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Preface

We would like to present, with great pleasure, the inaugural volume-7, Issue-2, February 2021, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

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Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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(Managing Editor)

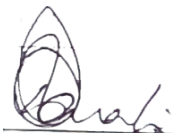

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Table of Contents

Estimation of DNA Fragmentation, Mutagenicity and Biochemical Changes in Mice Exposed to Diazinon and Its Commercial Formulation

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Abstract— *The aim of the present study was to determine the effect of organophosphorus (OP) insecticide diazinon (DZN) and its formulation (DZNF) in bone marrow and germ cells, DNA fragmentation and biochemical changes induced in Swiss albino male mice. The mice were randomly divided into 7 groups (10 mice each), the 1st group served as control, the 2nd, 3rd and 4th groups, as well, 5th, 6th, and 7th groups are treated with 1/10LD50 (6.5 mg/kg bw), 1/20 LD50 (13mg/kg bw) and 1/40 LD50 (26mg/kg bw) of DZNF and DZN i.p with single dose/week for 4 weeks, respectively. At the end of treatment all animals were sacrificed by cervical dislocation after 24 h of the last treatment. Bone marrow and spermatocyte cells were subjected to chromosomal analysis. As well, liver and brain tissues were collected from all animals for DNA fragmentation and biochemical analyses. Cytogenetic analysis revealeda significant increase (more than 2 folds) in structural aberrations (Chromatid and chromosomal gaps, breaks, deletions, centromeric attenuation and end to end)as well as numerical variations in DZNF treated groups than DZN treated groups in a dose dependent manner. A significant increase (about 3 folds) also was found in DZNF treated groups in structural and numerical aberrations of spermatocyte cells than DZN treated mice in a dose dependent manner. Levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA) and DNA fragmentation were significantly increased (about 2 fold) in mice exposed to DZNF than DZN exposed groups. In conclusion, our findings demonstrate that DZNF is more genotoxic than DZN as assessed by cytogenetic analysis of both somatic and germ cells of mice and had adverse effects on DNA and biochemical parameters. These results suggested that the effects of DZNF and DZN are dose dependent and the treatment with DZNF is more hazardous and toxic than DZN and it is important to avoid toxicities induced by organophosphate insecticides, take a high level of caution and minimize its agricultural and household uses.*

Keywords— Diazinon, formulation, mutagenicity, DNA fragmentation, biochemical changes, mice.

I. INTRODUCTION

Diazinon (DZN) (*O*,*O*-Diethyl*O*-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate) is an organophosphate (OP) insecticide which is the most commonly used to control cockroaches, silverfish, ants, and fleas. It has been extensively used in agriculture (including fruits, vegetables and nut trees) and horticulture for controlling insects in crops all over the world [1]. Commercial diazinon formulations (DZNF)are often more toxic than the pure pesticide compound, as they contain surface active ingredients, dyes, stabilizers, activity enhancers, and organic solvents with unknown or poorly characterized toxicity, which raises concern about the current assessment of genotoxicity induced by this pesticide and calls for a high level of caution in agricultural and household uses [2]. It had been used extensively in home and garden applications, in commercial formulations designed to prevent such pests as crickets or cockroaches from infesting homes or offices, and in pet collars. Residential application methods included aerosol cans, spray equipment, and granular spreaders. Due to the emerging health and ecological risks posed by diazinon, manufacturers agreed to phase out and cancel all residential products, so its use is minimized (USEPA 2006) [3].

It has been reported that DZN insecticide have negative effects on different tissues and organs such as the liver, brain, cardiac, kidney, pancreas, immune system, reproductive system, and vascular walls and can induce liver toxicity, neurotoxicity, cardiotoxicity, genotoxicity or cytotoxicity, and apoptosis. Various biochemical and hematological adverse changes in the body can be induced by OP compounds [4]. Moreover, different studies have shown that DZN could induce oxidative damage by increasing the formation of reactive oxygen species (ROS), depletion of the antioxidant enzymes, protein and lipid peroxidation (LPO) and DNA fragmentation in the cells [4,5].

The clastogenic and aneugenic potential of this pesticide was reported, where a significant percentage of chromosome aberrations in bone-marrow cells of the mouse was found [6,7]. Genotoxic effects are considered among the most serious side effects of diazinon which found to increased significantly ($P \leq 0.05$) the level of chromosomal aberrations (7.5 ± 1.04) in albino male rats including gap chromosomes (10%), break chromosomes (7%), fragment chromosomes (5%) and deletion chromosomes (8%) [8]. DZNF also induced increases in the frequency of micronucleated (MN) cells and DNA damage in human peripheral blood lymphocytes [2].

ALT (alanine aminotransferase) and AST (aspartate aminotransferase) are important indicators of liver damage in clinic finding. These enzymes were secreted to blood in hepatocellular injury and their levels increased [9]. A significant increase in ALT and AST were detected from the 2nd to 4thweek in the DZN - exposed rats [10]. The increased ALT and AST values were also found by Kalender *et al.*, [9], who stated that changes in these enzymes level might differ depending on exposure time and dose in adult male Wistar rats treated orally via gavage for 7 weeks. Organophosphorus insecticides treatment caused an increase in the activities of ALT and AST enzymes in the serum of male and female rats [11, 12]. They concluded that the increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane. As well a significant increase of malondialdehyde (MDA) levels in the liver associated with a decrease in antioxidant enzyme was found [11].

Therefore, the present study is designed to assess the effect of DZN and one of its formulations on DNA fragmentation, chromosomal aberrations in both somatic and germ cells and biochemical changes in Swiss albino male mice (*Mus musculus*).

II. MATERIALS AND METHODS

2.1 Chemicals

Diazinon insecticide was obtained from ADWIA 60 EC (Emulsifiable concentrate), Cairo, Egypt. A commercial formulation (DZNF) obtained from (Basudin 60EM®, Syngenta, Basel, Switzerland) containing 630 g of DZN per liter of the product. It was diluted in deionized water for final concentration. Malondialdehyde (MDA); phosphate-buffered saline (PBS), thiobarbituric acid (TBA) were purchased from Sigma chemical company (Sigma, St. Louis, MO, USA). Alanine transaminase (ALT), aspartate transaminase (AST) kits were purchased from Biorexfars, UK.

2.2 Animals

Adult Swiss albino male mice (*Mus musculus*) were purchased from the laboratory animal colony of the National Research Center, Dokki, Giza, Egypt. They were housed in appropriate conditions, and acclimatized for two weeks prior to initiation of the study. All mice were caged, and allowed free access to food and water. The mice were randomly divided into 7 groups (10 mice each) and were treated by oral gavage, once a week for four weeks as follow: 1) control group received corn oil, orally; 2) 1/10 LD_{50} (6.5 mg/kg bw) of DZN-treated group; 3) 1/20 LD_{50} (13 mg/kg bw) of DZN-treated group; 4) 1/4 LD_{50} (26mg/kg bw) of DZN-treated group; 5) 1/10 LD_{50} (6.5 mg/kg bw) of DZNF- treated group; 6) 1/20 LD_{50} (13 mg/kg bw) of DZNF-treated group; 7) 1/40 LD_{50}(26 mg/kg bw) of DZNF-treated group. The LD50 were selected according to Koltzsche [13], and El-Shenawy *et al.* [14]. At the end of treatment, all animals were sacrificed by cervical dislocation after 24 h of the last injection. Bone marrow and testis were subjected to chromosomal analysis (5 animals/each). As well, blood samples and liver and brain tissues were collected from all animals for DNA fragmentation, MDA and biochemical analyses.

2.3 Chromosomal analysis

2.3.1 Chromosomal analysis in somatic cells

Mice were subjected to cytogenetic analysis from bone marrow cells using the method of Preston *et al*. [15]. Briefly, mice were treated intraperitoneally (I/P) with Colchicine (0.05 mg/kg) for two and a half hours before sacrifice. Animals were sacrificed and femoral bone marrow cells were flushed with isotonic solution (0.9% NaCl). Hypotonic solution (0.56% KCl) was added to the cell pellet and incubated at 37°C for 30 minutes the solution was fixed, slides were air dried and stained with 10% Giemsa stain for 20 minutes. 50 metaphases were studied per animal scoring different types of chromosomal aberrations (structural and numerical aberrations).

