# Rehabilitation of Kerrib Lands in Upper Atbara River using Indigenous Trees and Water Harvesting Techniques

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**Abstract:** Trials were conduct at Elshowak area during2003 season to test the use of "U" shape and "L" shape micro water harvesting technique to grow *Acacia tortilis* subspecies *raddiana, Acacia mellifera, Acacia seyal, Zisyphus spina christi, Moringa oleifera* and *Balanites aegyptiaca* in an area affected by gully erosion. Survival rate trials showed that these species except *Moringa oleifera* can be established using micro water harvesting technique. Field trials showed that "U" shape micro-catchment have better results in terms of survival rate than control through increasing moisture content. Also field trial showed promising results that, 30%, 21.4% and 15.2% survival rates for *A.tortilis A.mellifera* and *Zizyphus spina christi* respectively when compared to control that obtained zero percent.

**Keywords:** Survival rate, gully erosion, micro-catchment, run off, U shape, Zero percent

## Introduction

Water erosion is dominant in semi arid zones. Sudan is suffering from water erosion especially in Eastern Sudan and water eroded soils in Sudan was estimated to be about 8 million hectares (Akhtar and Menching, 1993).

Gully erosion is a serious hazard to agricultural lands in Gedarif area, especially around Elshowak. Kerrib lands or gully erosion is one of the most important problems that faces farmers in upper Atbara River in addition to reduction of farm areas, it complicates planting and harrowing as indicated by farmers (Seid Ahmed, 2001). Water harvesting techniques were used in Sudan for increasing agricultural production Omer et al (1997). For trees also Salih and Iinanga (1997) tried this for increasing production of sorghum and gum. The removed soil is carried to increase siltation problems of khashum Elgerba dam. Areas affected by gully erosion reached 2904 $km^2$ in 1999 (Fadul *et al*, 1999*).* The rate of soil loss was 14.3 km during 1985-1987 and 9.8 km during 1987-1990 (Fadul *et al,* 1999*).* The initial capacity of Khashum Elgerba dam was 1.2 millions $m^3$ but is now reduced to only 650000 $m^3$. Annual washing of silt cost a lot of money and efforts (Seid Ahmed, 2001).

The vegetation cover percent at Elshowek area was found to be 3% (Seid Ahmed, 2001) The main reducing effect of vegetation on flooding and run off water erosion is that vegetation roots act as a binding agent to soil particles. In addition, litter and roots which decay in the soil act as a

cementing agent that improves soil structure and that vegetation canopy intercepts rain drops and reduces soil erosion caused by rain drop splash (Alaktar and Aga, 1995). Vegetation also reduces runoff speed and thus reduces its capacity to detach and remove soil particles. The alterations it makes to soil through combination of actions such as root binding effect, leaf and litter humus formation, the cover delay and reduce volume of flood and intercept rain drops (Alaktar and Aga, 1995). Forest cover may probably cut at least the peak flow by 80% and run off volume by 40%, besides removal of shrubs and grasses cause potential erosion risk (Meunive, 1996). Young (1989) reported that 60 -75 % of ground cover maintained through the rainy season was effective against erosion of vertisols in Australia. Also Meunive (1996) indicated that with 40% cover grown mainly in gully reduced sediment yield as much as 92.5 % even during storms. Mechanical means for erosion control can be used such as cut –off drain technique or other methods can be used to reduce erosion. But it should be complemented by plant cover for ever lasting and sustainable protection. Plant cover protects soil from direct rain drops and roots of plants bind soil particles and falling litter latter improves physical and chemical soil properties.

The objectives of this study were to evaluate the controlling effect of different tree species on reducing gully erosion, restoration of vegetation cover of degraded lands by indigenous multipurpose tree species and to evaluate micro-catchments (mainly U shape micro-catchment) as water harvesting techniques for raising trees.

## Materials and Methods

Experiments were conducted at khor Abu Gara near Elshowak town (longitude $35^0$ $22^-$ and latitude$14^0$ $24^-$ and 1672 feet's above sea level in wet season 2003. It is one of the most affected areas by gully erosion. The experimental area was one feddan (4200 $m^2$) .Two types of micro catchments were selected. "U" shape and "L" shape. Split plot design with 4 replicates was used. Micro-catchments were the main plots and tree species were the subplots. The dimensions were 1.5 x1.5 m for L shaped micro-catchments and U shaped micro–catchments. The radius of 1.1 m depth of catchment was 30 cm in both systems. Soil was taken from the corner of the catchment depth and accumulated on sides to give them strength. Six species were tested namely Talih, (*Acacia seyal),* Kitir (*Acacia mellifera),* Higlig (*Balanites aegyptiaca*), Seyal (*Acacia tortilis* subsp *Raddiana*), Sidir (*Zizyphus spina christi*) and Moringa (*Moringa oleifera)*. Spacing between trees was 3 x3 m. The tree species were planted after the experimental area had received 140 mm of rainfall. Control treatment planted on flat lands without making micro-catchment shapes. Soil moisture content was expressed on percentage basis as gravimetric soil moisture content method. Four random samples of soil were taken by auger from four soil depths as follows 0-20, 20-40, 40-60, and 60-80 cm. The samples were taken in tightly closed metal containers. Then containers were the weighed before and after drying to calculate moisture content on dry basis during the seasons 1996, 1997 and 1998. As field trials four villages were selected in the affected gully erosion

areas. About ten farmers were chosen randomly to represent each village. The farmers were given seedlings of all three tree species to grow in their farms and survival rates were evaluated after rainy season each year.

