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Roots Extractivism in Indigenous Malaria Control in Ekiti State, Nigeria

By J. Kayode & M.A. Omotoyinbo

Ekiti State University, Nigeria

Abstract- A combination of field surveys and direct observation was used to identify botanicals whose roots are extracted for anti-malaria purposes. A total of 33 botanicals were identified as having their roots being exploited for anti-malaria utilization in the study area. 14 of these species, representing 42% of the botanicals, were being cultivated while 58% were not cultivated. Most of the uncultivated species were indigenous tree species that has forest as their primary source. With the increasing deforestation, there is the need for conservation of these species. Strategies that would enhance the sustainable utilization of the species and make them available to the present and future generations were proposed.

Keywords: conservation, ethnobotanocals, roots extrac-tivism, malaria.

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ROOTS EXTRACTIVISM IN INDIGENOUS MALARIA CONTROL IN EKITI STATE, NIGERIA

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Roots Extractivism in Indigenous Malaria Control in Ekiti State, Nigeria

J. Kayode [α] & M.A. Omotoyinbo [σ]

Abstract- A combination of field surveys and direct observation was used to identify botanicals whose roots are extracted for anti-malaria purposes. A total of 33 botanicals were identified as having their roots being exploited for anti-malaria utilization in the study area. 14 of these species, representing 42% of the botanicals, were being cultivated while 58% were not cultivated. Most of the uncultivated species were indigenous tree species that has forest as their primary source. With the increasing deforestation, there is the need for conservation of these species. Strategies that would enhance the sustainable utilization of the species and make them available to the present and future generations were proposed.

Keywords: conservation, ethnobotanocals, roots extractivism, malaria.

I. Introduction

Malaria is a life-threatening parasitic disease caused by *Plasmodium* parasites that are transmitted by female anopheles mosquitoes. Estimates revealed that over 60% of hospital attendance in Nigeria health care facilities was due to malaria. The disease is also known for lowering mental and manual productivities as well as contributing to the level of poverty in the country.

There abounds a wide indigenous knowledge about the disease and its cure most especially among the aboriginal communities in the rural areas of the country (Kayode 2002). Indeed a myriad of myths abound on the various diseases in the rural areas of Ekiti State, Nigeria. For example, it is believed that malaria may be caused by staying and/or working too long in the sun, by drinking contaminated water and by witchcraft. Also abounds is a wide knowledge of the medicinal values of botanicals in their neighborhoods. Over 70% of the population in the state resides in rural areas. Kayode (2004) asserted that most of these people depend on the environment for the maintenance of their health.

In the recent time, there has been a resurgence of interest on the use of botanicals for health management and maintenance in Nigeria (Kayode 2006). This is considered necessary particularly now that the forest that constituted the primary source of these botanicals is seriously been threatened. Nigeria, a country in the heart of the tropical rainforest belt, has lost most of its total forest cover. The rate of deforestation in the country has been estimated to be at an average of about 3.5 per cent per annual. Deforestation at this rate translates to loss of 350,000 to 400,000 ha of Nigeria's forest land per year (Ladipo 2010).

Consequent on the above, attempts are being made to document botanicals that have medicinal value with a view to determining their abundance, identifying the endangered species and evolving strategies that would conserve the identified the rare species thus enhancing their sustainability for the present and future generations.

II. Materials and Methods

The methods of Lipp (1989, Kayode 2002 and 2005) which consisted of a combination of social surveys and direct field observation was used in this study. Ekiti State was divided into three zones based on the existing political delineation. In each zones, 10 communities that were still relatively free from urban influence were selected.

In each community, 10 indigenes that had maintained continuous domicile for a period of 10 years and above were selected and interviewed with the aid of a semi-structured questionnaire matrix. The interviews were conducted with a fairly open framework that allowed for focused, conversational and two-way communication as suggested by Moniar (1989).

Botanicals whose roots were extracted and used as anti-malaria were identified and their voucher specimens were collected. The sources of such botanicals and their methods of utilization were also identified. The abundance of the species was also determined using the time taken to physically come in contact with the sample of the botanical within the aboriginal plant communities in the community. Where a sample was sighted within 20 minutes, it was considered as very abundant, it was abundant when sighted within 21-60 minutes but rare when it takes more than 60 minutes. The voucher specimens were later identified and deposited at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

Key informants that were made up of officials of Health Department of the Local Governments Authorities, Ministry of Health, Hospital and other

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stakeholders, were interviewed. Also, in each community, group interviews were also carried out. Each group consists of a minimum of 5 respondents. This was done to attain group consensus on the suitability of the species identified at the individual level as advocated by Kayode and Omotoyinbo (2009).

III. Results and Discussion

A total of 33 botanicals were identified as having their roots being exploited for anti-malaria purpose. 14 of these species that belong to 11 families (Table 1) were being cultivated in the study area. The species were cultivated for other purposes; most of them were fruit trees that also served as viable source of income (Table 2). These include *Anacardium occidenttale, Bligha sapida, Carica papaya, Citrus aurantifolia, Citrus sinensis, Elaeis guineensis* and *Mangifera indica* thus household farms and household areas constituted the primary and secondary sources of these species. Other species cultivated were meant to serve as shade provider and sometimes for control of wind as wind breakers. These include *Ficus thonningi, Ficus ptatyphylla* and *Senna siamea* thus they were primarily planted in household areas and secondarily in common areas such as schools, churches and markets premises. *Corchorus olitrius* and *Vernonia amydgalinia* were cultivated to provide vegetables primarily in household farms while *Zingiber officinalle* was meant primarily to serve as spice. All these cultivated species were found either as very abundant and/or as abundant. Previous study by Kayode (2005) had asserted that the use of botanicals for medical purposes in the study area had always been byproduct rather than been a main product.

The non-cultivated botanicals consist of 19 species that belonged to 14 families (Table 3). They were mostly indigenous tree species whose wildlings were preserved in the study area. These species were primarily sourced from the forest and secondarily from household farms of individuals with high land holdings. These species include *Alstonia boonei, Allablackia floridunda, Axonopus bonduc, Chrysophyllum albidum, Eniada Africana, Enantia chlorantha, Khaya invorensis, Lecaniodiscus cupanioides, Mallotus oppositifolius, Melanthera scadens, Milicia excelsa, Millattia thonningi, Morinda lucida, Olax subscorpioidea* and *Pterocarpus crinaceus.*

Field observations revealed that a number of reasons served as disincentives to their cultivation (Table 4). These ranged from the long gestation period required for them to grow into maturity, land tenure, inadequate silvicultural knowledge on the species, ignorance, lack of accurate data on the demography of the species, unfavourable legal environment and prevailing agricultural methods practiced in the study area.

Information from respondents revealed that each of these indigenous species takes over 20 years to grow into maturity hence it is considered unwise to invest already scare resources, such as time and labour, on tree cultivation. Elsewhere in Islamabad, Shinwari and Khan (2000) had made similar observation and asserted that most perennials required prolonged period of growth with considerable number of years required to reach flowering and fruiting stage, thus minimizing their regeneration possibilities. Already land fragmentation is now a common phenomenon; hence the utilization of the available land was skewed towards food production. Though field observation revealed that respondents possessed indigenous technical knowledge about these species but such appeared to be limited to their utilization rather than their silviculture. Most respondents did not consider tree production as necessary particularly when they were of the opinion that the issue of biodiversity loss has been exaggerated. Kayode (2006) had observed that respondents in the aboriginal communities of Ekiti State were ignorant of the consequences of biodiversity loss.

At present, there is an apparent lack of individuals and institutions with accurate data on the population of the species in the study area. Estimates were based merely on observation rather than been scientific. Similarly the use of tariff, a form of tax, on trees was considered as unfair and discriminatory thus putting forest production at a disadvantage. No such tax is imposed on crop production in the study area. This observation is similar to that of Kayode (2011). The prevailing agricultural method in practice is shifting cultivation where the cultivator uses a field (land) for one or two growing seasons after which he moves to another. The continuous rotation of land is laborious hence with increasing cost and/or scarcity of labour involved in land preparation, the involvement of fire in land preparation is on the increase, thus eliminating tree wildlings in the process. The problems are further compounded by the fact that most of the indigenous species were high light demanders; hence their seeds remained dormant in the soil for a long time. Thus they are poorly represented in the sapling stage (Kayode 2010).

Results obtained from this study revealed that ten of the uncultivated species were rare on the abundant scale (Table 5). These species are principally timber species in the study area. Extractions of the roots are annihilative and predatory. In fact, Fasola and Egunyomi (2002) described root extraction as one of the highest destructive extractive techniques commonly observed in Nigeria. Kayode and Omotoyinbo (2008) asserted that such extraction usually resulted in scarcity of species, thus, extraction *ad infinitum* could not be guaranteed (Homma 1992). There is therefore the need to examine how the species could be conserved. The indigenous technical knowledge of the respondents on

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these species could be classified into ecology and reproductive biology of the species. The knowledge could be utilized for the conservation of the species. Table 6 enumerates the implications of the indigenous knowledge on the conservation of the species.

In conclusion, the conservation of the rare species would enhance sustainability in the supply of the species. Thus there is the need to domesticate some of these species by planting them in household areas. Communities should be encouraged to own forests. Some of these species could be planted in common areas, such as school compounds, market areas, churches and mosque premises, road sides, wastelands among others. The enlightenment of the populace, previously advocated by Kayode (2006), on the dangers of biodiversity loss is equally relevant. The establishment of botanical gardens in each zones of the state is also necessary. Moore (1990) had described such establishment as a sustainable *in situ* method as such will maintain the original self-perpetuating populations. It is expected that the ready availability of these botanicals will help in the control of malaria in developing areas such as the area where this study was conducted.