2.3.2 Chromosomal analysis in germ cells

Spermatocyte cells were prepared according to Russo [16]. Briefly, chromosomes were spread on clean glass slides by the gradual fixation/air-drying method. The preparations were stained with 2% Giemsa (Merck, Darmstadt, Germany) in PBS (pH 6.8) for 10 min for conventional chromosome analysis. Aberrations are scored in metaphase chromosomes of dividing cells. Fifty metaphase spreads per animal were analyzed for studying the chromosome aberrations.

2.4 Blood and tissue sampling

After 24 hr of the last dose of administration, animals were euthanized and their blood was obtained. Serum was separated and used for biochemical experiments. Liver and brain tissues were dissected quickly and washed with cold saline and were homogenized (1:10 w/v) in phosphate buffered saline (PBS) (50 mM sodium phosphate buffer, pH=7.4). Homogenate tissues were centrifuged at 10,000 rpm, for 15 min at 4°C, and supernatants were used for determination of MDA (lipid peroxidation indicator).

2.5 Biochemical assays and analysis

Enzyme activities of ALT, AST were assessed in the blood serum described in section 2.4, using kits and were expressed as international units per liter (IU/L).

2.6 DNA fragmentation analysis

To evaluate genotoxicity induced by DZN and its commercial formulation (DZNF), DNA fragmentation was assessed in combined liver and brain samples using spectrophotometer (Farag *et al.*, 2021) [17]

2.7 Determination of lipid peroxidation (LPO) in tissues

Malondialdehyde (MDA), as the main marker of lipid peroxidation, was measured in the brain and liver tissues. Levels of MDA were measured according to the method of Fernandez *et al.*[18], using the spectrophotometric measurement of color developed by reaction of MDA with thiobarbituric acid (TBA).

2.8 Statistical analysis

All values were expressed as mean±SE. Differences between groups were determined using one-way analysis of variance (ANOVA) and Tukey's as *post hoc* testing was performed for comparisons between groups. Values were regarded as significantly different at $P<0.05$.

III. RESULTS

The novelty of our study are the DZNF effects on DNA fragmentation, chromosomal aberrations and biochemical alterations induced in exposed mice, because there were a few reports on this subject. In the present study, DZNF and DZN insecticide were investigated for their toxicity in exposed Swiss albino male mice by genetic (chromosomal analysis in both bone marrow and spermatocyte cells), DNA damage analysis (DNA fragmentation) and biochemical analysis (AST, ALT and MDA assays). Cytogenetic analysis (Table 1) of bone marrow cells revealed that a significant increase (more than 2 folds) instructural aberrations (Chromatid and chromosomal gaps, breaks, deletions, CA and end to end) and total structural aberrations (33.60 ± 0.87^{g}, 26.40 ± 0.74^{f} , 14.80 ± 0.37^{d} and17.20 ± 0.48^{f} , 11.20 ± 0.20^{c} , 7.80 ± 1.31^{b}, for 1/10 , 1/20 and 1/40 LD_{50} of DZNF and1/10 , 1/20 and 1/40 LD_{50} of DZN, respectively) as well in numerical (N±1) and total numerical variations (17.40 ± 0.67^{g}, 14.60 ± 0.60^{f} , 11.60 ± 0.50^{e} and 8.40 ± 0.67^{d} , 4.80 ± 0.58^{c} , 2.80 ± 0.37^{b} , for 1/10 , 1/20 and 1/40 LD_{50} of DZNF and1/10 , 1/20 and 1/40 LD_{50} of DZN, respectively) in DZNF treated groups than DZN treated groups in a dose dependent manner.

TABLE 1
MEAN FREQUENCY OF CHROMOSOMAL ABERRATIONS IN MICE BONE MARROW CELLS EXPOSED TO DZN AND ITS COMMERCIAL FORMULATION (DZNF)

Treatment	Structural aberrations							Numerical variations		Total numerical variations
	Chromatid gap	Chromosomal gap	break	deletion	CA	End to end	Total structural aberrations	Hypoploidy	Hyperploidy	
Control	0.40±0.24	0.20±0.20	0.00±0.00	0.20±0.20	0.60±0.24	0.00±0.00	1.40±0.74[a]	0.00±0.00	0.20±0.20	0.20±0.20[a]
DZN (1/10 LD_{50})	3.20±0.20	3.20±0.20	3.20±0.37	3.20±0.20	2.60±0.40	1.80±0.20	17.20±0.48[f]	4.40±0.24	4.00±0.44	8.40±0.67[d]
DZN (1/20 LD_{50})	2.00±0.00	2.00±0.00	2.00±0.00	1.80±0.20	1.60±0.24	1.80±0.20	11.20±0.20[c]	2.20±0.37	2.60±0.24	4.80±0.58[c]
DZN (1/40 LD_{50})	0.60±0.24	1.20±0.37	1.20±0.20	1.60±0.24	1.80±0.37	1.40±0.24	7.80±1.31[b]	1.20±0.20	1.60±0.24	2.80±0.37[b]
DZNF (1/10 LD_{50})	3.00±0.00	9.60±0.50	2.60±0.24	6.20±0.37	4.40±0.24	7.80±0.37	33.60±0.87[g]	8.20±0.48	9.20±0.37	17.40±0.67[g]
DZNF (1/20 LD_{50})	4.80±0.20	7.20±0.58	6.60±0.40	4.00±0.31	2.20±0.20	2.20±0.20	26.40±0.74[f]	5.40±0.24	9.20±0.37	14.60±0.60[f]
DZNF (1/40 LD_{50})	1.80±0.20	2.80±2.20	3.60±0.24	3.20±0.20	2.80±0.32	2.60±0.24	14.80±0.37[d]	5.20±0.20	6.40±0.40	11.60±0.50[e]

DZN: diazinon insecticide; DZNF: diazinon commercial formulation; CA: centromeric attenuation .
Values in the same column with different superscript letters are differing significantly (p<0. 05).

TABLE 2
MEAN PERCENTAGE OF CHROMOSOMAL ABERRATIONS IN SPERMATOCYTE CELLS OF MICE EXPOSED TO DZN AND ITS COMMERCIAL FORMULATION (DZNF).

Treatment	Number of examined cells	Structural aberrations								Total structural aberrations		Numerical variations				Total numerical variations	
		Chain		Ring		x-y univalent		Autosomal univalent				N-1		N+1			
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Control	250	1	0.4	1	0.4	0	0.0	0	0.0	2	0.8	1	0.4	0	0.0	1	0.4
DZN (1/10 LD_{50})	250	8	3.2	10	4	6	2.4	8	3.2	32	12.8	9	3.6	8	3.2	17	6.8
DZN (1/20 LD_{50})	250	7	2.8	6	2.4	3	1.2	3	1.2	19	7.6	6	2.4	5	2	11	4.4
DZN (1/40 LD_{50})	250	3	1.2	5	2.0	2	0.8	1	0.4	11	4.4	4	1.6	3	1.2	7	2.8
DZNF (1/10 LD_{50})	250	28	11.2	35	14.0	10	4.0	11	4.4	84	33.6	19	7.6	16	6.4	35	14.0
DZNF (1/20 LD_{50})	250	25	10.0	28	11.2	4	3.6	10	4.0	72	28.8	16	6.4	13	5.2	29	11.6
DZNF (1/40 LD_{50})	250	16	6.4	18	7.2	8	3.2	9	3.6	51	20.4	11	4.4	10	4	21	8.4

DZN: diazinon insecticide; DZNF: diazinon commercial formulation

Table (2) showed a significant increase (about 3 folds) in DZNF treated groups than DZN treated in structural aberrations (chain, ring, x-y and autosomal univalents), (33.6, 28.8, 20.4% and 12.8, 7.6, 4.4% for 1/10 , 1/20 and 1/40 LD_{50} of DZNF and1/10 , 1/20 and 1/40 LD_{50} of DZN, respectively) and numerical variations (N ±1) (14.0, 11.6, 8.4% and 6.8, 4.4, 2.8 % for 1/10 , 1/20 and 1/40 LD_{50} of DZNF and 1/10 , 1/20 and 1/40 LD_{50} of DZN, respectively) of spermatocyte cells in mice in a dose dependent manner.

