer in the farming & agriculture are very high. Farmers were happy of getting increased yield in agriculture in the beginning, but slowly chemical fertilizer started displaying their ill-effect such as: leaching out, polluting water basin, destroying micro-organisms and damaging the plant, hence reduce the crop yield, organic matter form soil leads to soil acidification also excess amount of nitrogen used in chemical fertilizer can cause to the release of greenhouse gases like carbon dioxide and nitrous oxide.

*Keywords:* bio-fertilizer, cow's dung & urine, compost, insecticide, germinator.

*Classification:* FOR code: 279999

*Language:* English



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# Rejuvenation of Bio-Fertilizer: An Alternative Source

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## ABSTRACT

Indian scenario shows the infertility of chemical fertilizer is increasing day by day as compare to natural or bio-fertilizer so we are facing the symptoms of illness like birth defect, neurological condition like attention deficit, hyperactivity disorder (ADHD), chlorine illnesses like diabetes and degenerative diseases like cancer also, the effect of chemical fertilizer in the farming & agriculture are very high. Farmers were happy of getting increased yield in agriculture in the beginning, but slowly chemical fertilizer started displaying their ill-effect such as: leaching out, polluting water basin, destroying micro-organisms and damaging the plant, hence reduce the crop yield, organic matter from soil leads to soil acidification also excess amount of nitrogen used in chemical fertilizer can cause to the release of greenhouse gases like carbon dioxide and nitrous oxide.

Bio-fertilizers help to colonize the rhizosphere or the interior of the plant and increasing the growth of the plant by maintaining the availability of micro-organisms. The micro-organisms can regain the soil's natural nutrient cycle and established soil biological matter. Continuous use of bio-fertilizers makes the soil rich in essential nutrients, which promotes good yield. The bio-fertilizer can be needed to reduce the use of synthetic or chemical fertilizer and pesticide. Bio-Fertilizer can be prepared by both ways in solid as well as in liquid form.

This paper is underline In view of the shifting focus towards production methodology, uses as well applications of bio compost, insecticides,

Germinator at homemade and also large scale production. so public awareness programmers to enhance the extra potential of sustainable agriculture development as well as encouraging private organization and policy makers to take interest in this field.

**Keywords:** bio-fertilizer, cow's dung & urine, compost, insecticide, germinator.

**Author:** Department of Chemical Engineering, Sir Visvesvaraya Institute of Technology, Nashik.

## I. INTRODUCTION

Bio-Fertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrient in a form easily assimilated by plants.

Bio-fertilizers are selective live micro-organism like bacteria, fungi and algae. They provide a cost effective, eco-friendly & renewable source of nutrients. Bio-fertilizers improve the nutrient availability to the crops in which biological process is involved. They play a vital role in improving soil fertility and ensure maintaining long term sustainability.

Use of bio-fertilizer is one of the important component of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of bio-fertilizer.

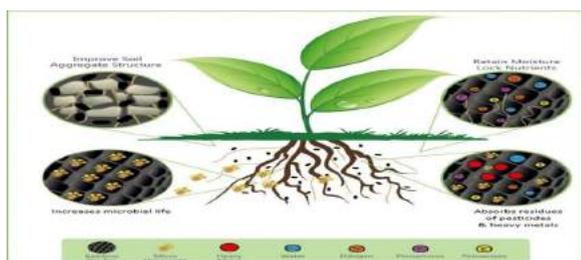


Figure 1: Working of Bio-Fertilizer

Bio-fertilizers become popular to counter the negative impact of indiscriminate use of chemical fertilizers. Chemical fertilizers and pesticides have played an important role in boosting the agricultural production for past 50 years in India, since their introduction during green revolution. Their immediate action and low cost resulted in the widespread acceptance and inclusion in cultivation practices. However their long term application contributed in loss of soil fertility along with addition of salts to the soil.

## II. METHODOLOGY

Bio-fertilizer is basically divided into three categories such as compost, insecticide and Germinator. The ingredients which are required for making this bio-fertilizer are easily available in the rural area.

### 2.1 Compost

Table 1: Component of Compost with quantity

Name of Component	Quantity (kg)
1. Cow's Dung	15
2. Cow's Urine	15
3. Wasted Jaggery	1
4. Wasted Dal Flour	1
5. Soil of Pimple or Banyan's Bud	1

Mix all the components in one drum in cold place away from the sunlight and hot places for 15days. Open the drum on 16<sup>th</sup> day we found it in dried form. Add 200lit water with this compost and spread it over 1Acer land for better illness control.