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Table 1 : Cultivated botanicals whose roots are exploited for anti-malaria in Ekiti State, Nigeria

S/N	Botanical	Vernacular Name	Family	Sources* 1^0	2^0	3^0
1.	*Azadirachta indica*	Dongoyaro	Meliaceae	HHA	CA	-
2.	*Anacardium occidentale*	Kaju	Anacardiaceae	HHF	HHA	-
3.	*Bligha sapida*	Ushin	Sapindaceae	HHF	HHA	-
4.	*Carica papaya*	Ibepe	Caricaceae	HHF	HHA	-
5.	*Citrus aurantifolia*	Osan wewe	Rutaceae	HHF	HHA	-
6.	*Citrus sinensis*	Osan didun	Rutaceae	HHF	HHA	-
7.	*Corchorus olitorius*	Ewedu	Titiaceae	HHF	HHA	-
8.	*Elaeis guineesis*	Ope	Arecaeae	HHF	HHA	-
9.	*Ficus thonningii*	Odan	Moraceae	HHA	CA	-
10.	*Ficus ptatyphylla*	Agbagba	Moraceae	HHA	CA	-
11.	*Mangifera indica*	Mangoro	Anacardiaceae	HHF	HHA	-
12.	*Senna siamea*	Kasia	Caesalpiniaceae	HHA	CA	-
13.	*Vernonia amydgalinia*	Ewuro	Asteraceae	HHF	HHA	-
14.	*Zingiber officinale*	Ajo	Zingiberaceae	HHF	PH	-

* HHA: Household Area, HHF: Household Farm, CA: Common Area, PH: Purchased

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Table 2 : Products derived from cultivated botanicals whose roots are exploited for anti-malaria in Ekiti State, Nigeria

S/N	Botanicals	Major product(s) that enhance cultivation
1.	*Azadirachta indica*	Shade and Wind control
2.	*Anacardium occidentale*	Fruits and Cash income
3.	*Bligha sapida*	Fruits and Cash income
4.	*Carica papaya*	Fruits and Cash income
5.	*Citrus aurantifolia*	Fruits and Cash income
6.	*Citrus sinensis*	Fruits and Cash income
7.	*Corchorus olitorius*	Vegetable
8.	*Elaeis guineesis*	Fruits and Cash income
9.	*Ficus thonningii*	Shade and Wind control
10.	*Ficus ptatyphylla*	Fruits and Cash income
11.	*Mangifera indica*	Fruits and Cash income
12.	*Senna siamea*	Fruits and Cash income
13.	*Vernonia amydgalinia*	Vegetable
14.	*Zingiber officinale*	Spice

Table 3 : Non- cultivated botanicals whose roots are exploited for anti-malaria in Ekiti State, Nigeria

S/N	Botanical	Vernacular Name	Family	Sources		
				1^0	2^0	3^0
1.	*Alstonia boonei*	Ahun	Apocynaceae	FR	HHF	-
2.	*Allablackia floridunda*	Eku	Clusiaceae	FR	HHF	-
3.	*Axonopus bonduc*	Idi	Poaceae	FR	HHF	-
4.	*Bridelia ferruginea*	Ira	Euphorbiaceae	FR	HHF	-
5.	*Caesalpinia bonduc*	Ayoo	Caesalpiniaceae	FR	HHF	-
6.	*Chrysophyllum albidum*	Agbalumo	Sapotaceae	HHF	FR	-
7.	*Chromolaena odorata*	Akintola	Asteraceae	CA	HHH	HHF
8.	*Eniada africana*	Ogube	Annonaceae	FR	HHF	-
9.	*Enantia chlorantha*	Awopa	Annonaceae	FR	HHF	-
10.	*Khaya invorensis*	Oganwo	Annonaceae	FR	HHF	-
11.	*Lecaniodiscus cupanioides*	Akika	Sapindaceae	FR	HHF	-
12.	*Mallotus oppositifolius*	Orokoro	Moraceae	FR	HHF	-
13.	*Melanthera scadens*	Ako yurinyun	Asteraceae	CA	HHF	-
14.	*Milicia excelsa*	Iroko	Clusiaceae	FR	HHF	-
15.	*Millettia thonningi*	Ito	Papilionaceae	FR	HHF	-
16.	*Morinda lucida*	Ohiho	Rubiaceae	FR	HHF	-
17.	*Olax subscorpioidea*	Ifon	Olacaceae	FR	HHF	-
18.	*Piper guineensis*	Iyere	Piperaceae	HHF	-	-
19.	*Pteocarpus crinaceus*	Osun	Papilionaceae	FR	HHF	-

* HHA: Household Area, HHF: Household Farm, CA: Common Area, PH: Purchased, FR: Forest

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Table 4 : Identified disincentives to tree cultivation in Ekiti State, Nigeria

S/N	Disincentives	Proportion (%) of Respondents that Identified the Disincentive
1.	Long gestation period required for trees to grow into maturity	98
2.	Land tenure	96
3.	Inadequate silvicultural knowledge on indigenous trees	85
4.	Ignorance on the implication of biodiversity loss	95
5.	Lack of accurate data on tree demography	94
6.	Unfavourable legal environment	93
7.	Prevailing agricultural methods	92

Table 5 : Abundance of identified anti-malaria botanicals in Ekiti State, Nigeria

Abundance	Cultivated Species	Non-cultivated
Very Abundant	*A. indica, A. occidentale* *B. sapida, C. papaya,* *C. sinensis C. olitorius* *E. guineensis, M. indica* *S. siamea, V. amydgalinia*	*C. odorata, P. guineensis*
Abundant	*C. aurantifolia, F. thonningii* *F. ptatyphylla, Z. officinale*	*A. bonduc, B. ferruginea* *C. albidum, C. bonduc* *E. chlorantha, M. lucida* *P. crinaceus,*
Rare	-	*A. boonei, A. floridunda,* *E. africana, M. thonningi* *M. oppositifolius, M. scadens* *M. excelsa, O. subscorpionidea*

Table 6 : Implication of the indigenous knowledge on the conservation of the identified rare species

Feature	Indigenous Knowledge	Conservation Implication	Botanicals
Ecology	(a) Thrive well in both forest and savanna vegetations	Suitable for cultivation in all parts of the state	*K. invorensis,* *L. cupanioides,* *O. subscorpioidea* *M. excelsa* *M. scadens* *M. thonningi*
	(b) Thrives well in forest vegetation	Suitable for cultivation in southern part of the state	*A. boonei,* *A. floridunda,* *M. oppositifolius*
	(c) Thrives well in savanna northern part of the state	Suitable for cultivation in	*E. africana*
Reproductive Biology	(d) Flowers during the dry season	Seeds are available for cultivation during rainy season	*A. boonei, A. floridunda* *E.africana, K. invorensis,* *L. cupanioides,* *M.thonningi,M. scadens* *M. excelsa* *O. subscorpioidea*
	(e) Flower throughout the year	Seeds available throughout the year	*M. oppositifolius*

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Establishment of Regeneration Protocol for Canola (*Brassica napus* L.)

By Sabrina Shameen Alam, Laila Khaleda & Mohammad Al-Forkan

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Abstract- The study was conducted with an aim of developing a genotype independent efficient in vitro regeneration system for Canola (Brassica napus L.) that is not grown in Bangladesh. Plant regeneration was achieved through embryogenic callus production from hypocotyls and cotyledonary leaves with petioles. The optimum medium for callus induction was found with 0.5 mgl-1 2, 4- dichlorophenoxyacetic acid (2, 4-D). The best shooting medium for canola contained 3.0 mgl-1 benzyl amino purine (BAP), 0.1 mgl-1 naphtalene acetic acid (NAA), and 5.0 mgl-1 AgNO3 and produced maximum number of shoots when kinetin was used at a concentration of 0.5 mgl- 1. The profound positive effect of AgNO3 for Canola was found for callogenesis at 0.5 mgl-1 and for regeneration at 5.0 mgl-1. In vitro regenerated shoots of canola produced well developed root system on both the media with 2.5 mgl-1 NAA and 1.0 mgl-1 indole 3-butyric acid (IBA).

Keywords: canola, plant regeneration, and ms medium.

GJSFR-C Classification : FOR Code: 970106, 270000

ESTABLISHMENT OF REGENERATION PROTOCOL FOR CANOLA BRASSICA NAPUS L.

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Establishment of Regeneration Protocol for Canola (*Brassica napus* L.)

Sabrina Shameen Alam [α], Laila Khaleda [σ] & Mohammad Al-Forkan [ρ]

Abstract- The study was conducted with an aim of developing a genotype independent efficient *in vitro* regeneration system for Canola (*Brassica napus* L.) that is not grown in Bangladesh. Plant regeneration was achieved through embryogenic callus production from hypocotyls and cotyledonary leaves with petioles. The optimum medium for callus induction was found with 0.5 mgl^{-1} 2, 4-dichlorophenoxyacetic acid (2, 4-D). The best shooting medium for canola contained 3.0 mgl-1 benzyl amino purine (BAP), 0.1 mgl^{-1} naphtalene acetic acid (NAA), and 5.0 mgl^{-1} $AgNO_3$ and produced maximum number of shoots when kinetin was used at a concentration of 0.5 mgl^{-1}. The profound positive effect of $AgNO_3$ for Canola was found for callogenesis at 0.5 mgl^{-1} and for regeneration at 5.0 mgl^{-1}. *In vitro* regenerated shoots of canola produced well developed root system on both the media with 2.5 mgl^{-1} NAA and 1.0 mgl^{-1} indole 3-butyric acid (IBA).

Keywords: canola, plant regeneration, and ms medium.

I. Introduction

Canola is the oilseed crop of cold countries, specially, Canada, USA and Europe as cultivation of Canola requires cool temperature during growing season. Bangladesh has a remarkable demand for edible oil. Among the oilseed crops grown in this country, *Brassica* occupies first position in respect of area and production. In the year of 2003-04, the production was 2.11 lakhs metric ton (Mt), whereas the total oilseed production was 4.0 lakhs Mt (BBS, 2005). Bangladesh consumes around 1.73 million tons of oils per year of which about 1.6 million tons fully met up by import. Hence, it is necessarily important to increase the production of *Brassica* which can save the country from the huge import pressure. Among the *Brassica* species, canola is the most productive variety which is low in both erucic acid (<2% in the oil) and glucosinolates that differentiate it from earlier rapeseed varieties (Eskin and McDonald, 1991). However, this variety is not grown well in the country. So, it is necessarily important to introduce canola in Bangladesh for improving both dietary health and production.

One of the most challenging tasks of the plant breeders in this century is the production of crops with increased sustainability to fulfill the need of present and future in terms of both yield and quality. Though Conventional backcross technique is a very well established one for improving seed quality such as low erucic acid (Agnihotri *et. al.*, 2004), oil crop breeding is complex than breeding of cereals and legumes due to the requirement of simultaneous manipulation of different traits. Comparisons of traits between conventionally derived and genetically modified plants has been assessed (Ian and Adrian, 2003) and quality such as rooting efficiencies in *Agrobacterium*- mediated DNA transformed canola was seen to be increased (Cardoza and Stewart, 2003). However, breeders have developed varieties of canola that are shorter and more resistant to lodging and shattering (Salisbery and Wratten, 1999). The suitable time for harvesting local *Brassica sp.* in Bangladesh is very short and challenging. Hence, this study has been designed to establish an efficient *in vitro* regeneration system for Canola to utilize the *Agrobacterium*-mediated genetic transfer to introduce a user friendly Canola variety in Bangladesh.

II. Materials and Methods

Canola seeds were collected from University of Alberta, Canada. The explants collected from *in vitro* grown seedlings used for the experiments were: hypocotyls and cotyledons with petiole. MS (Murashige and Skoog, 1962) medium was prepared with 3% (w/v) sucrose and solidified with 0.4% (w/v) agar and was stored at 4°C. MS media was supplemented with different PGRs (Plant Growth Hormones) such as 2, 4-D, BAP and additives such as proline, casein hydrolysate (CH) and $AgNO_3$ in a different formulation to adopt with plantlets (callus, shoots, roots etc.) Plant materials were sterilized with 70% Ethanol followed by immersing in 0.1% $HgCl_2$. Explants were inoculated into callus induction media. For callus initiation from hypocotyls and cotyledons, MS basal medium was supplemented with 0.5, 1.0 and 2.0 mg l^{-1} of 2, 4 –D with or without $AgNO_3$ or proline or BAP alone or in combination at a concentration of 0.5 mg l^{-1}. After 28 days from the first inoculation, all embryogenic except non-embryogenic, brown, slow growth and rhizogenic calli were transferred to the regeneration media. The multiple shoots were separated from the calli after 20 days of shoot development and were transferred to separate jars containing respective 60 ml shooting media. Roots were initiated when shoots were placed on medium containing MS basal salts supplemented with 2.5 mg l^{-1} NAA, and 30 g l^{-1} (w/v) sucrose and semi-solidified with

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0.4% agar. The plantlets with sufficient rooting system were taken out of the culture vessels and the roots were washed under tap water. The *in vitro* grown rooted plants were then transferred into small pots containing moisture soil. Hardening was carried out by periodical exposure of the plants to natural environment.