The data showed that soil moisture content was higher in the treatments namely U shape micro catchments for all depths particularly at 60-80 cm when compared to control as indicated in table (1).

## Results

**Soil moisture content**

**Table (1): Mean soil moisture content in using U shaped micro-catchment at depths 0-20, 20-40, 40-60 and 60-80 cm and control at Elshowak in season 1998.**

| Depths (cm ) | Moisture content (%) | |
|---|---|---|
| Level | U-shape | Control |
| 0-20 | 12.53 | 8.38 |
| 20-40 | 18.74 | 14.28 |
| 40-60 | 18.99 | 15.87 |
| 60-80 | 21.45 | 13.77 |

## Tree species survival rates

The survival rates of the tree species were found to be higher in the first month after planting then decreased gradually with increase in time as shown in table 2.

**Table 2: Survival rates of six tree species (*Acacia seyal, Acacia mellifera, Acacia tiortilis, Balanites aegyptiaca, Zizyphus spina christi and Moringa oleifera*) at Elshowak, one, 8 and 15months after planting in seasons (1999- 2001)**

| Species | Survival rate % of trees at different times after planting | | |
|---|---|---|---|
| | 1month | months8 | months15 |
| *Acacia seyal* | 95.8% | 19.7% | 10.9% |
| *Acacia mellifera* | 65.3% | 21.45% | 21.45% |
| *Acacia tortilis* | 89.1% | %30 | %30 |
| *Balanites aegyptiaca* | 15.2% | 15.2% | 15.2% |
| *Zizyphus spinachristi* | 10.9% | 10.9% | 10.5% |
| *Moringa olifera* | 0% | 0% | 0% |

**Evaluation of trees species in farmers' field during seasons 2006-2007:**

On farm evaluation was carried out in four villages around Elshwak town at Eltomat, Merebea, Elshowak and Elmogata; the assessment indicated higher survival rates were found in Elmerebea village followed by Eltomat and lower rates were recorded in Elmogata and Elshowak villages (Table 3).

**Table 3: Evaluation of four tree species survival rates at Elshowak area in season 2006\2007**

| The village | No of seedlings distributed | Species | Survival rate % |
|---|---|---|---|
| Eltomat | 320 | *Acacia mellifera* (kitir), *Zizyphus spina christi* (Sidir), *Acacia tortilis (Seyal), Balanites aegyptiaca* (Higlig) | 19 |
| Merebea | 200 | *Acacia mellifera* (kitir), *Zizyphus spinachristi* (Sidir), *Acacia tortilis (Seyal), Balanites aegyptiaca* (Higlig) | 30 |
| Elshowak | 60 | *Acacia mellifera* (kitir), *Zizyphus spinachristi* (Sidir), *Acacia tortilis (Seyal), Balanites aegyptiaca* (Higlig) | 1.66 |
| Elmogata | 250 | *Acacia mellifera* (kitir), *Zizyphus spinachristi* (Sidir), *Acacia tortilis (Seyal), Balanites aegyptiaca* (Higlig) | 10.2 |

**Discussion**

Results showed that it is possible to grow some indigenous tree species using simple water harvesting technique such as U shaped micro catchment which increases the soil moisture content. This will help both in using water which other wise will cause erosion. This in line with Omer *et al* (1997) who found that water harvesting techniques increased agricultural production. Also Salih and Inanga (1997) reported that water harvesting techniques increased production of sorghum and gum Arabic.

The survival rates of tree species decreased by time due to effect of drought because the rainy season was very short and soil moisture content will decrease consequently.

Other tree species were prone to drought such as *Moringa oleifera* whereas *Acacia tortilis* is drought resistant than the other tested tree species.

The higher survival rates in some villages such as Elmogata was due to good management of the trees in the rainy season and also these trees were preferable for the villagers.

It can be concluded that these tree species are well known for the farmers except Moringa and they are widely used as multi purpose trees. Also these tree species can be used to rehabilitate the area which had been degraded by gully erosion because they are drought resistant. Besides water harvesting techniques especially U shape micro catchments can be introduced to rehabilitate this area.