### 2.2 Insecticides/Pesticide

Table 2: Compositions of Insecticides

Name of Component	Quantity (kg)
1. Cow's Urine	20
2. Neem's Leaves	2-2 1/2
3. Sitafal's Leaves	2-2 1/2
4. Calotropis Gigantean Leaves	2 1/2 - 3
5. Ipomoea Cornea Leaves	2 1/2 - 3
6. Tobacco's Leaves	1/2 - 1/3
7. Garlic	1/2
8. Chilies	1/2

Take a one drum above a 30lit. Crushed all the Leaves in 20lit urine and add garlic and chilies. Mix the entire component in well manner and boil it about a 30-40°C and cold it. After cooling separate the liquid mixture and scrap. We can use liquid insecticides with 200lit water for 1acer land to better control on insects.

### 2.3 Germinator (Before Sowing)

Table 3: Compositions of Germinator

Name of Component	Quantity (kg)
1. Cow's Dung	1
2. Cow's Urine	1
3. Lime Powder	1
4. Turmeric	1/4

Mix all above component, add seeds in it and keep in close pot for one night. Dry it in next day and use for sowing.

Apart from this bio-fertilizer if found another symptoms then we can use a following ingredient as a pesticide:

1. Bacterial cancer – Ash of wood or Cow's dung,
2. Leaf spot – 1lit goat milk with 15lit water for 1/3 Acer land,
3. Sawfly larvae - juice of sitafal and Neem,
4. For green leaf – Aloe Vera and cow's urine

### III. RESULT AND DISCUSSION

We discuss here about the result of bio-fertilizer on a crop. In the first table we can see an effect of bio-fertilizer and plant growth regulator on growth attributes of fenugreek and second table effect of bio-fertilizer and different sources of organic on wheat crop height (cm) at different growth stages on Mize under field condition.

Crop Height increased with enhancing chemical fertilizer application for non-inoculated seeds.

*Table 4:* Effect of bio-fertilizer and chemical fertilizer on wheat crop height (cm) at different growth stages on Mize under field condition

Days		15	30	45	60	75	90	105	120 (at harvest)
Wheat Crop Height (cm)	Bio-fertilizer	12.8	21.38	90.33	145.2	147.03	156.98	158.26	162
	Chemical Fertilizer	8.3	15.23	70	146.3	155.03	158	162	170

### IV. CONCLUSION

1. Bio-fertilizer (Azotobacter) increases the efficiency of nitrogen fertilizer, increases yield of chili and bring more profit to farmers and also reduce the cost.
2. Higher dose of nitrogen (100kg/ha) of bio-fertilizer produced taller plant, which is an important attribute for higher yield.
3. Higher dose of nitrogen and seeding inoculation of Azotobacter is associated with

There was no significant difference between control and 33% fertilizer, while 66 and 100% fertilizer application resulted in more yield than control. A similar result was observed when seeds were inoculated by PSB bio-fertilizer. Crop Height yield of plants from non-inoculated and inoculated seeds with PSB at all chemical fertilizers was not different.

higher fruit weight, more number of seeds per fruit and higher dry weight but physiological weight loss is problem.

4. Bio-Fertilizer increases the growth of micro-organisms as well as earthworm which increase nutrient availability, better drainage, and a more stable soil structure. This will happen by the use of bio-compost.

*Table 5:* Comparison of chemical fertilizer Vs. Bio-fertilizer

	Chemical fertilizer	Vs.	Bio-fertilizer
Features	Chemical Fertilizer		Bio-fertilizer
Raw material	Non-renewable		Renewable
Energy	Fossil fuel		Solar
Reductant	H <sub>2</sub>		Organic
Catalyst	Al, Fe, Mo oxides		Nitrogenizes enzyme
Temp. & Pressure	750°F, 200-600atmm		Ambient T, P
Energy required	680kj.mol <sup>-1</sup> .NH <sub>4</sub> <sup>+</sup>		355kj.mol <sup>-1</sup> .NH <sub>4</sub> <sup>+</sup>
Efficiency	40-45%		90%
Pollution Effect	Exists due to indiscriminate use		Pollution free
Cost	High cost input @Rs.6/KgN		Low cost input @Rs.0.20Kg
Soil Health	Deteriorates		Improves

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