In the present study DNA damage assay was evaluated in combined liver and brain samples by DNA fragmentation (Table 3). Administration of DZNF resulted in significant increase in DNA fragmentation (about 2 fold) in mice exposed to DZNF than DZN treated groups in a dose dependent manner, as well, a significant difference were found between all treated groups either by DZN or DZNF and the untreated control. For determination of liver and brain damage induced by DZN and DZNF treatment, the activity of the hepatic enzymes (ALT and AST) and brain damage biomarker MDA (lipid peroxidation indicator) were investigated. As shown in Table (3) regarding the level of MDA as lipid peroxidation indicator, and in Table (4) for serum AST and ALT levels, there was a significant increase (about 2 fold) in mice exposed to DZNF than DZN treated groups for these parameters. There was also a significant increase in ALT, AST and MDA in DZNF treated groups than DZN treated groups in a dose dependent manner.

TABLE 3
DNA FRAGMENTATION AND MDA LEVEL IN MICE TREATED WITH DZN AND ITS COMMERCIAL FORMULATION (DZNF).

Treatment	DNA Fragmentation	MDA	
		Liver	Brain
Control	7.85 ± 0.41^{a}	2.23 ± 0.34^{a}	4.02 ± 0.57^{a}
DZN (1/10 LD_{50})	22.24 ± 0.30^{d}	28.93 ± 0.48^{d}	34.83 ± 0.40^{d}
DZN (1/20 LD_{50})	17.96 ± 0.51^{c}	21.44 ± 0.23^{c}	26.25 ± 0.33^{c}
DZN (1/40 LD_{50})	13.86 ± 0.42^{b}	15.28 ± 0.30^{b}	16.87 ± 0.43^{b}
DZNF (1/10 LD_{50})	38.26 ± 0.41^{g}	54.22 ± 0.35^{g}	67.44 ± 0.22^{g}
DZNF (1/20 LD_{50})	31.92 ± 0.32^{f}	46.87 ± 0.43^{f}	53.90 ± 0.46^{f}
DZNF (1/40 LD_{50})	26.23 ± 0.34^{e}	36.25 ± 0.40^{e}	41.88 ± 0.44^{e}

DZN: diazinon insecticide; DZNF: diazinon commercial formulation; MDA: malondialdhyde. Values in the same column with different superscript letters are differing significantly (p<0. 05). Values represents means ± standard errors. Number of animals/group = 10.

TABLE 4
ALT AND AST LEVELS IN MICE TREATED WITH DZN AND ITS COMMERCIAL FORMULATION (DZNF).

Treatment	ALT (U/L)	AST (U/L)
Control	32.33 ± 0.28^{a}	33.78 ± 0.56^{a}
DZN (1/10 LD_{50})	47.70 ± 0.30^{d}	61.87 ± 0.43^{d}
DZN (1/20 LD_{50})	36.84 ± 0.41^{c}	55.52 ± 0.33^{c}
DZN (1/40 LD_{50})	30.26 ± 0.60^{b}	42.90 ± 0.46^{b}
DZNF (1/10 LD_{50})	84.87 ± 0.43^{g}	98.22 ± 0.35^{g}
DZNF (1/20 LD_{50})	75.22 ± 0.35^{f}	87.47 ± 0.29^{f}
DZNF (1/40 LD_{50})	51.95 ± 0.50^{e}	71.73 ± 0.51^{e}

DZN: diazinon; DZNF: diazinon commercial formulation; ALT: alanine aminotransferase; AST: aspartate aminotransferase. Values represents means ± standard errors. Number of animals/group = 10. Values in the same column with different superscript letters are differing significantly (p<0. 05).

IV. DISCUSSION

Results showed that DZNF had a higher mutagenic effect than DZN itself on both somatic and germ cells of mice. These results supported those of Aboul-Ela [6], Alabi *et al.* [7], Ahmed and Alwan [8], Altamirano-Lozano *et al.* [19], who recorded a significant percentage of chromosome aberrations and sister chromatid exchange in bone-marrow cells of the mice exposed to DZN and also in mammalian spermatogenic cells of mice [20]. DZNF were found more toxic than DZN as compared to control, as they contain surface active ingredients, dyes, stabilizers, activity enhancers, and organic solvents [2]. In addition, studies conducted by the National Institute of Hygienic Sciences in Japan, confirmed that DZN exposure increased the frequency of abnormal chromosomes in hamster [21], and in human blood cell cultures exposed to diazinon [22]. However WHO, found that the effects of DZN on human health and the environment, gave no evidence of a mutagenic potential [23].

The evidence for the genotoxicity of diazinon is strong and appears to operate in humans. Studies in experimental animals in vivo showed either DNA damage (oxidative DNA damage, DNA strand breaks) or chromosomal aberration. In vitro, human cell lines also showed DNA damage (DNA strand breaks) or chromosomal damage (micronucleus formation, sister-chromatid exchange) (IARC, 2017) [24]. The findings presented in this study were consistent with previous studies that associated the exposure to OP pesticides with DNA damage, because DNA is a target for mutagens and carcinogens, which induce changes in DNA structure giving rise to mutations and/ or cell death [25-27]. Free radical generated following insecticide exposure may lead to extensive DNA damage [26]. So, DZN and its formulation are capable of inducing chromosomal aberrations and DNA damage [28]. The increased DNA damage in mice exposed to DZNF and DZN in our study raises concern about the current assessment of the health risk posed by this pesticide and calls for a high level of caution in agricultural and household uses due to the extensive use of OP, in particular, by young workers.

These findings also support earlier reports on DZN-related DNA effects and point to the main mechanism of DZN action on the cell level: genotoxicity, which was established by DNA fragmentation and comet assays [10, 29]; because OP induced ROS (reactive oxygen species) which attack DNA, causing DNA damage and cell death [30]. The evidence that diazinon can induce oxidative stress is strong. Diazinon induced oxidative stress in human and mammalian cells in vitro, and in a variety of tissues in numerous studies in rodents in vivo. Diazinon induces oxidative stress through alteration of antioxidant enzyme activity, depletion of glutathione, and increasing lipid peroxidation (IARC, 2017) [24].

Biomarkers of oxidative tissue damage include AST, ALT in serum and MDA in liver and brain tissues; we found that DZN increases the levels of these biomarkers. In support to our study findings, treatment with DZN increased AST, ALT and MDA levels in male rats compared to control [12]. DZN and DZNF, commonly used as organophosphorus, were found to cause oxidative stress by increasing the level of reactive oxygen species (ROS) in several organs including liver and brain after acute and chronic exposure [5, 31]. Oxidative stress (OS) induced by reactive oxygen species may be a main mechanism, which can be associated with oxidation, membrane damage, sperm DNA damage, apoptosis, and subsequent male infertility, enzyme inactivation and biochemical alteration [12, 32].

V. CONCLUSION

These findings demonstrated that DZNF is more genotoxic than DZN as assessed by cytogenetic analysis of both bone marrow and germ cells in mice and had adverse effects on DNA and biochemical parameters (AST, ALT and MDA levels) and lead to chromosomal aberrations, DNA fragmentation and biochemical alterations. Therefore, the treatment with DZNF is more toxic and harmful than DZN and it is important to avoid toxicities induced by organophosphates, take a high level of caution and minimize its agricultural and household uses.