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# إعادة تأهيل أراضي الكرب بأعالي نهر عطبرة باستخدام الأشجار المحلية في محاصد المياه الصغيرة

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**المستخلص**

تمت هذه التجارب في منطقة الشواك بشرق السودان في الفترة من 1999 وحتي 2007م وتم فيها تجريب مساكات المياه الصغيرة في شكل U وشكل لـ لاستزراع أشجار السيال والكتر والطلح و السدر والهجليج والمورنقا في مناطق متأثرة بالتعرية المائية. معدلات الحياة أوضحت امكانية استزراع هذه المناطق بهذه الأنواع ما عدا المورنقا وأكدت تجارب المزارعين امكانية ذلك ساعدت مساكات المياه في زيادة رطوبة التربة لتأسيس الأنواع التي تم تجربتها أوضحت التجارب أن نسبة السيال كانت 30 % والكتر 21.4% والسدر 15.2% بينما كان الشاهد 0%.

# Detection of Endotoxins in Escherichia coli of Dairy Calves in Khartoum State, Sudan

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**Abstract:** A total of 260 *E. coli* were isolated from faecal samples collected from 300 diarrhoeic calves in different localities of Khartoum State. These isolates were subjected for further confirmation by using API 20E and VITEK2 systems. The ability of some *E. coli* to produce enterotoxins was detected by using Suckling Mouse Test (SMT) and Reversed Passive Latex Agglutination (RPLA) test. Ninety isolates were confirmed by API 20E strips. Different identification percentages ranging from 89.8% to 99.8% were obtained by API 20E strips. Twenty *E. coli* scored high probability percentages ranging from 96% to 99% by using VITEK2. This is the first report in Sudan of using VITEK2 automated identification system for identification of *E. coli*. Fifty *E. coli* were selected randomly and examined for their production of heat-stable enterotoxin (STa) by using SMT. Forty five isolates (90.0%) were positive. These 50 *E. coli* were subjected to LT enterotoxins production ability test by using RPLA kit. This is the first report in Sudan of detection of LT in *E. coli* isolated from diarrhoeic dairy calves by using RPLA kits.

**Keywords** *: E. coli*, Calves, enterotoxins.

## Introduction

*Escherichia coli* can be isolated and classified using traditional methods, i.e. identifying its biochemical or antigenic characteristics. The pathogenic mechanisms may be studied in cell cultures and animal method assays, as well as more up to date molecular biology methods for study and diagnosis. The latter have proven that genes are involved in pathogenesis (Rodriquez Angeles, 2002). The virulence factors of pathogenic strains of *E. coli* include capsules, endotoxin, structures responsible for colonization, endotoxins and other secreted substances (Quinin *et al*, 2011). Two types of enterotoxins, heat labile (LT) and heat stable (ST) have been identified and each type of enterotoxin has two subgroups. Many strains of enterotoxigenic *E. coli* (ETEC) from pigs produce $LT_1$ which induces hyper secretion of fluid into the intestine through stimulation of adenylate cyclase activity. Most ETEC isolates which produce $LT_1$ also possess K88 adhesins. A second heat-labile toxin, $LT_2$, has been demonstrated in some EC strains isolated from cattle. One of the heat-stable enterotoxin subgroups, $ST_a$ has been identified in strains of ETEC isolated from porcine, bovine, ovine and human specimens. This toxin induces increased guanylate cyclase activity in enterocytes and the resultant increase in intracellular

guanosine monophosphate stimulates fluid and electrolyte secretion into the small intestine lumin and inhibits fluid absorption from the intestine. The precise cytotoxic effect of the other heat-stable enterotoxin, $ST_b$, is not known (Quinin *et al*, 2011). The pathological effects of infection with pathogenic *E. coli*, other than those attributed to endotoxin, derive mainly from the production of enterotoxins, verotoxins or cytotoxic necrotizing factors. Unlike enteroxoxins which affect only the functional activity of enterocytes, verotoxic necrotizing factors can produce demonstrable cell damage at their sites of action. Enterotoxigenic *E. coli* (ETEC): is the most common enteropathogen that causes diarrhoea in newborn farm animals (Radostits *et al*., 2007). In Sudan Ellaithi (2004), Mohamed (2009) and Elgaddal (2009), reported the involvement of *E. coli* as a cause of diarrhoeal disease in calves. *E. coli* also causes haemorrhagic colitis and dysentery.

The objective of this research work was to characterize *E. coli* obtained from diarrhoeic dairy calves by using rapid and automated systems and to study the enterotoxins of some of the isolated *E. coli* by bioassay and Immunoassay.

## Materials and Methods

### API 20E (BIOMERIEUX, France)

API 20E is a standardized identification system for Enterobacteriaceae and other Gram-negative rods (Crichton and Taylor, 1995).

Pure *E. coli* isolates were subcultured on blood agar and incubated at $36C^o \pm 2$ for 18–24 hours. The test was conducted according to the manufacturer BIOMERIEUX. Homogeneous bacterial suspension was obtained by using API 20E medium. Both tubes and cubules were filled with the inoculated API 20E media. Anaerobiosis was ensured in the ADH, LDC, ODC, URE and $H_2S$tests by filling the cubules with sterile mineral oil to form a convex meniscus. The incubation boxes were closed and incubated at $36C^o \pm 2$ for 18–24 hours. Identification was obtained with the numerical profile.