III. Results and Discussions

To avoid contamination of explants, Canola seeds were germinated on half strength of MS medium, 70% ethanol and 0.1 % $HgCl_2$ solution were used for sterilization. However, the use of 0.2% $HgCl_2$ was seen to reduce the percentage of seed germination, delayed the time required for germination and affected the elongation of the seedlings (data not shown). The effect of light on seed germination and seedling development were measured by using half strength of MS with different concentration of sucrose and agar without any growth regulators. Germination was seen to be better under dark, 100% in some cases. Callus initiated from Canola hypocotyls on medium supplemented with 0.5 mg l^{-1}, 2, 4-D, 0.5 mg l^{-1} BAP and 0.5 mg l^{-1} $AgNO_3$, after 24 days of culture (Fig. 1.A.). Large callus was formed in that condition (Fig. 2. A.). However, no significant callus formed on MS medium supplemented with 0.5 mg l^{-1} 2, 4-D, 0.5 mg l^{-1} BAP but without $AgNO_3$ (Fig. 2. B). Browning of explants and little or no callus formation was observed on MS medium supplemented with 0.5 mg l^{-1} 2, 4-D, 0.5 mg l^{-1} $AgNO_3$ but without BAP (Fig. 2. C). Initiation of callus from Canola cotyledons was observed on the medium supplemented with 0.5 mg l^{-1} 2, 4-D, 0.5 mg l^{-1} BAP and 0.5 mg l^{-1} $AgNO_3$, after 24 days of culture (Fig. 1. B.). Using BAP on callogenesis was found to increase the size and percentage of calli formation (data not shown). Similar reports were provided by Ali *et. al.* (2007) and Khan *et. al.* (2002) that supports the present investigation. Again, the use of $AgNO_3$ was described to promote the growth of *Brassica* callus by Williums *et. al.* (1990). The effects of only IAA, and IAA and IBA in combination on callogenesis were also evaluated. In case of hypocotyls about 12% of the calli produced roots within 20 days of culture on medium containing IBA at 0.5 mg l^{-1}. In case of cotyledon, callus induction occurred within two weeks on both the medium and the induced calli were yellowish white. Whitish 1-2% of the cotyledonary explants produced roots in the medium containing IAA at 0.5 mg l^{-1} (Fig. not shown). To evaluate the effect of CH and proline independently on callus induction, BAP and $AgNO_3$ concentrations were kept constant, both at 0.5 mg l^{-1}. The callus was mostly green and overgrowth of callus was observed even when they were placed on shooting medium. Hypocotyls (100%) produced callus of embryogenic nature on this medium though the size of the callus were small and were dominant at the cutting edge of the explants (Fig. not shown). Callus production was better from hypocotyles than cotyledons on proline supplemented media in absence of $AgNO_3$, where the cotyledons swelled but produced very insignificant callus (Fig. 3).

Change in BAP concentration changed the frequency of shoot formation from cotyledonary and hypocotyl calli. Cotyledonary calli gave the best percentage of shoot induction and development (88.46%) when BAP concentration was 2.0 mg l^{-1}. Increasing BAP concentration to 2.5 and 3.0 mg l^{-1} lowered the shoot initiation potential of cotyledonary callus to 60% and 53.84%, respectively (Fig. 4). But increasing BAP concentration increased percentage of shoot production from hypocotyl calli from 53.46% to 75% (Fig. 4.). The initiation of shoots was earlier in cotyledonary calli (About 20% callli started shooting within 10-12 days).

To get a complete plantlet, the induction of root at the base of *in vitro* regenerated shoots is an indispensible step. Spontaneous root generation occurs sometimes on MS medium with hormonal supplements for the induction of shoots. These roots were not in direct connection with the developed shoots and they were developed from the calli placed on the regeneration medium. The plantlets with these roots were not found to be efficient to thrive in soil. Therefore, independent medium for the induction of roots from shoots were used in the experiment. The plant growth regulators (PGR) used for the purpose were NAA and IBA. Roots initiated and developed sufficient root system on medium containing 2.5 mg l^{-} NAA and 0.5 mg l^{-} BAP. Because of the root production efficiency of the calli in callus induction medium containing 0.5 mg l^{-1} IBA, this plant hormone was used in rooting medium at a concentration of 1.0 mg l^{-1}. Shoots produced efficient root system in this medium. The days required for root initiation also varied in different media (data not shown). However, finally, different stages of regeneration from canola callus were established in the study. The best initiation of shoots from hypocotyls was observed on regeneration medium supplemented with 0.5 mg l^{-1} Kinetin, 2.5 mg l^{-1} BAP and 5.0 mg l^{-1} $AgNO_3$ (Fig. 5. A.). Shoots elongated significantly after three weeks of inoculation (Fig. 5. B.). Shoots were developed from cotyledonary callus in the medium supplemented with 0.5 mg l^{-1} Kinetin, 2.5 mg l^{-1} BAP and 5.0 mg l^{-1} $AgNO_3$, (Fig. 5. C.) and canola plantlet were precisely regenerated in medium supplemented with 2.0 mg l^{-1} BAP, 0.1 mg l^{-1} NAA and 5.0 mg l^{-1} $AgNO_3$ (Fig. 5. D.)

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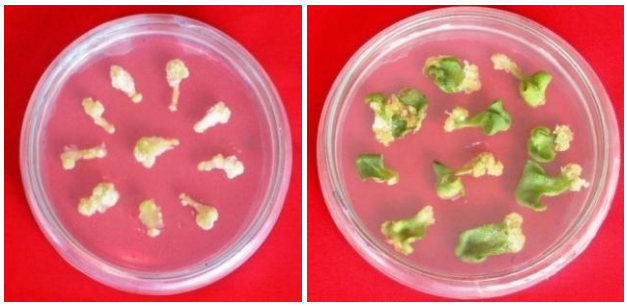
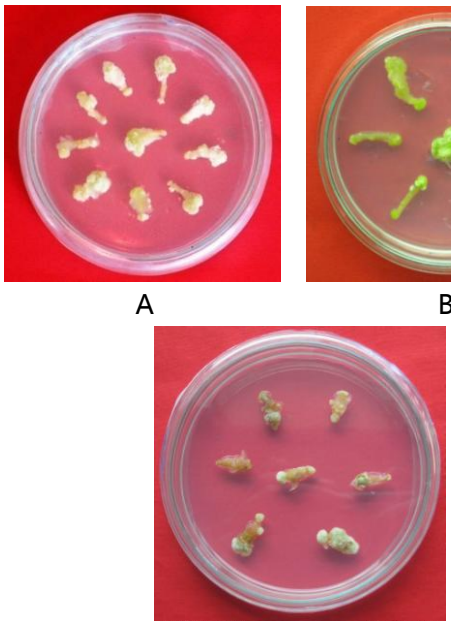


A B

Fig. 1 : Calli grown from canola hypocotyls (A) and cotyledons (B) on medium supplemented with 0.5 mg l^{-1}, 2, 4-D + 0.5 mg l^{-1} BAP + 0.5 mg l^{-1} $AgNO_3$, after 24 days of culture

A B

Fig. 3 : Effect of proline on callus induction and development from hypocotyls (A) and cotyledons (B) of Canola, after 24 days



A B C

Fig. 2 : Effect of $AgNO_3$ and BAP on callus induction and development from hypocotyls of canola. Changes according to the discussion are shown from panel A to C

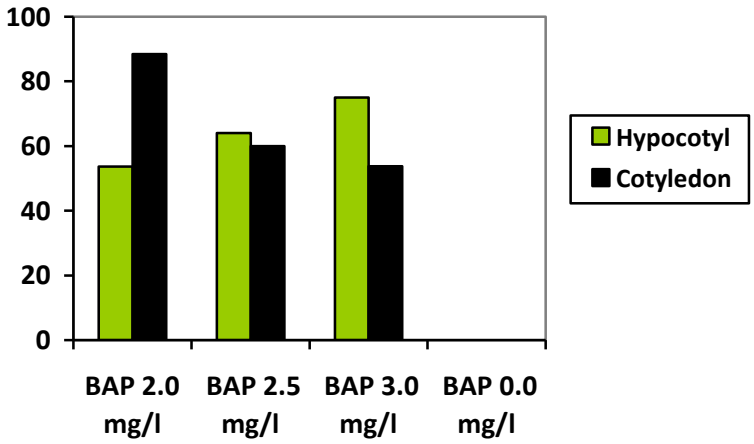


Fig. 4 : Changes in the percentage of shoot formation concentration changes shooting frequency from Canola on different media; changing BAP

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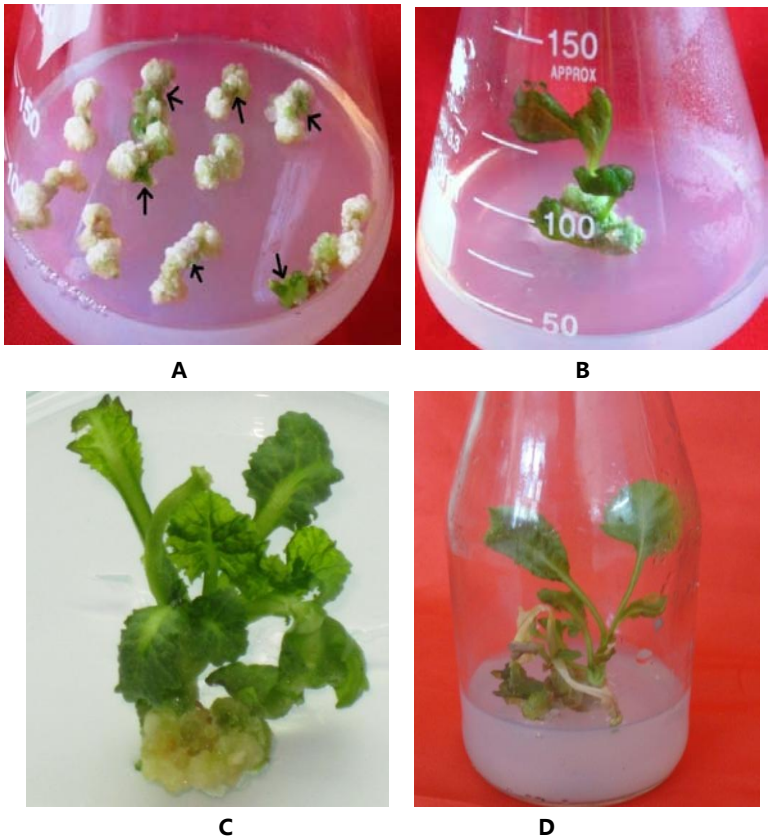

Fig. 5 : Different stages of regeneration from canola callus. (A) Initiation of shoots from Canola hypocotyls on regeneration medium supplemented with 0.5 mg l^{-1} Kinetin + 2.5 mg l^{-1} BAP + 5.0 mg l^{-1} $AgNO_3$, (B) Elongation of the shoots of Fig. A after three weeks of inoculation, (C) Development of shoots from Canola cotyledonary callus in the medium supplemented with 0.5 mg l^{-1} Kinetin + 2.5 mg l^{-1} BAP + 5.0 mg l^{-1} $AgNO_3$, and (D) Regenerated canola plantlet in medium supplemented with 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA + 5.0 mg l^{-1} $AgNO_3$

IV. Conclusion

A genotype independent efficient *in vitro* regeneration system for Canola (*Brassica napus* L.) has been established in the present study. Plant regeneration was achieved through callus production from hypocotyls and cotyledonary leaves with petioles. The optimum medium for callus induction and the best shooting medium has been described in the study. The positive effect of $AgNO_3$ for Canola was found for both callogenesis and regeneration. Cotyledonary calli were better in terms of shoot regeneration. *In vitro* regenerated shoots of canola produced rooting system on both the media with 2.5 mgl^{-1} NAA and 1.0 mgl^{-1} IBA.