ABBREVIATIONS

OP: Organophosphorus; DZN: Diazinon; DZNF: Diazinon formulation; DNA: Deoxyribonucleic acid; WHO: The World Health Organization; MDA: Malondialdehyde; PBS: Phosphate-buffered Saline; ALT: Alanine transaminase; AST: Aspartate transaminase; USEPA: US Environmental Protection Agency; NPIC: National Pesticide Information Center; ROS: Reactive oxygen species; LPO: Lipid peroxidation; ANOVA: Analysis of variance; IARC: International Agency for Research on Cancer.

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Effect of Tillage Practices on Selected Soil Properties in Sudan Savanna Agro-Ecology of Nigeria

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Abstract— *Field experiments were carried out in 2018 and 2019 cropping seasons to evaluate the effect of tillage practices on selected soil properties in Sudan Savanna Agro-ecology of Nigeria. Treatments consisted of zero tillage, flat beds and ridges, and were laid out in a randomized complete block design (RCBD) and replicated three times. Prior to experiment, surface (0-15 cm) soil samples were collected from eight points and bulked; post-harvest composite soil samples were also collected on the basis of treatments and were analyzed using standard analytical procedures. NCRIBEN-01M variety of sesame was used as the test crop for both cropping seasons. The data generated from the study were subjected to Analysis of Variance (ANOVA) using Genstat Release 10.3 DE after which significant means were separated using Least Significant Difference (LSD) at 5 % level of probability. Based on the findings of this study, there were significant effects of tillage practices with respect to most soil parameters studied in 2018 and 2019 cropping seasons. The effects of tillage practices on soil nutrients indicated that the zero tilled plots had higher nutrients and organic matter, followed by the flat beds while the ridged plots gave lower values for essential nutrients and organic matter in both cropping seasons. For conservation or retention of essential nutrients as well as organic matter in soil, zero tillage is recommended for the study area*.

Keywords— Soil properties, Sudan Savanna, Tillage Practices, Nigeria.

I. INTRODUCTION

Tillage is performed to loosen the soil and produce a good tilth. Tillage requirement of a crop is site, environment and soil type specific (Ojeniyi and Agboola, 1995). Tillage contributes up to 20 % amongst crop production factors (Adekiya and Ojeniyi, 2011). Tillage operations in various forms have been practiced from the very inception of growing crop plants (Sharma and Behera, 2008). To prepare a virgin or fallow land and use it for growing crops, tillage in any form is an indispensable practice even today. Tillage is one of the forms of management practices of soil, water, nutrient, crop and pests. Tillage helps to replace natural vegetation with useful crops and is necessary to provide a favourable edaphic environment for the establishment, growth and yield of crop plants (Sharma *et al.,* 2002). After harvest of the crop, soil becomes hard and compact. Beating action of rain drops, irrigation and subsequent drying, movement of inter-cultivation implements and labour cause soil compaction. Seeds need loose, friable soil with sufficient air and water for good germination. The field should be free from weeds to avoid competition with the crop. It should also be free from stubbles to facilitate easy and smooth movement of sowing implements. There have been conflicting reports on the influence of tillage on soil chemical properties; likewise contradictory reports as to the superiority of crops on tilled plots to those of no-till plots have been documented (Adekiya and Ojeniyi, 2011). Ridge tillage was found to increase growth of okra on ultisol of central Southwest, Nigeria relative to no-tillage (Ojeniyi and Adekayode, 1999).

However, manual tillage systems including ridges, heaps and flat beds have been reported to degrade soil quality and reduce chemical and biological qualities especially on alfisols in the rainforest areas of Southwest, Nigeria (Busari and Salako, 2013). The study of relative effect of ridging and no-tillage on soil properties and yield of sweet potato in guinea savanna zone (middle belt) of Nigeria showed that no-till gave higher tuber yield of sweet potato compared to ridging, which was adduced to have higher moisture content, N, P, K, Ca and Mg status (Ojeniyi, 1993). In Nigeria, farmers commonly till the soil to improve its physical, chemical, and biological characteristics that alter plant growth and yield (Agber *et al.,* 2017). Crops grown without tillage are stunted and show symptoms of water and nutrient deficiencies because of high surface bulk

density, low porosity, retarded infiltration, and low water holding capacity of the soil (Ali *et al.*, 2015). However, the conventional and traditional tillage methods have negative effects on soil life and increase mineralization of organic matter. A zero tillage system, on the other hand, is a conservation method that involves the use of crop residues that aid water infiltration, prevent erosion, and increase organic matter content and agricultural productivity (Ali *et al.*, 2015).

Soil properties describe the physical and chemical characteristic behaviour of soils including the nutrient status (Usman, 2017). The need for basic knowledge and assessment of changes in soil properties and their fertility status with time to evaluate the impact of various tillage practices has become necessary for sustainable agriculture in Nigerian savanna zones. Similarly, for sustainable soil nutrient management in these zones, there is also need for an understanding of how soil responds to tillage practices over time (Oyedele *et al.*, 2014). One of the major important components of agricultural management and sustainability as well, a goal of most farmers in the tropics is the maintenance of soil nutrients and qualities. This according to Mallo (2010) provides avenue for measuring levels of crop productivity. Soil properties reveal soil quality which measures the levels of soil fertility. This means that assessing soil quality also involves measuring and evaluating soil properties for optimum crop yield. Soil properties may have influence on various processes that are suitable for agricultural practices, though the dynamic soil nature describes the condition of a specific soil due to management practices. However, for sustainable crop production, there is need for adoption of improved tillage practices and proper soil management that would ensure optimum crop yield. The knowledge of tillage and soil properties in Nigerian savanna is necessary in addressing the problem of low crop productivity and to ensure optimum food production. Thus, the objective of this study was to provide documented information on the effect of tillage practices on post harvest soil properties of sesame fields in the study area.

II. MATERIALS AND METHODS

2.1 Experimental Site

Field experiments were carried out in 2018 and 2019 cropping seasons to evaluate the effect of tillage practices on post harvest selected soil properties of sesame fields at the Research Farm of the Federal College of Education (Technical), Potiskum, Yobe State-Nigeria. NCRIBEN-01M variety of sesame was used as the test crop for both cropping seasons. Treatments consisted of zero tillage, flat beds and ridges, and were laid out in a RCBD and replicated three times. The study location falls within the Sudan Savanna Agro-ecology of Nigeria with mean rainfall of about 800 mm per annum and temperature of 39 – 42 ^{0}C. It is located between latitude $11^0$42' N to $11^0$43' N and longitude $11^0$04' E to $11^0$06' E (YSGN, 2008).

2.2 Soil Data Collection and Analysis

Prior to experiment, surface (0-15 cm) soil samples were collected from eight points and bulked; post-harvest composite soil samples were also collected on the basis of treatments. The soil samples taken from each plot according to treatment were air dried; crushed and sieved using 2 mm sieve and analyzed using standard soil analytical procedures at the Department of Soil Science, University of Maiduguri, Nigeria. Particle size distribution was determined by the Hydrometer method (Bouyocous, 1951). Soil pH was measured with the glass electrode pH meter in soil solution ratio 1: 2 in 0.01 M $CaCl_2$. Soil organic carbon (OC) was determined by the Walkey and Black method. Total N by the macro-Kjeldahl digestion method (Bremner and Mulraney, 1982), Available P was determined by Bray and Kurtz (1945) extraction method. Exchangeable cations were extracted using NH_4OAC solution, K and Na were read using flame photometer, while Ca and Mg was determined using the Atomic Absorption Spectrophotometer (AAS). Effective cation exchange capacity (ECEC) was established as the summation of the exchangeable cations (K, Na, Ca, Mg) and exchange acidity. The data generated from the study were subjected to Analysis of Variance (ANOVA) using Genstat Release 10.3 DE after which significant means were separated using Least Significant Difference (LSD) at 5 % level of probability.