### VITEK 2 (BIOMERIEUX, France)

The test was done according to the procedure of Funke *et al*., (1998).

Pure colonies were suspended in 3.0 ml sterile saline with turbidity of 0.50- 0.63 MacFarland. The tested samples and the GN identification cards were placed into a cassette. The cassette was placed into a vacuum chamber station inside the vitek2 analyzer machine in which GN cards were inoculated with bacterial suspensions. The inoculated cards were passed by a mechanism, which cut off the transfer tube and sealed. The cassette was manually inserted in the viteck 2 reader-incubater module at $35.5C^o$ and every card automatically subjected to a kinetic fluorescence measurement every 15 minutes. The results were interpreted by the ID-GNB database after the incubation period.

### SUCKLING MOUSE TEST (SMT)

This biological method described by Dean *et al* (1972) and standardized by Giannella (1976). The principal of the test based on the injection of extracted STa preparation of *E. coli* into the stomach of 2-4 days old suckling mouse FOR accumulation of the fluid in the intestine. The inoculated mice per os were kept at room temperature for 4

hours and then decapitated or killed. The abdomen was opened, then the small intestines were examined for distension and then removed by forceps. The intestines were then weighed using a sensitive balance and the ratio of gut weight to the body weight was calculated. Ratios of less than 0.070 were considered negative, those in range of 0.070-0.090, were considered doubtful positive and those over 0.090 were positive.

**Detection of heat-labile (LT) enterotoxin**

Reversed Passive Latex Agglutination test was described by Carroll *et al*., (1990). Polystyrene latex particles were sensitized with purified antiserum taken from rabbits immunized with purified *Vibrio cholerae* enterotoxin. These latex particles agglutinated in the presence of *vibrio cholerae* enterotoxin (CT) or *E. coli* heat-labile enterotoxin (LT). If either toxin is present, agglutination occurs. It involved preparation and extraction of *E. coli* LT enterotoxin. The plate was arranged so that each row consisted of 8 wells. Each sample needed the use of 2 such rows, 25µl of diluents were added in each well of the two rows except for the first well in each row. 25µl of test sample was added to the first and second well on each row, 25µl was picked up and doubling dilutions was performed along each of the 2 rows. The $7^{th}$ well was left to contain diluents only, 25µl of sensitized latex was added to each well of the first row, 25µl of latex control was added to each well of the second row. The plate was rotated gently to mix the contents by hand, covered and put in moist place to avoid evaporation. The plates were left at room temperature for 24hours. The agglutination pattern was judged by comparison with the illustrated results classified as (+), (++) and (+++) and considered positive. Results in the row of wells containing latex control were negative.

**Rsults**

Different identification percentages ranged from 99.8% to 89.8% were scored by 90 *E. coli* isolates after using Api 20E identification system was shown table (1). According to interpretation of the tests, after using VITEK2 identification system for identification of 20 *E. coli* isolates. The isolates scored high probability percentages ranged from 99.8% to 89.8% (Table 2). The weight of intestines, weight of residual carcasses and calculated mean ratios for the tested 50 isolates which tested for their production of STa, are shown in table (3). A mean ratio of 0.09 or over was considered positive and the ratios less than 0.09 were negative results. Forty five isolates (90.0%) gave positive results with SMT test (Table 4). Ten isolates (20%) of the same Fifty *E. coli* isolates used in SMT test, gave positive results. i.e. agglutination was noticed as a net onto the bottom of the wells, after subjected to LT enterotoxins production ability test by using RPLA (Oxoid, TD 0920A) kit (Table 4).

**Table (1)**API 20E identification system results of the isolated *Escherichia coli*:

| Test | *E. coli* 4 | *E. coli* 27 | *E. coli* 49 | *E. coli* 57 | *E. coli* 86 | *E.coli* 117 | *E. coli* 135 | *E. coli* 165 | *E. coli* 198 | *E. coli* 212 |
|---|---|---|---|---|---|---|---|---|---|---|
| **ONPG** | + | + | + | + | + | + | + | + | + | + |
| **ADH** | - | - | + | - | - | - | - | - | - | + |
| **LDC** | + | + | + | + | + | + | + | + | + | + |
| **ODC** | + | - | + | - | - | - | + | + | - | + |
| **CIT** | - | - | - | - | - | - | - | - | - | - |
| **$H_2S$** | - | - | - | - | - | - | - | - | - | - |
| **URE** | - | - | - | + | - | + | - | - | + | - |
| **TDA** | - | - | - | - | - | - | - | - | - | - |
| **IND** | + | + | + | - | + | + | + | + | + | + |
| **VP** | - | - | - | - | - | - | - | - | - | - |
| **GE** | - | - | - | - | - | - | - | - | - | - |
| **GLU** | + | + | + | + | - | + | + | - | + | + |
| **MAN** | + | + | + | + | + | + | + | + | + | + |
| **INO** | + | - | - | - | - | - | + | - | - | - |
| **SOR** | - | - | + | + | - | + | + | - | + | + |
| **RHA** | + | - | + | + | - | + | + | + | + | + |
| **SAC** | - | + | + | + | - | - | + | + | - | - |
| **MEL** | - | + | - | + | - | - | + | + | - | + |
| **AMY** | + | - | - | - | - | - | - | - | - | + |
| **ARA** | - | + | + | + | + | + | + | + | + | + |
| **Ox** | - | - | - | - | - | - | - | - | - | - |
| **Id%** | **99.8** | **98.3** | **97.9** | **96.3** | **95.4** | **94.4** | **97.2** | **94.5** | **99.0** | **89.8** |