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Allelopathic Efeects of Aqueous Extracts of Plant Residues on Two Tropical Weeds of South Western Nigeria

By Modupe Janet Ayeni & Joshua Kayode

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Abstract- The allelopathic effects of aqueous extracts of plant residues from Zea mays (root and tassel) and Cajanus cajan (leaves and stem) were examined on the two weeds, Euphorbia heteropyhlla L. and Bidens pilosa L. The results obtained showed that the aqueous extracts retarded the germination and the initial growth of both weeds. The effects were concentration dependent as the degree of retardation increased with increase in the concentrations of the extracts. The retardation of germination of E. heterophylla seeds was more pronounced in seeds treated with extracts from C. cajan leaf. The % germination decreased from 18% in the 5g concentration to 10% in 25g/200mL concentration at 144hrs experimental time. Similarly, the retardation of germination of B. pilosa seeds was more pronounced in C. cajan stem extract treated seeds.

Keywords: allelopathy, cajanus cajan, euphorbia heterophylla, bidens pilosa.

GJSFR-C Classification : FOR Code: 060799

ALLELOPATHIC EFEECTS OF AQUEOUS EXTRACTS OF PLANT RESIDUES ON TWO TROPICAL WEEDS OF SOUTH WESTERN NIGERIA

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Allelopathic Efeects of Aqueous Extracts of Plant Residues on Two Tropical Weeds of South Western Nigeria

Modupe Janet Ayeni [α] & Joshua Kayode [σ]

Abstract- The allelopathic effects of aqueous extracts of plant residues from *Zea mays* (root and tassel) and *Cajanus cajan* (leaves and stem) were examined on the two weeds, *Euphorbia heteropyhlla* L. and *Bidens pilosa* L. The results obtained showed that the aqueous extracts retarded the germination and the initial growth of both weeds. The effects were concentration dependent as the degree of retardation increased with increase in the concentrations of the extracts. The retardation of germination of *E. heterophylla* seeds was more pronounced in seeds treated with extracts from *C.* cajan leaf. The % germination decreased from 18% in the 5g concentration to 10% in 25g/200mL concentration at 144hrs experimental time. Similarly, the retardation of germination of *B. pilosa* seeds was more pronounced in *C. cajan* stem extract treated seeds.

The % germination decreased from 52% in the 5g concentration to 20% in 25g/200mL concentration at 144hrs experimental time. The radicles of the two weeds were also retarded by the extracts. The retardation of radical lengths of *E. heterophylla* and *B. pilosa* seedlings were more pronounced in *Cajanus cajan* stem extract treated seeds as no radicle emerged until 72hrs experiment time. The radicle length of *E. heterophylla* seedling at 144hrs experiment was 0.93cm in the 5g concentration which reduced to 0.29cm in 25g/ 200mL extract concentration. The radicle length of *B. pilosa* seedling at 144hrs experimental time was 1.25cm in 5g concentration which decreased to 0.59cm in 25g/ 200mL concentration. The plumule lengths of the two weeds were also retarded by the extracts. The plumule length of *E. heterophylla* seeds were mostly retarded by extract from *C. cajan* stems. Plumule length in the 5g concentration was 0.53cm which decreased to 0.06cm in the 25g extract concentration. *B. pilosa* seedlings were retarded mostly by extracts from *C. cajan* leaves. Plumule length was 1.20cm in the 5g extract concentration which decreased to 1.03cm in 25g/200mL extract concentration.

Statistical analyses ($P < 0.05$) revealed that there were significant differences in the germination, radicle and plumule lengths obtained in the extract treated seeds when compared to the control especially at between 120 and 144hrs experimental time.

Keywords: allelopathy, cajanus cajan, euphorbia heterophylla, bidens pilosa.

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I. Introduction

The presence of weeds in agricultural fields greatly reduces crop yields. This has forced farmers to use herbicides as possible control measure. At present, these herbicides are highly expensive beyond the reach of resource-poor farmers. Quite often they are not available in the rural areas where majority of the farmers reside. Situations abounds where farmers procured resources from diverse sources and yet the herbicides were unavailable to purchase and when available, they could be adulterated and useless. Also the herbicides are environmentally unfriendly.

Consequent on the above, there has been a resurgent of interest in the search for sustainable weed control strategies that would address the problems stated above. Attempts made elsewhere included the use of allelopathy. In Nigeria, there abounds a gross dearth of studies on the allelopathic effects of crop residues until recently when allelopathic effects of some crop residues were considered on some agricultural crops and weeds by Ayeni *et al.,* 2010, Ayeni and Kayode (2012), Ayeni and Kayode (2013). The study being presented here examined the allelopathic effects of two common plant residues on two common weeds in Ekiti State, Nigeria.

II. Materials and Methods

Laboratory experiment was conducted in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria, in September 2010 to determine the effects of different concentrations of aqueous extracts from residues of maize tassels and roots as well as *Cajanus cajan* leaves and stems on the germination and growth of two tropical weeds (*E. heterophylla* and *B. pilosa).*

Z. mays' tassels and roots were obtained from the experimental farm of the Faculty of Agricultural Sciences of the Ekiti State University, Ado-Ekiti, Nigeria, after the corns had been harvested. *Cajanus cajan's* leaves and stems were obtained from a farmland in Ikere-Ekiti, a town located at about 15km from the campus of the University.

These materials were chopped into pieces and were air-dried for three weeks after which they were

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pounded using pestle and mortal. Seeds of *E. heterophylla* and *B. pilosa* were obtained within the University campus.

III. Extract Preparation

Portions of 5g, 10g, 15g, 20g, and 25g of each of the grounded samples of the crop residues were measured out using G&G Electric Top Loading Digital balance, JJ300Y, China. Each portion was soaked in 200ml distilled water in 500ml conical flasks. The mixtures were shaken intermittently for 24hrs at 25^0C ± 1^0C. The extracts for each crop residue was filtered and the filtrates were stored in a refrigerator for further usage.

IV. Allelopathy Bioassay

In each treatment, two layers of Whatman No. 1 filter papers were put in each Petri dish (with a diameter of 9cm). Five seeds, each of the weeds, were sown in the Petri dish and each replicated ten times for each extract concentration. The filter papers were moistened daily with the different extract concentrations using syringe and needle. Control experiments were set up for each extract residues and were replicated ten times. All the Petri dishes were arranged on germination tables at room temperature between 25-30^OC. The seeds were considered as germinated upon radicle emergence and the number that germinated was counted and recorded for six days. The radicle and plumule growth elongations were recorded at 24hrs interval. The data obtained from the experiments were compared to those obtained from the control using Analysis of Variance (ANOVA, $P < 0.05$).

V. Results and Discussion

a) Seed germination

The effects of aqueous extracts from the plant residues on the germination of seeds of the two weeds are shown in Tables 1and 2. The % germination of *E. heterophylla* seeds in all the four treatments was retarded (Table 1). It was observed that germination of seeds of *E. heterophylla* were retarded mostly by the extract from the *C. cajan* stem as no germination occurred until 72hrs experimental time (Table1 D). The effects of the extracts on the % germination (Table 1 A-D) increased with increase in the concentration of the extracts. This tends to show that the effects of the extracts were concentration dependent.

In *Z. mays* root extract treated seeds of *E. heterophylla* (Table 1 A), results obtained at 144hrs experimental time revealed that while the % germination in the control was 54%, those of 5, 10, 15, 20 and 25g/ 200mL concentrations were 34%, 24%, 24%, 24% and 20% respectively. In *Z. mays* tassel extract treated seeds (Table 1 B), results obtained at 144hrs experimental time was 54% in the control, those of 5, 10, 15, 20 and 25g/mL concentration were 54%, 40%, 32%, 18% and 10% respectively. Likewise, the % germination of *E. hereophylla* seeds in the *C. cajan* leaves aqueous extracts treated seeds (Table 1 C) was 28% in the control which decreased to 10% in 25g/200mL concentration. Also, in the *C. cajan* stem aqueous extract treated seeds (Table 1 D), % germination was 36% in the control which decreased to 6% in 25g/200mL Concentration.

The germination of *B. pilosa* seeds in the aqueous extracts of the four treatments were shown in Table 2. It was also observed that the aqueous extracts also brought a considerable inhibition in the germination of *B. pilosa* seeds. In *Z. mays* root extract treated seeds (Table 2 A), the % germination of *B. pilosa* seeds was 90% in the control experiment, those of 5, 10, 15, 20 and 25g/ 200mL were 74%, 52%, 48%, 32% and 32% respectively. In the *Z. mays* tassel extract treated seeds (Table 2 B), the % germination was 72% in the control which decreased to, 30% in 25g/200mL concentration. Likewise, the *C. cajan* leaf extract treated seeds (Table 2 C) had 90% germination in the control experiment which decreased to 46% in the in 25g/200mL concentration. In *C. cajan* stem extract treated seeds (Table 2 D), control experiment had 70% which decreased to 20% in 25g/200mL concentration. It was observed that extract from the *C. cajan* stem retarded the germination of *B. pilosa* seeds more than the others.

Statistical analysis($P<0.05$) showed that there were significant differences in the % germination of extract treated seeds between 96 and 144hrs experimental time in all the treatments except the extract from the *Z. mays* tassel in *B. pilosa* treated seeds where no significant different was observed in extract treated seeds and the control at low extract concentrations.

The study lend credence to the previous assertions of Oyun (2006) who reported that aqueous extracts from *Gliricidia sepium* caused a prolong delay of maize seeds germination. Also, Aisha *et al.* (2010) and Monica *et al.* (2011) reported the aqueous extracts of *Ascarum europaeum* L. inhibited the germination and growth of *Lycopersicum esculentum.*

VI. Radicle Length

The effects of the aqueous extract of the plant residues on the radicle lengths of the two weeds are shown in Tables 3 and 4. The results showed that the four plant residues retarded the radicle lengths of the weeds. *E. heterophylla* seeds treated with aqueous extract of *C. cajan* stem were mostly retarded (Table 3 D). At 144hrs experimental time, the radical length in the control experiment was 2.38cm, those of 5, 10, 15, 20 and 25g/ 200mL concentrations were 0.93cm, 0,83cm, 0.72cm, 0.51cm and 0.29cm respectively. Likewise, *B. pilosa* seeds treated with extract from *C. cajan* stem resulted in more inhibition of the radicle lengths

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(Table 4 D). Radicle length in the control experiment was 1.52cm, those of 5, 10, 15, 20 and 25g/200mL extract concentrations were 1.25cm, 0.99cm, 0.92cm, 0,83cm and 0.59cm respectively.