III. RESULTS AND DISCUSSION

3.1 Selected Soil Properties of the Experimental Site before Planting

The results of analysis of selected soil properties of the experimental site before planting are presented on Table 1. The results indicated that soils for both cropping seasons were sandy clay loam in texture. This texture is ideal for crop production as crops require soils that are well drained for optimum growth and yield. The high sand content of the soils in 2018 and 2019 respectively (67.10 – 65.00 %) was indicative of the low clay content (21.20 – 20.90 %) for both years which could be attributed to the soil separates sorting activities by organisms, clay eluviation, surface soil erosion, parent material

or a combination of these factors (Malgwi *et al.,* 2008). The slightly acidic pH of the soils (6.96 – 6.70) also indicate that the soils are suitable for crop production as this pH range is the optimum pH for most crops and microbial activities in soil.

The results of physical and chemical properties of the experimental site before planting (Table 1) indicates a poor soil fertility status that requires fertilizer application to replenish nutrients taken out from the soil through crop harvest and to supplement nutrients to boost yields (Olatunji and Ayuba, 2012). The values of SOM (1.95 and 0.98 %) for the two cropping seasons were below the average range of 2.5- 2.6 % considered for good crop growth (Aduayi *et al.,* 2002) in the study area. The results of the soil analysis thus indicated that soil amendment was required in line with earlier observation by Agboola (1975) who reported that farmers in Africa requires adequate soil amendment for good crop production as a result of low inherent soil fertility. In addition, the poor nutrient status of this soil is characteristic of many tropical soils where the slash and burn practice coupled with high insolation and rainfall prevents the build-up of organic matter which is the store house of most nutrients (Aduayi *et al.* (2002; Anjembe, 2004; Senjobi *et al.* (2013).

TABLE 1
SELECTED SOIL PROPERTIES OF THE EXPERIMENTAL SITE BEFORE PLANTING IN POTISKUM

Property	2018	2019
Chemical Property		
pH	6.96	6.70
Organic Carbon (%)	0.55	0.57
Organic Matter (%)	1.95	0.98
Total Nitrogen (%)	0.17	0.19
Available P ($mgkg^{-1}$)	3.15	3.30
Exchangeable Cation ($Cmol\ kg^{-1}$)		
Ca	3.10	2.83
Mg	2.80	2.60
K	0.24	0.22
Na	0.03	0.02
EB	6.17	5.67
EA	0.20	0.18
CEC	6.37	5.85
Base Saturation (%)	96.86	96.92
Particle Size Distribution		
Sand (%)	67.10	65.00
Silt (%)	11.70	14.10
Clay (%)	21.20	20.90
Textural Class	Sandy clay loam	Sandy clay loam

3.2 Main Effect of Tillage Practices on Selected Soil Properties

The main effects of tillage practices on selected soil properties are presented on Table 2a-c. The effect of tillage practices on soil properties also show significant difference in most of the soil parameters studied in 2018 and 2019 cropping seasons. Tillage operations are known to influence both the release and conservation of soil nutrients. The effects of tillage practices on nutrients indicated that the zero tilled plots had higher nutrients followed by the flat beds while the ridged plots had the least available in both years. The higher nutrient status of zero tillage can be attributed to the presence of mulch on the surface due to decomposed plant residues, which led to enhanced soil organic matter status and associated availability of nutrients (Agbede, 2008). Tillage systems that reduce soil disturbance and residue incorporation have generally been observed to increase soil organic matter content (Mrabet *et al*., 2001). Ismail *et al.* (1994) concluded that conservation tillage systems results in significant and positive effects on several chemical soil properties. Soil organic matter largely contributes to nutrient cycling and thus supplies of N, S and other elements as well (Saleque *et al.*, 2009).

TABLE 2 a

MAIN EFFECT OF TILLAGE PRACTICES ON SELECTED SOIL PROPERTIES IN POTISKUM

Tillage Practices	BS (%)		CEC (cmol kg^{-1})		Ca (cmol kg^{-1})		EA (cmol kg^{-1})		EB (cmol kg^{-1})		K (cmol kg^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Flat	88.17	91.76	6.40	6.48	3.54	3.57	0.47	0.52	5.93	5.96	0.27	0.34
Ridged	89.22	89.61	6.16	6.22	3.29	3.32	0.57	0.64	5.56	5.58	0.25	0.25
Zero	88.42	92.84	6.47	6.57	3.62	3.65	0.37	0.42	6.10	6.06	0.28	0.28
LSD (P≤0.05)	0.14	1.35	NS	0.19	0.22	0.16	0.08	0.05	0.39	0.32	NS	0.15

NS= Not Significant

TABLE 2 b

MAIN EFFECT OF TILLAGE PRACTICES ON SELECTED SOIL PROPERTIES IN POTISKUM

Tillage Practices	Mg (cmol kg^{-1})		N (%)		Na (cmol kg^{-1})		OC (%)		OM (%)		P (mg kg^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Flat	1.50	1.51	0.083	0.083	0.62	0.62	0.88	0.88	1.52	1.51	3.29	3.30
Ridged	1.45	1.45	0.079	0.085	0.57	0.57	0.86	0.86	1.49	1.48	3.91	3.92
Zero	1.83	1.56	0.083	0.079	0.64	0.64	0.89	0.89	1.54	1.54	3.30	3.32
LSD (P≤0.05)	0.22	NS	0.002	0.004	0.04	0.05	NS	0.12	NS	0.10	0.312	0.25

NS= Not Significant

TABLE 2 c

MAIN EFFECT OF TILLAGE PRACTICES ON SELECTED SOIL PROPERTIES IN POTISKUM

Tillage Practices	pH		Sand (%)		Clay (%)		Silt (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
Flat	6.57	6.62	71.78	71.73	15.07	15.07	13.15	13.20
Ridged	6.56	6.57	72.60	72.35	14.54	14.54	12.86	13.11
Zero	6.61	6.56	70.43	70.36	16.13	16.22	13.43	13.43
LSD (P≤0.05)	NS	NS	1.49	1.34	0.72	0.65	0.34	NS

NS= Not Significant

Several researchers observed an increase of soil organic matter and carbon with conservation tillage practices in the top soil layer (Bronick and Lal, 2005; Vogeler *et al.,* 2009; Powlson *et al.,* 2012; Schjonning and Tomsen, 2013). In general, tillage improves the decomposition of crop residues by facilitating contact between plant tissue and soil aggregate surfaces, the primary biome of soil microorganisms (Bronick and Lal, 2005). In addition, tillage and organic matter in the soil and improves the availability of nutrients for plant growth through the formation of clay humus complexes and the increase of charged surfaces for nutrient binding. Accumulation of considerable amounts of total nitrogen, phosphorus (P), and potassium with conservation tillage was observed (Calegari *et al.,* 2013; Spiegel *et al.,* 2007). This may be due to the fact that the land was not disturbed which increased the buildup of soil organic matter, resulting in high organic carbon which reflects a reduced rate of leaching in the soil profile in the soil studied. Tillage systems (zero tillage) that reduce soil disturbance and residue incorporation have generally been observed to increase organic C. Zero tillage has been reported to have resulted in increased in organic C content which in turn enhances soil quality and resilience (Abid and Lal, 2008). Differences in available N among tillage systems observed in the current study are in agreement with those of other studies (Martin-Rueda *et al.,* 2007). Available N was significantly higher in zero tillage treatment than in the other tillage systems.

Similarly, a study on Mollisols in Nebraska, available N was significantly greater under zero tillage than conventional tillage (Martin-Rueda *et al.,* 2007). In another study, soil available N content was also significantly increased under zero or minimum tillage (Martin-Rueda *et al.,* 2007). Higher Nitrogen in the zero tilled soils may be attributed to less loss through immobilization, volatilization, denitrification, and leaching (Malhi *et al.,* 2001). Available P and K as well as other essential nutrient elements were higher under zero treatment probably due to higher soil organic C level. Zibilske *et al.* (2002) reported that improvement of soil available P was due to redistribution or mining of P at lower soil depths. Also, work done by Redel *et al.* (2007) showed a high amount of P under zero tillage treatment compared to the conventional tillage and have attributed this to an increase in contact time between P and soil particles.