Key:

| | |
|---|---|
| **Id%** | Identification percentage |

**Table (2)**VITEK2 identification system results of the isolated *Escherichia coli*:

| Test | *E.*7 | *E.* 19 | *E.* 43 | *E.* 78 | *E.* 96 | *E.* 117 | *E.* 157 | *E.* 199 | *E.* 244 |
|---|---|---|---|---|---|---|---|---|---|
| **2 APPA** | - | - | - | - | - | - | - | - | - |
| **10 $H_2S$** | - | - | - | - | - | - | - | - | - |
| **17 BGLU** | - | - | - | - | - | - | - | - | - |
| **23 ProA** | - | - | - | - | + | - | - | - | - |
| **33 SAC** | + | + | + | + | + | - | + | + | + |
| **40 lLATK** | + | + | + | - | - | - | + | + | + |
| **46 GlyA** | - | - | - | - | - | - | - | - | - |
| **58 O129R** | + | + | + | + | + | - | + | + | + |
| **3 ADO** | - | - | - | - | - | - | - | - | - |
| **11 BNAG** | - | - | - | - | - | - | - | - | - |
| **18 dMAL** | + | + | + | + | + | + | + | + | + |
| **26 LIP** | - | - | - | - | - | - | - | - | - |
| **43 dTAG** | - | - | - | - | - | - | - | - | - |
| **41 AGLU** | - | - | - | - | - | - | - | - | - |
| **47 ODC** | - | - | + | + | - | + | - | - | - |
| **59 GGAA** | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **4-PyrA** | - | - | - | - | - | - | - | - | - |
| **12 AGLTp** | - | - | - | - | - | - | - | - | - |
| **19 dMAN** | + | + | + | + | + | + | + | + | + |
| **27 PLE** | - | - | - | - | - | - | - | - | - |
| **35 dTRE** | + | + | + | + | + | + | + | + | + |
| **42 SUCT** | + | + | + | + | + | + | + | + | + |
| **48 LDC** | + | + | + | + | + | + | + | + | + |
| **61 lMLTa** | - | - | - | - | - | - | - | - | - |
| **5 lARL** | - | - | - | - | - | - | - | - | - |
| **13 dGLU** | + | + | + | + | + | + | + | + | + |
| **20 dMNE** | + | + | + | + | + | + | + | + | + |
| **29 TyrA** | - | + | + | - | - | - | + | - | + |
| **36 CIT** | - | - | - | - | - | - | - | - | - |
| **43 NAGA** | - | - | - | - | - | - | - | - | - |
| **53 lHISa** | - | - | - | - | - | - | - | - | - |
| **62 ELLM** | + | + | + | - | - | - | - | - | - |
| **7 dCEL** | - | - | - | - | - | - | - | - | - |
| **14 GGT** | - | - | - | - | - | - | - | - | - |
| **21 BXYL** | - | - | - | - | - | - | - | - | - |
| **31 URE** | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **37 MNT** | - | - | - | - | - | - | - | - | - |
| **44 AGAL** | + | + | + | + | + | + | + | + | + |
| **56 CMT** | + | + | + | + | + | + | + | + | + |
| **64 lLATa** | - | - | - | - | - | - | - | - | - |
| **9 BGAL** | + | + | + | + | + | + | + | + | + |
| **15 OFF** | + | + | + | + | + | + | + | + | + |
| **22 BAlap** | - | - | - | - | - | - | - | - | - |
| **32 dSOR** | + | + | + | + | + | + | + | + | + |
| **39 5KG** | + | + | + | - | - | - | + | + | + |
| **45 PHOS** | - | - | - | - | - | - | - | - | - |
| **57 BGUR** | + | + | + | + | + | + | + | + | + |
| **Proba.** | **99 %** | **99 %** | **99 %** | **99%** | **99 %** | **97 %** | **97 %** | **96 %** | **96%** |