Statistical analysis (P<0.05) revealed that there were significant differences in the radical lengths of extract treated seeds between 72 and 144hrs experiment time in the three crop residues extracts except the extracts from *Z. mays* roots on radicle length on *B. pilosa* at the low extract concentrations (Table 4). The results obtained in this study corroborated the earlier assertions of Khan *et al.* (2011) who reported that litter from leaves and stem of *Rhazyastricta dence* significantly reduce the germination, radicle, plumule growth and number of roots of maize. Sisodia and Siddiqui (2010) reported that the radicle and plumule lengths of seedlings of test species were reduced significantly in response to the *C. bonplandianum* extracts.

VII. Plumuleat Length

The effects of aqueous extracts of plant residues on the plumule growth of the two weeds were shown in Tables 5 and 6.The results also showed that the four aqueous extracts of the plant residues retarded the plumule length of the two weeds. The results revealed that the plumule length of *E. heterophylla* seeds treated with the aqueous extracts from *Z. mays* root was 2.83cm at 144hrs experimental time in the control experiment. The plumule lengths of the 5, 10, 15, 20 and 25g/200mL extract concentrations were 1.99cm, 1.40cm, 1.30cm, 0.84cm and 0.64cm respectively (Table 5 A). Also in the *Z. mays* tassel extract treated seeds, the plumule length of *E. heterophylla* in the control was 2.23cm, those of 5, 10, 15, 20 and 25g/200mL concentrations were 1.79cm.1.47cm, 0.91cm, 0.90cm and 0.81cm respectively (Table 5 B). It was observed that extracts from *C. cajan* stem retarded the plumule lengths of *E. heterophylla* mostly with 1.31cm in the control which decreased to 0.06cm in 25g/200mL concentration (Table 5 D).

The results also revealed that the plumule lengths of seedlings emerged from *B. pilosa* extract treated seeds were retarded by the aqueous extracts of the plant residues. In the *Z. mays* root extract treated seeds (Table 6 A), plumule length in the in the control experiment was 1.52cm. Those of 5, 10, 15, 20 and 25g/200mL concentrations were 1.20cm, 1.17cm, 1.06cm, 0.93cm and 0.38cm respectively. Also in the *C. cajan* stem extract treated seeds (Table 6 D) plumule length was 2.17cm in the control experiment which decreased to 1.03cm in 25g/200mL concentration.

Statistical analyses (P<0.05) revealed that significant differences abound in results obtained from *E. heterophylla* seeds treated with the aqueous extracts of the residues, most especially at higher extract concentrations, between 96 and144hrs experimental time. The results obtained from this study were in accordance with the work of Seerjana *et al.* (2007) who reported that the leaf aqueous extracts of *Parthenium hysterophorus* L. exhibited significant inhibitory effects on seed germination and seedling growth of all test species in cruciferous species. Abu-Romman (2010) also noted that allelochemicals released into the surrounding might inhibited or retarded root or radicle and shoot or coleoptile of plants. Aisha *et. al.* (2010) and Yarnia *et. al.* (2009), Kaul and Bansal (2002) also demonstrated similar results in their studies.

In conclusion, the study revealed that the root and tassel of *Z. mays* contain some allelochemicals which might be responsible for the inhibitory effects exhibited on the two weeds examined in this study. According to Sanchez- Moreiras *et. al.* (2004), *Z. mays* tassels' allelopathy was attributed to hydroxamic acid. Also, An *et al.* (2003) and Alberto *et al.* (2012) reported that *Z. mays* root allelopathy contained 2, 4- dihydroxy-7-methoxy-2*H* -1, 4- benzoxazin-3(4*H*) – one (DIMBOA). Nulifer (2006) revealed the phenolic acid in *Cajanus cajan* to include ptotocatechic, p- hydroxyl benzoic acid. All these chemicals might be responsible for the retardation in the germination and the initial growth of the two weeds examined here. It is hereby recommended that there is need for further studies on the potentials of turning these crop residues from waste materials to wealth.

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Table 1 : Effects of aqueous extracts of *Zea mays* (root and tassel) and *Cajanus cajan* (leaf and stem) on the germination of seeds of *E. heterophylla*

Extracts g/200mL	Experimental Time (Hrs)					
	24	48	72	96	120	144
A.						
Z. mays root 0	0a	8a	42a	54a	54a	54a
Z. mays root 5	0a	6a	24b	34b	34b	34b
Z. mays root 10	0a	4a	16b	24b	24b	24b
Z. mays root 15	0a	4a	16b	24b	24b	24b
Z. mays root 20	0a	2a	14b	24b	24b	24b
Z. mays root 25	0a	0a	10b	20b	20b	20b
B						
Z. mays tassel 0	0a	14a	30a	52a	54a	54a
Z. mays tassel 5	0a	10b	30a	38ab	42ab	54a
Z. mays tassel 10	0a	4b	18ab	32abc	36ab	40ab
Z. mays tassel 15	0a	2b	10b	24bc	32ab	32ab
Z. mays tassel 20	0a	2b	6b	18bc	18b	18b
Z. mays tassel 25	0a	0b	4b	10c	10b	10b
C						
C. cajan leaf 0	0a	12a	14a	18a	28a	28a
C. cajan leaf 5	0a	6a	12a	14a	18ab	18ab
C. cajan leaf10	0a	4a	10a	12a	14ab	14ab
C. cajan leaf 15	0a	2a	10a	12a	14ab	14ab
C. cajan leaf 20	0a	2a	8a	10a	12ab	14ab
C. cajan leaf 25	0a	2a	6a	10a	10b	10b

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D						
C. cajan stem 0	0a	2a	14a	36a	36a	36a
C. cajan stem 5	0a	0b	10ab	16b	16b	16b
C. cajan stem 10	0a	0b	8ab	12b	16b	16b
C. cajan stem 15	0a	0b	8ab	8b	8b	8b
C. cajan stem 20	0a	0b	4ab	6b	6b	6b
C. cajan stem 25	0a	0b	0b	6b	6b	6b

Means followed by the same letter with the column for each treatment are not significantly different at (P< 0.05)

Table 2 : Effects of aqueous extracts of *Zea mays* (root and tassel) and *Cajanus cajan* (leaf and stem) on the germination of seeds of *B. pilosa*

Extracts g/200mL	Experimental Time (Hrs)					
	24	48	72	96	120	144
A.						
Z. mays root 0	0a	36a	82a	86a	90a	90a
Z. mays root 5	0a	20b	50b	66a	74a	74a
Z. mays root 10	0a	12b	32bc	40b	48b	52bc
Z. mays root 15	0a	12b	28c	38b	46b	48c
Z. mays root 20	0a	4b	20c	24b	32b	32c
Z. mays root 25	0a	4b	20c	24b	32b	32c
B						
Z. mays tassel 0	0a	32a	42a	50a	72a	72a
Z. mays tassel 5	0a	14b	42a	50a	72a	72a
Z. mays tassel 10	0a	9b	38a	46a	58ab	60ab
Z. mays tassel 15	0a	2b	34a	46a	50ab	58ab
Z. mays tassel 20	0a	0c	30a	32a	44bc	46bc
Z. mays tassel 25	0a	0c	22a	28a	30c	30c
C						
C. cajan leaf 0	0a	54a	64a	72a	72a	90a
C. cajan leaf 5	0a	14b	56ab	70ab	70ab	78ab
C. cajan leaf10	0a	10b	48ab	54abc	64abc	64abc
C. cajan leaf 15	0a	4b	32bc	40bc	64abc	64abc
C. cajan leaf 20	0a	2b	20c	40bc	62abc	62abc
C. cajan leaf 25	0a	0c	12c	28c	46c	46c
D						
C. cajan stem 0	0a	34a	14a	56a	64a	70a
C. cajan stem 5	0a	12b	10ab	36b	50ab	52ab
C. cajan stem 10	0a	6b	8ab	28b	34bc	46b
C. cajan stem 15	0a	6b	8ab	22b	28bc	34b
C. cajan stem 20	0a	4b	4ab	20b	20bc	28b
C. cajan stem 25	0a	0b	0b	14b	16c	20b

Means followed by the same letter with the column for each treatment are not significantly different at (P< 0.05)

Table 3 : Effects of aqueous extracts of *Zea mays* (root and tassel) and *Cajanus cajan* (leaf and stem) on the radicle length (cm) of *E. Heterophylla*

Extracts g/200mL	Experimental Time (Hrs)					
	24	48	72	96	120	144
A.						
Z. mays root 0	0.00a	0.03a	0.64a	1.17a	2.00a	3.68a
Z. mays root 5	0.00a	0.00a	0.36b	1.01a	1.74ab	2.46b
Z. mays root 10	0.00a	0.00a	0.31bc	0.75ab	1.72ab	2.05bc
Z. mays root 15	0.00a	0.00a	0.19bcd	0.72ab	1.23c	1.69bcd
Z. mays root 20	0.00a	0.00a	0.06cd	0.33c	0.72cd	1.19cd
Z. mays root 25	0.00a	0.00a	0.00d	0.00c	0.41d	0.76d

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B						
Z. mays tassel 0	0.00a	0.00a	0.33a	0.70a	0.89a	2.41a
Z. mays tassel 5	0.00a	0.00a	0.06a	0.27a	0.89a	1.16b
Z. mays tassel 10	0.00a	0.00a	0.06a	0.20a	0.77a	1.10b
Z. mays tassel 15	0.00a	0.00a	0.02a	0.17a	0.35a	0. 79b
Z. mays tassel 20	0.00a	0.00a	0.01a	0.15a	0.31a	0.78b
Z. mays tassel 25	0.00a	0.00a	0.00a	0.12a	0.21a	0.26b
C						
C. cajan leaf 0	0.00a	0.11a	0.58a	1.48a	2.13a	3.07a
C. cajan leaf 5	0.00a	0.10a	0.22ab	0.59b	1.16b	1.75b
C. cajan leaf10	0.00a	0.06ab	0.18ab	0.29b	0.49b	0.68bc
C. cajan leaf 15	0.00a	0.03ab	0.09b	0.29b	0.46b	0.63bc
C. cajan leaf 20	0.00a	0.00b	o.o2b	0.20b	0.29b	0.52c
C. cajan leaf 25	0.00a	0.00b	0.01b	0.16b	0.28b	0.35c
D						
C. cajan stem 0	0.00a	0.00a	0.69a	1.61a	1.95a	2.38a
C. cajan stem 5	0.00a	0.00a	0.17b	0.59b	0.88ab	0.93b
C. cajan stem 10	0.00a	0.00a	0.08b	0.48b	0.75b	0.83b
C. cajan stem 15	0.00a	0.00a	0.02b	0.43b	0.61b	0.72b
C. cajan stem 20	0.00a	0.00a	0.00b	0.28b	0.40b	0.51b
C. cajan stem 25	0.00a	0.00a	0.00b	0.17b	0.25b	0.29b

Means followed by the same letter with the column for each treatment are not significantly different at (P< 0.05)

Table 4 : Effects of aqueous extracts of *Zea mays* (root and tassel) and *Cajanus cajan* (leaf and stem) *on the radicle length of B. pilosa*