Cultivation also stimulates soil carbon losses due to accelerated oxidation of soil carbon by microbial action. Hence when organic matter is lost the associated nutrients are also lost. Yin and Vyn (2002) also observed more soil nutrients in case of no-tillage as compared to deep tillage. The least values of essential nutrients recorded by the ridged plots compared with the zero tilled plots could be due to inversion of top soil during soil preparation, which brought less fertile subsoil to the surface in addition to possible leaching (Ali *et al.,* 2006) as well as rapid mineralization and uptake of nutrients by the crops (Adekiya *et al.,* 2009).

Similarly, Alam *et al.,* (2014) reported that tillage practices showed positive effect on soil properties and crop yields, Bulk and particles densities were decreased due to tillage practices having the highest reduction of these properties and the highest increase of porosity and field capacity in zero tillage. The highest total N, P, K and S in their available forms was recorded in zero tillage. Therefore, zero tillage was found to be suitable for soil health and achieving optimum crop yields.

IV. CONCLUSION AND RECOMMENDATIONS

Based on the findings in this study, there were significant effects of tillage practices with respect to most parameters studied in 2018 and 2019 cropping seasons. The effects of tillage practices on nutrients indicated that the zero tilled plots had higher nutrients and organic matter, followed by the flat beds while the ridged plots gave lower values for essential nutrients and organic matter in both cropping seasons. For conservation or retention of essential nutrients as well as organic matter in soil, zero tillage is recommended in the study area.

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Assessing the Impact of Urban Growth on the Forest Degradation in Musanze District

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Abstract— *As the days are passing there are changes in development in many districts of the country where the urbanization is growing in term of expansion every day. This expansion is due to the population pressure where the population need to satisfy their needs and trying to accommodate themselves with all those acts the forests are damaged by population through constructions and by cooking and which leads to forest degradation. It is in this context that the present study was conducted in order to assess the impact of urban growth on the forest degradation in Musanze. The study had the Specific objectives which were the analyzing of the trend of forest degradation; the assessment of urban growth status; and to establish the relationship between urban growth and forest degradation. The researcher used data (orthophoto) from Rwanda Land Management and Use Authority of the period 2009-2019. The Arc GIS, total station TS06, Differential GPS, have been used for accomplishing this research. Results show that forests lost at rate of 3.3 % every year due to the heavy urban growth which is not monitored and this implicate that within 30 years there will not be any forest in Musanze district. It is recommended that the district should deliver the education and training courses to local communities; should organize special campaigns about importance of forests to the comminutes and the society, should mobilize the population and other stakeholders to plant many trees in whole city and also make the forestation.*

Keywords— Musanze district, Urban growth, Forest degradation, City development.

I. INTRODUCTION

The human population grew at unprecedented rate during the last two decades globally as well as in the Asia-Pacific region. The region accounts for more than half of the world's population. During the last 25 years, the population of the region grew by more than a billion (2.45 billion in 1980 to 3.6 billion in 2005), and is likely to reach to 4.2 billion by 2020. However, population growth changed its character and form in the twentieth century with the advancement of technology and medical facilities. This has resulted in major demographic transitions, such as changes in rates of births and deaths, age composition and rural population growth rate. The impact of demographic changes on forests and the environment is often discussed in terms of biological carrying capacity, i.e. the maximum number of individuals that a resource can sustain (Schaffartzik, et al., 2014). However, many factors influence carrying capacity, such as economic development, socio-political processes, and trade, technology, and consumption preferences. Many studies have already shown that demographic changes in conjunction with the other factors have affected natural resources in general and forests in particular (Misselhorn, 2005). Forests provide an array of goods and services critical to economic development and human well-being. The demands of a growing population on forests are increasing in both magnitude and variety. However, the impacts of demographic changes are not widely understood beyond the very broad and superficial relationship that population growth increases demand for goods and services provided by forests. In this context, a thematic study on the impact of predicted demographic changes on forests and forestry has been conducted as part of the Asia Pacific Forestry Sector Outlook Study II (APFSOS II). Some of the important demographic factors that could affect forests and forestry by 2019 include population size and growth, population distribution and population structure (Corvalan, et al., 2005).

As the days are passing there are changes in development in many districts of the country where the urbanization is growing in term of expansion every day and every time. This expansion is due to the population pressure where the population need to satisfy their needs and trying to accommodate themselves with all those acts the forests are damaged by population through

constructions and by cooking and which leads to forest degradation. The present study was conducted in order to assess the impact of urban growth on the forest degradation in Musanze.

II. MATERIALS AND METHODS

2.1 Description of the study area

The study was conducted in Northern Province in the district of Musanze. Musanze District was selected as a site according to its relatively convenient location and rapid infrastructure development and rapid urban growth due to large number of population coming in town to search for job and to extend their business. Its ecological importance to biodiversity associated with the forests located within its boundaries is also a good aspect for this study. Musanze district covers a planned area of 530 km^2 according to the recent approved master Plan and elaborated for implementation in 2014. The district comprises seven (15) Sectors including Busogo, Cyuve, Gacaca, Gashaki, Gataraga, Kimonyi, Kinigi, Muhoza, Muko, Musanze, Nkotsi, Nyange, Remera, Rwaza and Shingiro. The livelihood of the city depends on the environment and natural resources such as forests, water, land, air, plants and animals.

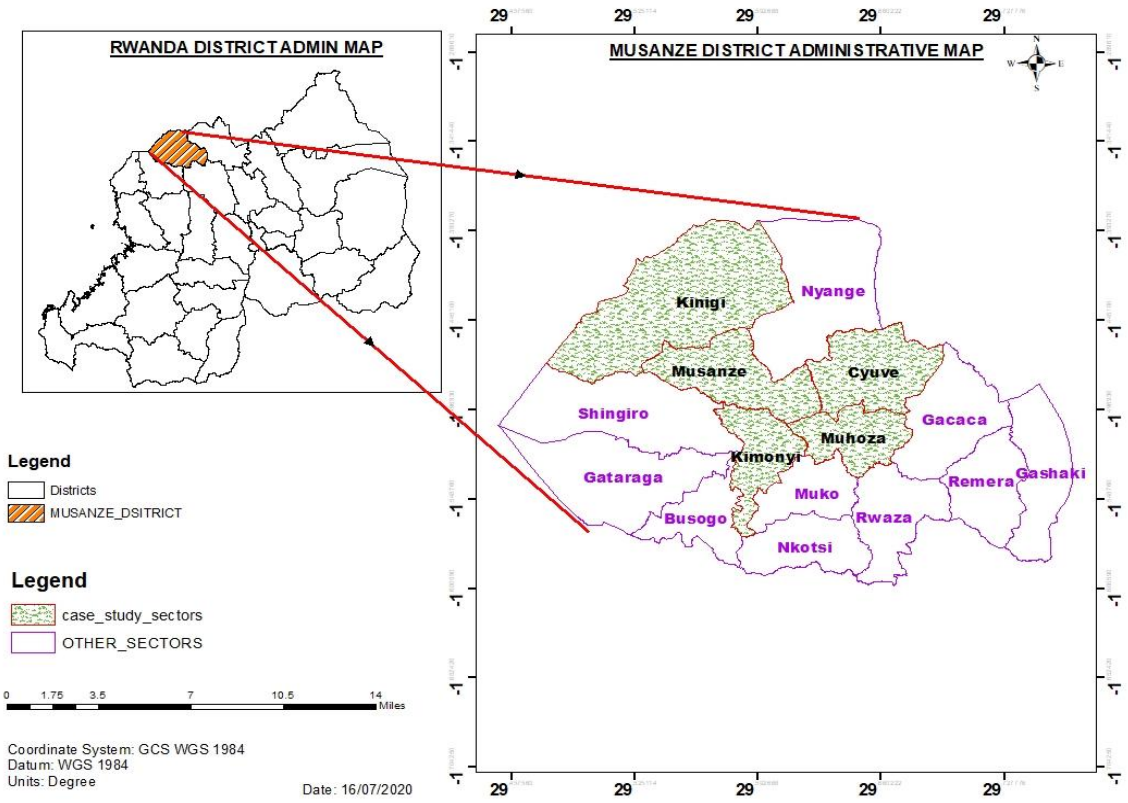

FIGURE 1: Map of the study area

2.2 Data collection and data processing Methods

Data collection is constituted by the primary data and the secondary data; where by the orthophotos have been given by the Rwanda Land Management and Use Authority, those orthophotos showed the housing and land use from the year of 2009. The orthophotos are the ones which contain the information of the forests in 2009 and it shows how the forests were in 2009. The orthophotos of Musanze district have been added to ArcGIS and the shape file of Musanze administrative boundary has been added to the ArcGIS. The sectors on Musanze district have been labeled in ArcGIS in order to know the location of sector of case study and to see the forests in each sector of case study in order to choose randomly two forests to be digitalized. After the choosing of the forests to be analyzed, they have been digitalized and the data have been projected to ITRF 2005 and after that, they have been exported to shape file. After the exporting, the areas of forests in are identified in the table of content and check the attribute table of the forests in order to know the areas of the digitalized forests. After the projection of the forests of 2008 the topographic survey has been conducted by setting out the control points with the differential global positioning system (DGPS) and from those control points, the total station took place by being oriented to the reference made by the differential global positioning system and after the survey of the forest has been done by taking all