**Key:**

| | |
|---|---|
| ***E.*** | ***E. coli*** |
| **Proba.** | **Probability** |

**Table (3)** Detection of STa enterotoxin produced by *E. coli* isolates using suckling mouse test (SMT):

| ***E. coli*** **isolates** | **Intestinal Weight (g)** | **Carcass Weight (g)** | **Ratio** | ***E. coli*** **Isolates** | **Intestinal Weight (g)** | **Carcass Weight (g)** | **Ratio** |
|---|---|---|---|---|---|---|---|
| **1** | 0.096 | 1.000 | 0.096 | **26** | 0.124 | 1.00 | 0.124 |
| **2** | 0.216 | 1.304 | 0.165 | **27** | 0.224 | 1.274 | 0.176 |
| **3** | 0.101 | 1.224 | 0.083 | **28** | 0.146 | 1.144 | 0.128 |
| **4** | 0.082 | 1.620 | 0.078 | **29** | 0.188 | 1.144 | 0.164 |
| **5** | 0.238 | 1.476 | 0.161 | **30** | 0.242 | 1.416 | 0.170 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **6** | 0.180 | 1.240 | 0.145 | **31** | 0.224 | 1.200 | 0.187 |
| **7** | 0.332 | 1.692 | 0.196 | **32** | 0.332 | 1.242 | 0.267 |
| **8** | 0.212 | 1.468 | 0.212 | **33** | 0.146 | 1.000 | 0.146 |
| **9** | 0.260 | 1.376 | 0.189 | **34** | 0.202 | 1.058 | 0.191 |
| **10** | 0.322 | 1.322 | 0.244 | **35** | 0.990 | 1.086 | 0.091 |
| **11** | 0.264 | 1.616 | 0.163 | **36** | 0.124 | 1.132 | 0.109 |
| **12** | 0.240 | 1.084 | 0.221 | **37** | 0.174 | 1.124 | 0.154 |
| **13** | 0.275 | i.668 | 0.165 | **38** | 0.258 | 1.138 | 0.227 |
| **14** | 0.356 | 1.532 | 0.232 | **39** | 0.231 | 1.146 | 0.201 |
| **15** | 0.212 | 1.432 | 0.170 | **40** | 0.291 | 1.168 | 0.249 |
| **16** | 0.264 | 2.080 | 0.127 | **41** | 0.243 | 1.296 | 0.187 |
| **17** | 0.292 | 1.486 | 0.197 | **42** | 0.027 | 1.184 | 0.022 |
| **18** | 0.660 | 1.312 | 0.503 | **43** | 0.091 | 1.192 | 0.076 |
| **19** | 0.146 | 1.230 | 0.119 | **44** | 0.175 | 1.300 | 0.135 |
| **20** | 0.218 | 1.276 | 0.171 | **45** | 0.188 | 1.378 | 0.136 |
| **21** | 0.149 | 0.998 | 0.150 | **46** | 0.099 | 1.225 | 0.080 |
| **22** | 0.184 | 1.044 | 0.176 | **47** | 0.100 | 1.301 | 0.077 |
| **23** | 0.175 | 1.214 | 0.144 | **48** | 0.120 | 1.299 | 0.092 |
| **24** | 0.146 | 1.016 | 0.144 | **49** | 0.083 | 1.300 | 0.064 |
| **25** | 0.202 | 1.152 | 0.175 | **50** | 0.162 | 1.205 | 0.134 |

**Table (4)** Detection of STa and LT enterotoxins elicited by E. *coli* isolates using CMT and RPLA in different localities of Khartoum State:

| Locality | No. of tested isolates | STa positive Isolates | LT positive isolate |
|---|---|---|---|
| Bahry & East Nile | 20 | 19 (95.0%) | 5 (25.0%) |
| Omdurman | 17 | 15 (88.2%) | 3 (17.6%) |
| Khartoum & Gabal Awleia | 13 | 11 (84.6%) | 2 (15.4%) |
| Total/ % | 50 | 45 (90.0%) | 10 (20.0%) |

## Discussion

The 90 *E. coli* on API 20E strips showed different identification percentages ranging from 99.8% to 89.8%. Slight variations in the biochemical behavior of the microorganism may be attributed to variations in the genetic constituents of different stains resulting in different phenotypic characteristics. These genetic variations may be of chromosomal or plasmid origin. In both circumstances, transfer of genetic material between strains of the same species does occur through different mechanisms. These findings generally fulfilled the API 20E requirements for identification as *E. coli* (Holmes *et al*., 1978; Willis and Cook, 1975). Twenty *E. coli* were selected randomly and inserted into VITEK2 automated identification system. They scored high probability percentages and there were slight variations in the results of their biochemical tests. Although the results of biochemical reactions revealed many differences between *E. coli* strains, they fulfilled the requirements for identification as *E. coli* by VITEK2 system (Shetty *et al*., 1998; David, 2005). The present findings showed that Vitek2 as an automated system provided very good and trustable accuracy and reproducible results as shown in repeated samples of same source. Economic studies estimate a good future for automated systems in microbiology lab (Simoons-Smit and Maclaren, 1994; Ling *et al*., 2001; Ling *et al*., 2003; O'Hara and Miller, 2003; Funke, 2004; Otto-Karg *et al*., 2009). This is the first report in Sudan of using VITEK2 automated identification system for identification of *E. coli*. Isolation of *E. coli* doesn't necessarily means the presence of the disease unless, virulence factors are identified i.e. toxins and/or fimbriae (Nakazawa *et al.*, 1987; Hirsh, 2004; Quinin *et al*., 2011). In this study 50 randomly selected *E. coli* isolates were tested by the Suckling mouse test (SMT) for production of heat-stable (STa) enterotoxin. Forty five isolates (90%) gave positive results with SMT test. This finding agrees with Ellaithi (2004) who found that 85.7% of isolated *E. coli* produced STa enterotoxin. The reliability of the suckling mouse was also confirmed by Dean *et al*. (1972). This study disagrees with Gyles (1971) who stated that the infant mouse test is unsatisfactory as a method for detection of STa enterotoxin.