Extracts g/200mL	Experimental Time (Hrs)					
	24	48	72	96	120	144
A						
Z. mays root 0	0.00a	0.52a	0.79a	1.02a	1.48a	1.89a
Z. mays root 5	0.00a	0.15b	0.46b	0.75ab	1.18ab	1.85a
Z. mays root 10	0.00a	0.09bc	0.40b	0.69ab	1.00b	1.86a
Z. mays root 15	0.00a	0.03c	0.36bc	0.50bc	0.86b	1.77a
Z. mays root 20	0.00a	0.02c	0.21bc	0.43bc	0.81bc	0.50b
Z. mays root 25	0.00a	0.01c	0.09c	0.22c	0.37c	0.47b
B						
Z. mays tassel 0	0.00a	0.95a	0.32a	0.95a	1.42a	2.20a
Z. mays tassel 5	0.00a	0.07ab	0.22ab	0.56b	1.21ab	2.11ab
Z. mays tassel 10	0.00a	0.02bc	0.16b	0.54b	1.11ab	1.77ab
Z. mays tassel 15	0.00a	0.00c	0.09bc	0.42bc	0.99ab	1.70ab
Z. mays tassel 20	0.00a	0.00c	0.08bc	0.32bc	0.89ab	1.59ab
Z. mays tassel 25	0.00a	0.00c	0.00c	0.17c	0.72b	1.29b
C						
C. cajan leaf 0	0.00a	0.20a	0.41a	0.95a	1.27a	1.89a
C. cajan leaf 5	0.00a	0.19a	0.26b	0.86ab	1.23ab	1.63ab
C. cajan leaf10	0.00a	0.15a	0.11c	0.61abc	0.99abc	1.41ab
C. cajan leaf 15	0.00a	0.10b	0.05c	0.54bc	0.98abc	1.39ab
C. cajan leaf 20	0.00a	0.00b	0.04c	0.32c	0.79bc	1.32ab
C. cajan leaf 25	0.00a	0.00b	0.02c	0.24c	0.59c	1.05b
D						
C. cajan stem 0	0.00	0.00a	0.42a	0.76a	1.05a	1.52a
C. cajan stem 5	0.00a	0.00a	0.23ab	0.67ab	0.98a	1.25ab
C. cajan stem 10	0.00a	0.00a	0.12b	0.39bc	0.69ab	0.99ab
C. cajan stem 15	0.00a	0.00a	0.10b	0.31cd	0.64ab	0.92ab
C. cajan stem 20	0.00a	0.00a	0.01b	0.25cd	0.55ab	0.83ab
C. cajan stem 25	0.00a	0.00a	0.00b	0.04d	0.39b	0.59b

Means followed by the same letter with the column for each treatment are not significantly different at (P< 0.05)

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Table 5 : Effects of aqueous extracts of *Zea mays* (root and tassel) and *Cajanus cajan* (leaf and stem) on the plumule length (cm) of *E. heterophylla*

Extracts g/200mL	Experimental Time (Hrs)					
	24	48	72	96	120	144
A.						
Z. mays root 0	0.00a	0.00a	0.08a	0.49a	1.27a	2.83a
Z. mays root 5	0.00a	0.00a	0.05a	0.42a	1.23a	1.99ab
Z. mays root 10	0.00a	0.00a	0.04a	0.37a	1.01ab	1.40bc
Z. mays root 15	0.00a	0.00a	0.02a	0.35a	0.78ab	1.30bc
Z. mays root 20	0.00a	0.00a	0.00a	0.28ab	0.53bc	0.84c
Z. mays root 25	0.00a	0.00a	0.00a	0.00b	0.36c	0.64c
B						
Z. mays tassel 0	0.00a	0.00a	0.10a	0.80a	1.48a	2.23a
Z. mays tassel 5	0.00a	0.00a	0.05a	0.60a	0.90ab	1.79a
Z. mays tassel 10	0.00a	0.00a	0.00a	0.38ab	0.74ab	1.47a
Z. mays tassel 15	0.00a	0.00a	0.00a	0.01b	0.48ab	0. 91b
Z. mays tassel 20	0.00a	0.00a	0.00a	0.01b	0.30b	0.90b
Z. mays tassel 25	0.00a	0.00a	0.00a	0.00b	0.21b	0.81b
C						
C. cajan leaf 0	0.00a	0.00a	0.07a	0.73a	1.53a	2.26a
C. cajan leaf 5	0.00a	0.00a	0.06a	0.61a	1.36a	2.02b
C. cajan leaf10	0.00a	0.00a	0.05a	0.26a	0.90a	1.47b
C. cajan leaf 15	0.00a	0.00a	0.00a	0.25a	0.90a	1.40b
C. cajan leaf 20	0.00a	0.00a	0.00a	0.17a	0.58b	0.98b
C. cajan leaf 25	0.00a	0.00a	0.00a	0.08b	0.48b	0.63b
D						
C. cajan stem 0	0.00a	0.00a	0.15a	0.62a	1.03a	1.31a
C. cajan stem 5	0.00a	0.00a	0.01b	0.21ab	0.45ab	0.53b
C. cajan stem 10	0.00a	0.00a	0.00b	0.06b	0.12b	0.16b
C. cajan stem 15	0.00a	0.00a	0.00b	0.04b	0.08b	0.12b
C. cajan stem 20	0.00a	0.00a	0.00b	0.03b	0.07b	0.10b
C. cajan stem 25	0.00a	0.00a	0.00b	0.03b	0.05b	0.06b

Means followed by the same letter with the column for each treatment are not significantly different at ($P < 0.05$)

Table 6 : Effects of aqueous extracts of *Zea mays* (root and tassel) and *Cajanus cajan* (leaf and stem) on the plumule length of *B. pilosa*

Extracts g/200mL	Experimental Time (Hrs)					
	24	48	72	96	120	144
A.						
Z. mays root 0	0.00a	0.45a	0.69a	0.91a	1.09a	1.52a
Z. mays root 5	0.00a	0.00b	0.28b	0.56b	0.88ab	1.20ab
Z. mays root 10	0.00a	0.00b	0.12bc	0.51bc	0.83ab	1.17ab
Z. mays root 15	0.00a	0.00b	0.09bc	0.38bc	0.67bc	1.06ab
Z. mays root 20	0.00a	0.00b	0.03c	0.33bc	0.53bc	0.93b
Z. mays root 25	0.00a	0.00b	0.00c	0.18c	0.27c	0.38c
B						
Z. mays tassel 0	0.00a	0.00a	0.54a	0.87a	1.45a	1.96a
Z. mays tassel 5	0.00a	0.00a	0.32ab	0.66ab	1.04ab	1.83a
Z. mays tassel 10	0.00a	0.00a	0.21bc	0.45bc	0.95ab	1.32a
Z. mays tassel 15	0.00a	0.00a	0.02c	0.34bc	0.60b	1.17ab
Z. mays tassel 20	0.00a	0.00a	0.00c	0.23c	0.52b	1.01b
Z. mays tassel 25	0.00a	0.00a	0.00c	0.06c	0.45b	0.87b
C						
C. cajan leaf 0	0.00a	0.00a	0.49a	0.67a	0.89a	1.23a
C. cajan leaf 5	0.00a	0.00a	0.21b	0.86ab	0.79a	1.20a

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C. cajan leaf10	0.00a	0.00a	0.04c	0.61abc	0.58ab	1.02ab
C. cajan leaf 15	0.00a	0.00a	0.01c	0.54bc	0.58ab	0.91ab
C. cajan leaf 20	0.00a	0.00a	0.00c	0.32c	0.38b	0.82ab
C. cajan leaf 25	0.00a	0.00a	0.00c	0.24c	0.02b	0.60b
D						
C. cajan stem 0	0.00a	0.12a	0.74a	1.19a	1.65a	2.17a
C. cajan stem 5	0.00a	0.04b	0.31b	1.05a	1.55a	1.97ab
C. cajan stem 10	0.00a	0.04b	0.28b	0.93ab	1.31a	1.55ab
C. cajan stem 15	0.00a	0.03b	0.15b	0.74ab	1.03a	1.37ab
C. cajan stem 20	0.00a	0.00b	0.08b	0.39b	0.98a	1.31ab
C. cajan stem 25	0.00a	0.00b	0.06b	0.37b	0.79a	1.03b

Means followed by the same letter with the column for each treatment are not significantly different at ($P < 0.05$)

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Hiv-1 Matrix Protein P17 Initiates Virus Assembly

By Galina Vorkunova, Tatiana Gorodnicheva, Sergei Lupandin & Alissa Bukrinskaya

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Abstract - HIV-1 matrix protein (MA) is small multifunctional protein located on N terminus of Gag protein p55. MA posseses three transport signals: membranotropic, nucleophilic and the signal of nuclear export and functions in the cell as shuttle protein.

MA is cleaved from Gag precursor by viral protease early in infection and is transported into the nuclei where it associates with viral RNA (vRNA). The complex MA-vRNA is transported to plasma membrane – the place of HIV assembly - using MA membranotropic signal and phosphorilation.

Mutant MA (M4) prepared by Dr. Dupont (USA, Worchester, Medical School) used in association with vRNA lost membranotropic signal and can not move to the plasma membrane. It was located in the nuclei and cytoskeleton. It could be suggested that mutant MA "get stuck" during cellular transport. That localization unusual for wild HIV-1 could suggest that wild MA complex with vRNA delivers vRNA from the nucleus to the plasma membrane through cytoskeleton.

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HIV-1 MATRIX PROTEIN P17 INITIATES VIRUS ASSEMBLY

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Abstract- HIV-1 matrix protein (MA) is small multifunctional protein located on N terminus of Gag protein p55. MA posseses three transport signals: membranotropic, nucleophilic and the signal of nuclear export and functions in the cell as shuttle protein.

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I. Introduction

The assembly of HIV-1 and the pathogenesis of infection are still not fully understood. Some authors believe that in the case of retroviruses Gag protein penetrates into the nuclei and connects with vRNA (7-9). The other authors (10) show that Gag interacts with vRNA in the cytoplasm where the assembly of HIV-1 initiates.

Our data show that the interaction of vRNA with viral protein still takes place in the nucleus, but we also show that vRNA interacts in the nuclei not with Gag protein but with its part - matrix protein. Thus in the process of initiation of HIV infection is involved not Gag but its part - matrix protein (MA). This protein resides on the N terminus of Gag precursor, and a membranotropic signal within the matrix protein is responsible for Gag protein transport to the plasma membrane where the viral assembly takes place.

Early in infection MA is cleaved from Gag protein by viral protease (11) and due to the three transport signals (membranotropic, nucleophylic and the signal of nuclear export) MA is considered as the cell shuttle protein circulating between nucleus and cytoplasm.

We have shown that the cleavage of MA from Gag precursor by viral protease takes place very early after Gag synthesis (Fig.1) and cleaved MA is involved into viral assembly. With the help of the nucleophylic signal (4) MA penetrates into the nuclei and associates there with vRNA. Thus, it could be suggested that initiation of HIV-1 viral infection unlike the other retroviral infections takes place using not Gag protein but only its part -matrix protein.