points required for having the enough information in order to know how the forest were in 2018. The topographical survey has been done in all selected sectors for case study and with this topographical survey the points taken with total station has been saved in scv comma delimited in excel and after all the points have been added to Arc GIS and then after the points have been digitalized and projected to ITRF 2005 in order to get the shape file of forests in 2019 and after this the areas of forests in 2018 have been has been find in the attribute table of the forests in 2019 in the table of content, the two forests in each sector of the case study will be chosen randomly and will be surveyed accordingly. All those data are the one, which are used in this research.

During this research project, the following software's have been used, Arc GIS has been used for the production of the maps and for the analysis of the geospatial data. The total station TS06 and its accessories have been used to know the actual topographic situation of the forests of Musanze district in 2019.Differential GPS has been used for setting the control survey of the forest in 2018 and it has been used for doing the boundary survey of the forests. MS WORD has been to write the research report.MS EXCEL has been used to make the tables, diagrams and the charts in analyzing this research report.

III. RESULTS AND DISCUSSION

3.1 The trends of forest degradation

The trends of the forest degradation is characterized by the loss of the areas of the forests in Musanze districts

TABLE 1
THE FOREST DEGRADATION

Sector name	Forest 2009(m^2)	Forest 2014(m^2)	Forest 2019(m^2)
Cyuve	82844.708	56,845.638	19,322.47
Kimonyi	124,154.72	83,153.12	62,982.97
Muhoza	74,733.31	70221.45	65,820.89
Musanze	166,463	103,601.31	79,640.94
Kinigi	275,843.86	261,229.91	257,455.38
Total	**724,039.594**	**575,051.428**	**485,222.643**

The table1 shows the forest degradation from 2009 to 2019 where the forests have been lost especially in Cyuve, Kimonyi, Muhoza, Musanze and Kinigi sectors, the areas occupied by the forests have loss from 82844 m2, 124154 m2, 74733 m2, 166463 m2 and 275843.86 m2 to 19322 m2, 62982 m2, 65820 m2, 79640 m2, and 257 455 m2 respectively to Cyuve, Kimonyi, Muhoza, Musanze and Kinigi sectors. The table 2 shows clearly, how the forests in Musanze district are being lost slowly by slowly with the years.

3.2 The status of urban growth

Every year the increase of the urban growth is affecting the forests areas.

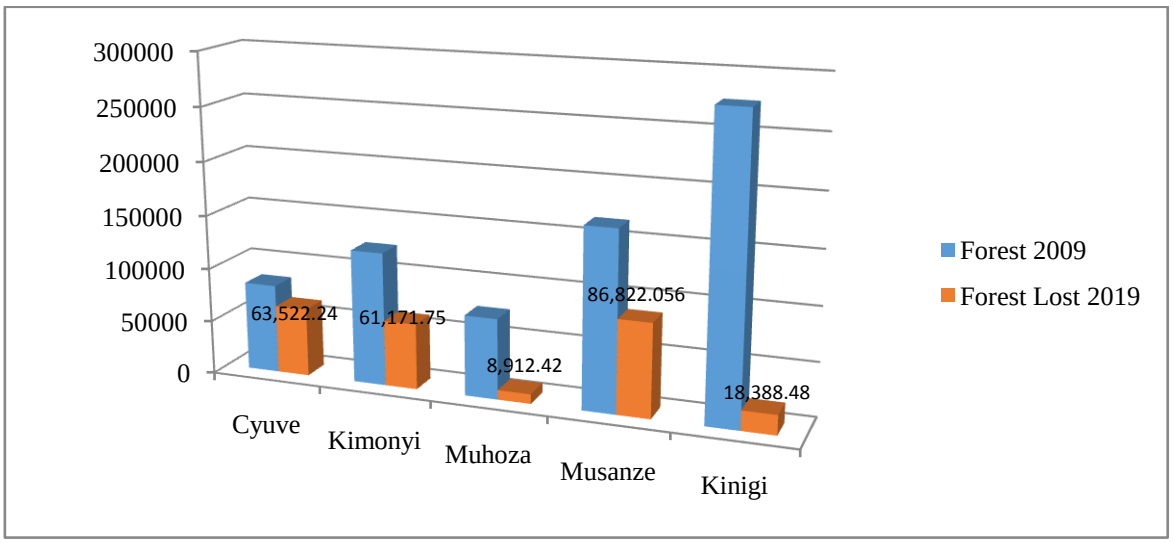

FIGURE 2: The urban growth in Musanze district

The figure 2 shows the areas of the forests in 2009 and it shows also the areas of the forests lost from 2009 to 2019. The very big areas lost have been made on the forests in Cyuve, Kimonyi and Musanze sectors due to the rate of the urban growth. The loss of the forests shows that there is a big increase of people in the all sectors of the case study. The urban growth is expressed in the times of the forest lost. The total area of urban growth from 2009 to 2019 is 238,816.95 m^2 all five sectors in Musanze city.

3.3 The relationship between the urban growth and the forest degradation

TABLE 2
URBAN GROWTH AND FOREST DEGRADATION RELATIONSHIP

Sector name	Forest 2009 (m^2)	Forest 2019 (m^2)	Forest lost 2009-2019 (m2) As indicator of urban growth
Cyuve	82,844.708	19,322.47	63,522.24
Kimonyi	124,154.72	62,982.97	61,171.75
Muhoza	74,733.31	65,820.89	8,912.42
Musanze	166,463	79,640.94	86,822.06
Kinigi	275,843.86	257,455.38	18,388.48
TOTAL	**724,039.594**	**485,222.643**	**238,816.95**

The table 2 shows the relationship between urban growth and forest degradation where the urban growth is characterized by areas of forests lost from 2009 to 2019 and the forest degradation is characterized by the areas changes of forest in 2009 and final areas of forests in 2019. As the forest changes by diminishing as there is urban growth.

IV. CONCLUSION AND RECOMMENDATIONS

This study focus was to assess the impact of urban growth on the forest degradation in Musanze district, from 2009-2019. To facilitate and guide the decision making for the urban planners for taking into account the forest degradation, and the good land use planning to sustain urban growth in Musanze District. To provide a useful understanding on how government policies should be implemented for improving the quality of urban life for city residents

The result shows that there are big quantities of forests lost every delay the rate of 3.3 % every year due to the heavy urban growth as it characterized in time of the forest loss which is not monitored and this can implicate that within 30 years there will not be any forest in this city. Recommendation

Based on the results found for this research, several recommendations were formulated to facilitate the better urban planning and land use management:

- Delivering education, training and bridging courses to local communities. The contact of city residents with urban and forest officers is the main source from which urban residents receive advice to avoid the big rate of forests loss,
- To organize special forest campaigns about the good importance of forests to the communities and the society,
- To improve a strong collaboration between city residents and other stakeholders involved in urban planning and development,
- To mobilize the population and other stakeholders to plant many trees in whole city and also make the forestation where the forest has been degraded.