The same Fifty *E. coli* isolates used in SMT test, were subjected to LT enterotoxins production ability test by using RPLA kit. Ten isolates (20%) gave positive results, i.e. agglutination was noticed as a net onto the bottom of the wells. These ten isolates were also proved to possess STa by SMT test. Same findings were reported by Salih *et al* (1998) who detected LT in 8 (27.6%) of 29 *E. coli* isolated from camel calves and also by Elgaddal (2009) who detected LT in 16.6% *E. coli* isolated from camel calves. This is the first report in Sudan of detection of LT in *E. coli* isolated from diarrhoeic dairy calves by using RPLA kit. Also 10 *E. coli* isolates reported to have both STa and LT enterotoxins and according to Quinn *et al*., (2011), the possession of the two enterotoxins increases the bacterium virulence.

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# التعرف على السموم الداخلية للاسكريشيا الكولونية في عجول الألبان في ولاية الخرطوم – السودان

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**المستخلص**

من 300 عينة براز لعجول مصابة (*Escherichia coli*)تم عزل 260اسشيريشيا قولونية بحالات الاسهال فى محليات ولاية الخرطوم المختلفة. تلى ذلك تاكيد التعريف لعدد 90 معزولة و قد تم التاكيد بنسب تراوحت بين 89,8% و 99,8%. Api 20E باستخدام نظام (VITEK2 autoanalizer) جرى تاكيد تعريف 20 من معزولات الاسشيريشيا باستخدام جهاز كاول تقريرمن نوعه على مستوى السودان, تراوحت فيه نسب التاكيد بين 96,0% و 99,0%. تم اختبار قدرة 50 من الاسشيريشيا القولونية المعزولة على انتاج الزيفانات المقاومة للحرارة و ذلك باستخلاص هذه الزيفانات و حقنها فى الفئران الرضيعة. خمسة و اربعون (90,0%) معزولة اثبت انتاجها للزيفانات. اخضعت نفس معزولات الاسشيريشيا القولونية الخمسون لاختبار انتاج الزيفانات المتاثرة بالحرارة و ذلك بواسطة طقم التلازن المطاطى السلبى المعكوس. وجدت عشر معزولات فقط 20,0% تنتج هذا النوع من الزيفانات. وهذه العشر معزولات معزولات كانت فى الاصل ممتلكة للزيفانات المقاومة للحرارة. عليه تعتبر هذه الدراسة هى الاولى من نوعها فى السودان من حيث استخدام طقم التلازن المطاطى السلبى فى اختبار امتلاك معزولات الاسشيريشيا القولونية المعزولة من عجول مصابة بالاسهال للزيفانات المتاثرة بالحرارة.

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**Short Communication**

# First Report on Parasitoids on Fruit Flies (Tephritidae: Diptera) in Sudan

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**Abstract:** Laboratory experiments were conducted at Kassala and Hudieba Agricultural Research Stations and also at the Plant Protection Directorate (PPD) South Kordofan Station. Consecutive rearing of fruit flies from fruits of guava, (*Psidium guajava*), mango, (*Mangifera indica*) and sidir, (*Zizyphus* spp) collected from Khartoum, Gezira, River Nile, South Kordofan, Kassala and Sinnar states led to isolation and identification of the parasitoids *Psyttalia cosyra* and *Euryotma* sp., for the first time in Sudan, from a guild of fruit flies including the mango fruit fly, *Ceratitis cosyra,* medfly, *C. capitata* the guava fruit fly, *C. quinaria.* The parasitoids *Psyttalia concolor* and *Euryotma* sp emerged from pupae of Zizyphus fruit fly, *C. incompleta.*

**Keywords:** Tephritid, kiobionts, parasitic hymenoptera, fruits

## Introduction

Fruit flies are the most damaging pests of horticultural crops worldwide. In Sudan, the introduction of the invasive fruit fly, *Bactrocera in vadens* increased damage associated with fruit flies, and increased their importance as national pests.

Parasitoids are efficient natural enemies that incapacitate and regulate populations of insect pests. Thirty-two parasitoids were introduced into Hawaii between 1947 and 1952 to control fruit flies (Wharton et al, 2000). In general parasitoids lay their eggs in the eggs, maggots or pupae of fruit flies and abort subsequent development.

The parasitoid *P. cosyrae*, initially described as *Opius cosyrae* was later included in the *Opius* subgenus *Psyttalia* and subsequently elevated to the generic rank (Wharton, 1987).