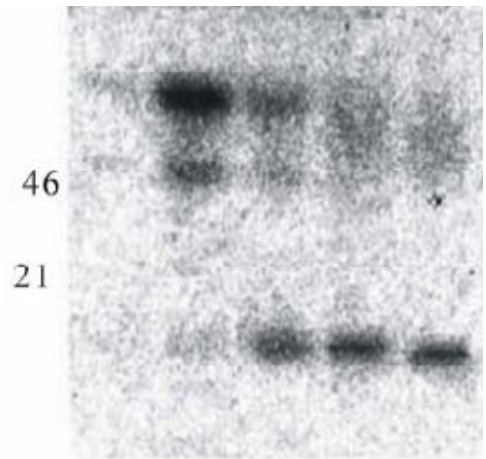

Fig.1

Intracellular localization of Gag and MA in cytoplasmic fraction of MT4 cells. Pulse-chase experiment. MT4 cells were infected with HIV-1 (strain MVP- 899). 14C leucine was added 20 hours after cell infection for 20 min. (pulse). Then the cells were washed with phosphate buffer (PBS) and the label incubated for 1,2 and 3 hours (chase). After that the cells were washed and fractionated on cytoplasm, membranes and nuclei as described (3, 4). As seen on figure 1, the amount of Gag is cytosol is smaller after 1 hour while the amount of MA (p17) is significantly increased during 2-4 hours after infection. This suggests that MA is cleaved from Gag protein in the cytoplasm very soon after its synthesis. The nuclei during this time did not contain the viral proteins.

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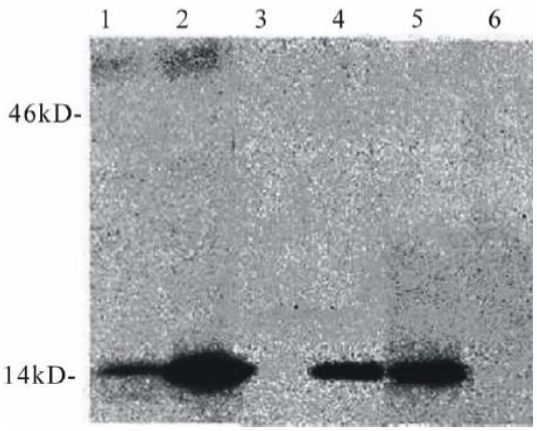

Fig. 2

It was shown earlier that in the cell membranes and in the nuclei MA is associated with vRNA (4, 5). Fig. 2 shows the MA localization in T cells 24 hours after transfection by plasmids containing MA of wild type (lanes 1, 3, 5) or by mutant MA type M4 (2, 4, 6) which had no membranotropic signal. The mutant was prepared by S. Dupont. The cells infected with mutant MA could not induce HIV-1 infection, and was not found in cellular membrane but in the nuclei and cytoskeleton. We suggest that the mutant get stuck in the earlier points of the cell transport and that the complex wild MA-vRNA is transported to the plasmamembrane through cytoskeleton.

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Water Quality Assessment of Tulashi Tank of Kolhapur District (M.S.), India

By K. B. Koli & D. V. Muley

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Abstract- Water of Tulashi tank is utilized for pisciculture, irrigation and domestic purposes. It is an urgent need to assess the water quality. The Physico-chemical parameters of Tulashi tank were studied during January to December, 2011. The results discovered that there was a significant seasonal variation in some physico-chemical parameters. Water temperature ranges from 22.16 to 28.64°C, turbidity 0.68 to 2.95 NTU. Transparency 32.45 to 44.56 cm. The pH 7.10 to 8.85, DO 6.48 to 8.52 mg/l, CO2 1.89 to 5.98 mg/l, Alkalinity 126.42 to 162.42 mg/l, Total Hardness 82.46 to 156.26 mg/l. Phosphate 0.19 to 1.94 mg/l, Nitrates 2.17 to 12.45 mg/l and Chlorides 37.26 to 43.48 mg/l. Above values are within the acceptable limits of BIS standard for drinking water therefore the water is potable and suitable for pisciculture.

Keywords : water quality; tulashi tank; kolhapur district.

GJSFR-C Classification : FOR Code: 960608

WATER QUALITY ASSESSMENT OF TULASHI TANK OF KOLHAPUR DISTRICT M.S., INDIA

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K. B. Koli [α] & D. V. Muley [σ]

Abstract- Water of Tulashi tank is utilized for pisciculture, irrigation and domestic purposes. It is an urgent need to assess the water quality. The Physico-chemical parameters of Tulashi tank were studied during January to December, 2011. The results discovered that there was a significant seasonal variation in some physico-chemical parameters. Water temperature ranges from 22.16 to 28.64°C, turbidity 0.68 to 2.95 NTU. Transparency 32.45 to 44.56 cm. The pH 7.10 to 8.85, DO 6.48 to 8.52 mg/l, CO2 1.89 to 5.98 mg/l, Alkalinity 126.42 to 162.42 mg/l, Total Hardness 82.46 to 156.26 mg/l. Phosphate 0.19 to 1.94 mg/l, Nitrates 2.17 to 12.45 mg/l and Chlorides 37.26 to 43.48 mg/l. Above values are within the acceptable limits of BIS standard for drinking water therefore the water is potable and suitable for pisciculture.

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I. Introduction

Water is very important life supporting material. Every living organism needs water, without which neither the life nor any development is possible. Thus it is very much essential for a healthy growth. But it may become harmful for life, if one uses water polluted with harmful or with toxic substances and poor sanitation. (Gupta and Gupta, 1997) Water quality parameters provide the basis for judging the suitability of water for its designated uses and to improve existing conditions. For optimum development and management for the beneficial uses, current information is needed which is provided by water quality programmes (Lloyd, 1992). We depend on water for domestic needs, irrigation, sanitation and disposal of wastes. The quality and quantity of surface water bodies like lakes and tanks depend upon the climate, catchments, geography of the area and the inputs and outputs both natural and manmade (Gray, 1994). The water quality of lakes can be degraded due to microbiological and chemicals contaminants. In water natural impurities are in very low amounts. Lakes, dams, rivers are important source of fresh water.

The quality of water is described by its physical, chemical and microbial characteristics. But, if some correlations were possible among these parameters, then significant ones would be fairly useful to indicate the quality of water (Dhembhare,1997) The study of important, because fluctuation in the water quality has an influence on the biotic communities (Aher and Mane,2007). This information is important to be communicated to the general public and the Government in order to develop policies for the conservation of the precious fresh water resources (Ali et al.., 2000). The aims of this study were designed to monitor seasonal variation in water quality parameter of Tulashi tank so as to assess its status and suitability through the potability and aquaculture point of view and to compare observed levels of studied parameters with the corresponding WHO and BIS guidelines values for drinking-water quality and pisciculture.

II. Materials and Methods

Tulashi tank was artificial earthen (with masonary spillway) type of dam. It is situated about 48 km from Kolhapur city, towards the south-west side and constructed in 1966 – 1969. It is situated at Latitude 16° 31'15 " East and longitude 74° 01' 00 " North. Its average length is 1522 meter and average height is 48.68 m. The Catchment area of the project is 34.92 Sq. Km and Water storage capacity is 91.92 M.cum. Average rainfall in this region is 1.778 m. The scope of the scheme is a lift irrigation project on Tulashi river near Burambali (Tal-Radhanagari) for irrigating land in Tulashi valley only. Tulashi Irrigation Project construction was completed in 1977-78 (Govt Gazette of Kolhapur) by Maharashtra Krishna Valley Development Corporation Pune. The water is utilized for irrigation, pisciculture as well as domestic purposes.

Study of physico-chemical parameters were carried out during the period of January, 2011 to December, 2011. By considering the morphometry and human activities sampling sites were selected at different places along the tanks, From all the four stations of tank, water samples were collected from the periphery at about 1 to 1.5 meters depth fortnightly. The water samples were collected in plastic cans of 2-3 litapacity and brought to the laboratory to study various parameters.

Temperature and pH were recorded at the time of sample collection using portable kit. The determination of dissolved oxygen, free carbon dioxide, hardness, chlorides, total alkalinity, inorganic phosphate and nitrate were analyzed in the laboratory as per the standard procedure and metrology

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described by Gelt man et al. (1978), Trivedi and Goel (1984) and APHA, AWWA, WPCF (1998).

III. Results and Discussion

The results of physical properties of water from Tulashi tank was given in table 1 and the results of chemical properties of water from Tulashi tank was shown in table 2.

Table 1: Physical parameters of Tulashi Tank, Kolhapur District

Month	Temperature (^{0}C)	Turbidity (NTU)	Transparency (cm)	pH
Jan	22.16	2.25	40.10	7.10
Feb	22.48	2.90	32.45	7.65
Mar	24.56	2.95	35.46	7.45
Apr	26.48	1.20	32.48	7.80
May	28.64	1.45	30.40	8.10
Jun	27.45	1.65	42.56	8.28
Jul	26.47	1.74	39.42	8.13
Aug	25.12	1.56	38.85	8.12
Sept	25.47	1.90	35.42	7.90
Oct	24.18	0.85	44.56	8.08
Nov	23.56	0.68	45.26	8.10
Dec	22.58	1.56	46.18	8.26

Table 2 : Chemical parameters of Tulashi Tank, Kolhapur District (values are in mg/l)

Month	Dissolved Oxygen	Free CO_2	Alkalinity	Hardness	Phosphate	Nitrate	Choloride
Jan	7.35	2.52	132.45	83.42	0.35	2.17	38.12
Feb	6.48	2.98	126.42	82.46	0.31	2.45	39.23
Mar	6.88	2.13	132.46	96.43	1.2	2.22	37.26
Apr	8.14	2.78	128.46	107.12	1.06	2.43	38.10
May	8.52	1.56	142.45	156.26	0.82	2.46	42.26
Jun	8.46	2.47	131.47	145.72	0.19	2.17	41.15
Jul	8.42	4.23	152.47	110.49	0.89	2.45	43.48
Aug	8.26	4.23	158.45	102.45	0.67	6.48	42.45
Sept	8.10	4.59	148.46	98.24	1.46	12.45	41.27
Oct	8.49	5.58	162.42	95.47	1.85	11.56	40.48
Nov	8.10	5.98	158.46	91.46	1.92	6.42	42.26
Dec	8.13	6.12	152.47	87.48	1.94	4.23	39.45

Surface water temperature was fluctuated between 22.28 ± 0.32 ^{0}C to 28.96 ± 0.65 ^{0}C at Tulashi tank. The minimum temperature was recorded in the month of January (22.16^{0}C) and maximum in May (28.64^{0}C). The seasonal pattern in temperature fluctuation was recorded as low in winter season, while high in summer season. Swaranlatha and Rao (1998) have recorded minimum water temperature during winter and maximum during summer (May, June) of Banjara Lake.

Turbidity is an important limiting factor in the productivity of freshwater ecosystems. It was recorded within the range from 0.68 to 2.95 NTU. The minimum turbidity was observed in the month November of maximum in March. Arvindkumar (1995) observed that maximum transparency value was in December and minimum in August at freshwater tropical wetland of santhal Pargana (Bihar).

Transparency is the property of water by which it allows light to pass. Light is an essential factor in freshwater environments for survival of hydrophytes and acts source of oxygen as a byproduct of photosynthesis. Transparency was varied from 32.45 cm to 44.56 cm. Lower transparency values were observed in February and higher in October. This pattern in transparency was also observed by Agarwal and Thapliyal (2005) in Bhilangana River from Tehri Dam reservoir of Uttaranchal.

The pH of the water samples was alkaline throughout study period. pH was recorded within the range from 7.10 to 8.28. Minimum pH was observed in the month of March and maximum in the month of June. Altindag et al (2009) also reported that pH was fluctuated between 8.18 to 8.21 in Karaman stream of Antalya, Turkey. pH range within 6.0 to 8.5 range is good for culturing tropical fish species (Huett, 1977)

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and, it is the recommended levels for drinking water (WHO, 1984): Federal Environmental protection Agency (FEPA) recommended pH 6.5-8.0 for drinking and 6.0-9.0 for aquatic life. The cheap rate of photosynthetic activities reduces the assimilation of carbon dioxide and bicarbonates which are eventually responsible for increase in pH, the low oxygen values coincided with high temperature during the summer month. The factors like temperature bring about changes in the pH of water. The higher pH values observed suggests that carbon dioxide, carbonate- bicarbonate equilibrium is affected more due to change in physico- chemical condition (Karanth, 1987; Tiwari,1988). Change in pH is due to discharge of agricultural wastes, human anthropogenic activities and surface runoff.

The dissolved oxygen was recorded within the range from 6.48 to 8.52 mg/l Minimum dissolved oxygen was recorded in the month of February and maximum in the month of May. The amount of dissolved oxygen in water was not constant but fluctuates, depending on temperature, depth, wind and amount of biological activities such as degradation (Adeniji, 1986; Ibe, 1993): Naz and Turkmen (2005) have reported that in summer, dissolved oxygen concentration decreased due to high temperature. The dry season showed a significantly higher oxygen content than the wet at Kangimi reservoir, Kaduna state Nigeria.

The free carbon dioxide content in the water samples of Tulashi tank was within the range from 1.89 to 5.98 mg/l. High content of free carbon dioxide was recorded in the month of November and minimum in May (Sikabira, 2010) also recorded the similar results regarding free CO2 content from freshwater bodies.

Alkalinity in the water samples of Tulashi tank varied from 126.42 to 162.42 mg/l. Minimum alkalinity was recorded in February and maximum in October. Similar observations have been made by Holden and Green (1960) and Tailing and Rzoska (1967) on Rivers Sokoto and Nile in Egypt respectively.

The hardness was fluctuated from 82.46 to 156.26 mg/l. The minimum hardness was recorded in the month of February and maximum in May. The similar results were obtained by Ravichandran et al. (2009) from the study of ponds from Phosphate content was fluctuated between 0.19 to 1.94 mg/l. It was observed as minimum in June and maximum in December. The phosphate content in any water body was quite opposite in relation to dissolved oxygen and phytoplankton population. Many earlier workers have also reported similar findings (Ghavzan et al., 2006).

Nitrate content in the water was recorded within the range from 2.17 to 12.45 mg/l. Minimum nitrate content was observed in the month of January and maximum in September.

The chloride content in water samples was recorded from 37.26 to 43.48 mg/l. Minimum chloride content was recorded in the month of March and maximum in July. Chourasia and Adoni (1985) also found similar behavior of chlorides in their studies on Sagar Lake with summer maxima and winter minima.

Almost all parameters during the present study meet the water quality norms as per BIS New Delhi. Now it is clear that, the tank is not polluted by human anthropogenic activities and agricultural runoff from surrounding areas. Hence, it is appropriate for human consumption. Moreover, tank is used for irrigation, fishery and domestic purposes. As water is not contaminated, it is potable.

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The Effect of Chromium on Glycogen Content of Certain Tissues of Freshwater Fish, Channa Gachua

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Abstract- The study was carried out to assess the impacts of Chromium on glycogen content of certain tissues of freshwater fish, Channa gachua. For this purpose we have analysed the experimental data. After analysed we have concluded that the effect of the presence of chromium on different organs act differently and the effect depends on time also.

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THE EFFECT OF CHROMIUM ON GLYCOGEN CONTENT OF CERTAIN TISSUES OF FRESHWATER FISH, CHANNA GACHUA

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The Effect of Chromium on Glycogen Content of Certain Tissues of Freshwater Fish, Channa Gachua

Salahuddin [α] & R. K. Khola [σ]

Abstract- The study was carried out to assess the impacts of Chromium on glycogen content of certain tissues of freshwater fish, Channa gachua. For this purpose we have analysed the experimental data. After analysed we have concluded that the effect of the presence of chromium on different organs act differently and the effect depends on time also.

I. Introduction

Water pollution (Kawade *et al.*, 2012) is the biggest threat of urbanization, industrialization and modern agricultural practices. It leads to variation in physical, chemical and biochemical properties of water bodies. The aquatic environment has always been subjected to different types of pollutants (Mance, 1987; Farkas *et al.,* 2000). Beaumont *et al*, (2000) and Braunbeck (2000) reported that rapid industrialization in India has resulted into a consequential increase in the liquid wastes, which are traditionally being liberated into open land or nearby natural water, causing a number of problems to plant and animal (Ramona *et al.*, 2001). The problems of environmental pollution and its harmful effect on aquatic biota, including fish is receiving focus during the last few decades (Jagadeesan *et al.,* 2001. Zikic & Stajn, 2001). Industrial discharges containing toxic and hazardous substances, including heavy metals contribute hugely to aquatic ecosystem (Ghem *et al.*, 2001; Woodling *et al.,* 2001). Heavy metals are natural trace components of the aquatic environment, but their levels have been increased due to domestic, industrial and agricultural movements. It poses greatest threat to the health of Indian ecosystem (Rani *et al.*, 2001; Desai *et al.*, 2002; Joshi *et al.*, 2002; Saxena, 2002). Discharge of heavy metals into the aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and incremental behavior. Aquatic organisms including fish collects metals many times higher than present in water (Madhusudan *et al.,* 2003; Surec, 2003; Olaifa *et al.*, 2004), thus causing an adverse effect on the fish metabolism (Ohe *et al.,* 2004). Studies proved that, fish subjected to metal toxicity shows that biochemical and histopathological alterations in different target tissues like gills, liver and kidney. Histopathological lesions and increase in size of gills was reported in fishes exposed to heavy metals (Devlin, 2006; Gupta & Ashwini, 2006). Necrosis and rupture of gills of *Labeo rohita* on exposure to Copper was reported by Kalele and Dhande (2006). Effect of sub *J. Recent Trends Biosci.,* 2(1): 29-37, 2012 29 lethal concentration of zinc on histological changes and bioaccumulation of zinc in kidney of fish *Channa punctatus* (Bloch) have been studied by Gupta and Srivastava, (2006) and Loganathan *et al*, (2006). Impact of cadmium on the biochemical constituents of *Oreochromis mossambicus* was studied by Hameed *et al*, (2006). Athikesavan *et al*, (2006) studied histopathological changes in the gills, liver, intestine and kidney of nickel treated freshwater fish *Hypopthalmichthys molithrix* (Valenciennes). Among heavy metals, Chromium is being used in industries like organic chemicals, electroplating, iron and steel works electrical works, etc. Chromium is an essential trace element in humans as well as in aquatic organisms. It is listed among 25 hazardous substances that pose threat to human health. Electroplating industrial effluent containing chromium is known to alter the protein, glycogen and lipid composition in fishes (Muley *etal.,* 2007).

Glycogen in the tissue is also considered to be the immediate source of energy to adapt to the environmental conditions. Several workers have reported the impact of various heavy metals on the biochemical constituents of different aquatic organisms (Kharat *et al.*, 2009). However, the research work related to the chromium effect on *Channa gachua* is scanty. Hence the present study was undertaken to evaluate the toxicity of Chromium on the glycogen levels of gills, liver, kidney and gonads (ovary and testis) of freshwater fish, *Channa gachua*, for 24, 48,72 and 96 hrs of exposure.

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II. Materials and Methods (Kawade et al., 2012)

Adult live fish, *Channa gachua* were collected from the local market and brought to laboratory. Only healthy fishes (Length: 12-15 cms, Weight: 50-56 g) were acclimatized in glass aquaria for 15 days and were fed with earthworms and water in the aquaria was replaced by fresh water at every 24 hrs. Stock solution of Sodium dichromate was prepared by dissolving appropriate amount of Na2 Cr2 O7 as Cr salt in distilled water. The fish *Channa gachua* were exposed to Cr (as Na2 Cr2 O7) to know the acute toxicity at 24, 48, 72 and 96 hrs. For selection of test concentration, some pilot tests were carried out. The range of concentration was selected between 0 to 100% mortality. In order to maintain the concentration of chromium, the water in the aquaria was changed every 24 h during the exposure. The mortality rate of *Channa gachua* was recorded at 24, 48, 72 and 96 h exposure to the heavy metal. The percentage for corrected mortality was calculated using the Abbott's formula (1952).

Table 1: Glycogen content(mg) of different tissues of *Channa gachua* after 24 to 96 hrs exposures to Na2 Cr2 O7

Organ / Time	Gills	Liver	Kidney	Ovary	Testis
Control	5.24	7.39	5.8	4.56	4.67
24 Hrs	4.73	5.97	4.61	4.21	4.15
48 Hrs	3.99	5.63	4.49	3.93	3.98
72 Hrs	3.76	4.61	3.93	3.71	3.59
96 Hrs	3.15	3.59	3.2	3.15	3.09

III. Analysis the Data

We have analysis the data experimented by (Kawade *et al.*, 2012). First we have analysed the data using Pearson Correlation Matrix and after that we have concluded the result involving two way anova.

Correlation matrix was prepared within the studied parameters tabulated in Table 2. The strong positive correlation-ship between the changes of Glycogen level of these parameters could be due to Chromium use.

Table 2 : Pearson Correlation Matrix

	GILLS	LIVER	KIDNEY	OVARY	TESTIS
GILLS	1.000				
LIVER	0.971	1.000			
KIDNEY	0.958	0.995	1.000		
OVARY	0.983	0.984	0.972	1.000	
TESTIS	0.974	0.998	0.990	0.992	1.000

Now we have used the statistical tool anova for further analysis.

Let us take the Hypothesis that there is no signaficance difference of Glycogen content in the presence of Chromium between the Organs and Times.

First we compare the variance of Organs with the variance of residual.

F =26.00 tabulated in Table 3.

The table value of F for υ_1=4 and υ_2=16 at 5% level of significance is 3.006917.

The calculated value is greater than table value and we have concluded that the Glycogen content in the presence of chromium of different organs differ significantly. That is Glycogen content at different Organs in the presence of Chrimium are different.That is Glycogen content depends on Organs in the presence of Chromium.That is chromium effects different organs differently.

Now , let us compare the variance according to Times with the variance of residuals.

F= 14.12444 tabulated in Table 3.

The table value of F for υ_3=4 and υ_2=16 at 5% level of significance is 3.006917.

The calculated value is greater than table value and we conclude that Glycogen content in the presence of Chromium of different Organs changes according to times. That is Glycogen content in the presence of chromium depends on times.

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Table 3 : Mean squares and F values

Sources of value	Sum of squares	Degrees of freedom	Mean square(variance)	F	F critical (Tabular value of F)
Between Organs	14.86118	4	3.715296	26	3.006917
Between G Level	8.073304	4	2.018326	14.12444	3.006917
Residual	2.286336	16	0.142896		
Total	25.220824	24			

IV. Conclusion

It is concluded that Chromium effects the Glycogen level of different organs differently and it also acts according to time.

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

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The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

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- Never confuse figures with tables - there is a difference.

Approach

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- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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