Also Wharton in 1987 concluded that *P. concolor* and *P. cosyrae* are similar but the ovipositor of the former is distinctly longer. These two species were native to Africa; they were reported from Congo, Kenya, Egypt, Uganda, Zaire, Tanzania, Nigeria, Sierra Leone and South Africa (Wharton *et al*, 2000 and El-heneidy *et al*, 2001). Kimani -Njogu *et al*, 2001 reported that various Kenyan populations of *Psyttalia spp* are capable of successfully developing on medfly, *C. capitata*. Opiinae braconids were reared on *C. anonae, C. cosyra, C. fasciventris* and *C. rosa* (Copeland *et al*, 2009). Under laboratory conditions, *P. cosyrae* is capable of producing viable, female offspring through several generations when hybridized with *P. concolor*. Using molecular data, a broader selection of *Psyttalia* populations included *P. cosyrae* from Kenya and Benin proved to be genetically relatives (Rugman-Jones *et al*, 2009). *P. cosyrae* has been reared on *C. cosyra* infesting mangoes

and a few wild host plants in Kenya and Tanzania (Copeland *et al*, 2009). In general *Psyttalia* spp prefer to oviposit in later instar larvae of its host (Wharton and Yoder, 2011). Their ability to attack and successfully develop *in C. cosyra* and *C. capitata* in the laboratory was previously reported however they were reported to be encapsulated by *C. rosa*, *C. fasciventris*, *C. anonae*, and *B. cucurbitae* (Mohamed *et al*, 2003). The present study was therefore set to investigate the presence of parasitoids of dominant fruit flies in Sudan.

**Materials and Methods**

A guild of fruits showing symptoms of fruit fly eggs oviposition were collected from fields and markets.

Fruits of mango were collected from Wad Medani, Khartoum and Sinnar areas and fruits of Guava were collected from Khartoum, Sinnar and River Nile States. Fruits of *Zizyphus* spp were collected from Shambat, Elfaki Hashim (Khartoum State), Kassala and Abu Karshoula (South Kordofan State) during 2008, 2009 and 2010.

The fruits were separately kept for 2 weeks inside rectangular ventilated plastic containers (30x20x20cm) placed on 10 cm moist sand soil layer. The set up were observed daily for pupation over a period of 10-14 days. Pupae, transferred to empty cages, placed each on 10 cm layer of moist sandy soil, were observed for parasitoids emergence. The emergent parasitoids, preserved in vials containing 70% ethanol, were identified by comparison with samples of the Insect Collection Unit of the Agricultural Research Corporation (ARC) at Wad Medani- Sudan.

**Results and Discussion**

*C. capitata, C. cosyra, C. quinaria* and *B. invadens* were emerged from fruits of guava and mango collected from Khartoum, Gezira, River Nile, South Kordofan States, while only *C. incompleta* was identified from fruits of *Zizyphus* species collected from the states.

*Eurytoma* sp. emerged from *C. capitata* on guava fruits from the River Nile State, and a guild of fruit flies comprising of *C. capitata, C. cosyra* and *C. quinaria* on fruits of guava collected from Elfaki Hashim and Sinnar. *P. cosyrae* emerged from pupae of *C. cosyra, C. capitata,* and *C. quinaria* on guava fruits collected from Khartoum, Gezira and River Nile States *P. concolor* and *Eurytoma* sp emerged from pupae of *C. incompleta* on *Zizyphus* spp. Parasitism of *C. incompleta by P. concolor and Eurytoma* was 20% and 5% respectively.

Studies regarding susceptibility and incapacitation of fruit flies by parasitoids and the consequence reduction in fruit damage are imperative. Susceptibilities of fruit flies, especially B. invadens, to paras-itoids may provide an effective means for reduction of the damage and consequently economic losses. A program of mass rearing of parasitoids as bio agents for control of fruit flies is of significance from the econ-omic point of view and for environmental safety.

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## معلومة مختصرة عن المتطفلات على ذباب الفاكهة
## ( جنس تفرتيدي : رتبه ذوات الجناحين) في السودان

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**المستخلص**

أجريت تجارب معملية بمحطتي بحوث كسلا و الحديبه وبالا داره العامة لوقاية النباتات بمحطة جنوب كردفان، تم إستخراج ذباب الفاكهة من ثمار كل من المانجو و الجوافة و نوعين من السدر وذلك للبحث عن وجود متطفلات علي ذباب الفاكهة في ولايات الخرطوم , الجزيرة, نهر النيل , جنوب كردفان , كسلا و سنار بالسودان. أثبتت النتائج وجود *Psyttalia cosyra* and *Euryotma* sp للمرة الأولي في السودان كمتطفل علي الأطوار اليرقيه لكل من ذبابه البحر الأبيض المتوسط *Ceratitis capitata* و ذبابه المانجو *C. cosyra* و ذبابه الجوافة *C. quinaria* التي تم إستخراجها من ثمار المانجو و الجوافة كذلك تم تسجيل *Psyttalia concolor* و *Euryotma* sp كمتطفلات علي *C. incompleta* التي تم إستخراجها من السدر بنوعيه.