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Manurial value Assessment of Coir Pith through Field Study

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Abstract— *Pot culture studies with coir pith compost indicated that this material in combination with soil and sand can be a suitable for farm yard manure in potting mixtures. When used alone also, coir pith compost was found to be a good medium for the container cultivation. The only practical problem is the use of coir pith alone appears to be the anchorage. A part of this study with nutrient supplementation had indicated that the only major plant nutrient element among N, P, and K that is deficient in composted coir pith is nitrogen.*

Keywords— Coirpith, potting mixture, NPK.

I. INTRODUCTION

Use of composted coir pith for agricultural use as a substitute for common organic manures had been tried experimentally decomposed coir pith has very high moisture retention capacity and its wetability is much better than peat (Evans and Stamps, 1996). The decomposition of lignin present in coir pith results in the formation of humic fractions (Kndalli et al., 2001) It had been often suggested that this product may also be used as a component of potting mixtures for container cultivation and even as an exclusive medium for soilless cultivation of indoor ornamental plants. Being a plant product, coirpith is expected to contain all plant nutrient elements and have release all of these on decomposition. However, the level of nutrient availability in this organic manure requires to be assessed. The present study was taken up to assess these aspects and the specific objectives are listed below.

1) Assess the suitability of coir pith compost as a component of potting mixture for the container cultivation

2) Test of use of coirpith compost as on exclusive medium for soil less cultivation of plants.

Evaluate the nutrient status of this product when used alone and also in combination with sand and soil.

II. MATERIALS AND METHODS

This pot trial had factorial combinations of four different types of potting mixture and four types of nutrient supplementation. Statistical design was CRD with four replications. There were a total number of 80 plants. The test crop selected Tomato var: Vellayani Vijay and the crop period were from August to December. The treatments were the following.

- Media
- Coir pith compost alone
- Soil:Sand in 1:1 ratio
- Soil:Sand:FYM (Farm Yard Mannure) in 1:1:1 ratio

- Sail:Sand:CPC (Coirpith Compost) in 1:1:1 ratio

2.1 NPK treatments

These include supply of three fertilizer nutrients N, P and K. There was one treatment that was received all the three nutrients and another that had none. The levels of nutrients were as per recommendations and calculated on per plant basis. One set of four bags were considered as control in which no fertilizer element was added. In addition to soil application as above, foliar spray of 0.1% nutrient carriers – urea, sodium dihydrogen orthophosphate, Murite of potash were given at weekly intervals as per treatments starting from 14 days after planting in order to ensure the growth was not affected for the need of respective nutrients at any stage. Observations on growth, yield and yield components were taken using standard procedures at periodical intervals and data processed by analysis of variance technique.

TABLE 1
GROWTH, YIELD AND YIELD COMPONENTS OF TOMATO

Types of Media	Plant Height at first harvest (cm)	Spread of leaves (Cm)	Days to first flowering	Days to first harvest	Total No of Fruits	Total weight of fruits
Coir pith compost alone (B1)	58.60	28.95	16.10	43.35	28.0	747.27
Soil:Sand in 1:1 ratio (B2)	50.25	21.95	20.30	45.70	21.0	466.68
Soil:Sand:FYM in 1:1:1 ratio (B3)	56.90	24.43	18.65	43.40	27.9	754.26
Soil:Sand:CPC (B4)	63.30	22.58	16.30	42.80	28.95	702.53
CD(0.005)		20.26	2.259		3.031	55.27
Fertilizers						
No Fertilizers	54.63	19.50		46.19	17.25	46.13
Nitrogen	56.75	25.56		43.44	34.56	863.57
Phosphorous	59.63	29.75		43.56	21.85	600.48
Potassium	56.06	20.06		43.81	21.38	550.67
NPK	59.25	27.50		42.06	37.31	901.15
CD (0.05)		2.262		2.45	3.39	61.794

III. RESULT AND DISCUSSION

Data on the growth, yield and yield components were subject to statistical analysis and it was found that the interaction between the two sets of factors, media composition and nutrient supplementation was not statistical significant. As such, mean values only are presented and discussed (Table 1). The differences between individual factors was statistical significant and on mean values relating to the four media indicate an inferiority on the medium with soil and sand only, which received no organic supplement. Those with coir pith compost were at par with the standard medium of farm yard manure, soil and sand. The proportion of CPC in the mixture did not appear to be very critical and interestingly the one having CPC alone was as good as any other medium including the traditional soil: sand:FYM in equal proportions. The possibility of substituting

FYM in potting mixture is clearly indicated. Another important indication is the possibility of using CPC as the sole medium for container cultivations and there for using it for soilless cultivation of plants. The only practical problem with the use of CPC alone was the poor anchorage at the later stage of the growth of the crop and necessity for the staking the plants when required. The pH of coco peat is closer to the optimum for the growth of most plants than that of sphagnum peat, which is highly acidic. So, replacing sphagnum peat with coco peat can also result in considerable saving, as lot of lime is required for bringing down pH of sphagnum peat (Cresswell, l997). The importance of identifying porous organic substrates a substitute of Sphagnum moss had been reported (Bonita Kristine Lowry, 2015) and coir pith compost appears to be one possibility.

Among the nutrients supplemented viz: Nitrogen, Potassium and Phosphorous the only having a significant deficiency in coir pith appears to be nitrogen. Advantage of supplementation of this nutrient element is clear both in growth and yield. The factors not much affected by the medium composition are plant height and days to flowering and fruiting.

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Analysis of Physicochemical Parameters in Wastewater and Heavy Metals in Soils of Flower Farms in Ethiopia

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Abstract— *Floriculture is a young and fast-growing industry in Ethiopia. The sector has created employment opportunity and contributed to our country's economic development. But it is blamed for causing environmental pollution. Therefore, the aim of this study is to determine the concentration of pollutants in wastewater and soils of flower farms located in Ethiopia which were selected using purposive sampling. Wastewater and soil samples required for the determination of physico-chemical parameters and heavy metal concentrations were collected from the flower farms from April 1 to May 25, 2019. Physicochemical parameters including pH, electrical conductivity, total dissolved solids, phosphate, sulfate and chemical oxygen demand in wastewater, and concentrations of lead, cobalt, and zinc in soil were determined. Accordingly, the pH values of the four flower farms (Farm 1, Farm 2, Farm 4 and Farm 5) were slightly acidic and below minimum pH value (6) allowed for wastewater effluent set by Ethiopian Environmental Protection Authority. Electrical conductivity at all farms, total dissolved solid at Farm 4, chemical oxygen demand at Farm 3, and 4, sulphate at Farm 4, and phosphate at Farm 2 and 4 were above the provisional standard set by EPA. This study revealed that wastewater sample collected from Farm 4 doesn't comply with EPA standard in all study parameters. While, the mean concentrations of cobalt and zinc of soil samples varied from 2.8 to 46.6 mg/kg and 54.4 to 111.1 mg/kg, respectively. Conclusively, the wastewater quality discharged from flower farms is not at a level it cannot cause harmful effect. Therefore, there is a need to ensure that wastewater is properly treated before discharged into the environment. Also, the authors recommend that further holistic investigation should be carried out on socio-economic and soil pollution of the floriculture industry in Ethiopia.*

Keywords— Floriculture, Heavy metal, Physicochemical, Soil, Wastewater.

I. INTRODUCTION

Floriculture is a discipline of horticulture concerned with the cultivation of flowering and ornamental plants for gardens and for floristry, comprising the floral industry. It can also be defined as "The segment of horticulture concerned with commercial production, marketing, and sale of bedding plants, cut flowers, potted flowering plants, foliage plants, flower arrangements, and noncommercial home gardening" (Getu, 2009; Tilahun, 2013).

Floriculture is a young and fast-growing industry in Ethiopia. Since the industry is export-oriented, it serves to generate foreign exchange. According to Arefaynie (2009), the major factors that have contributed to the development of horticulture industry in Ethiopia include suitable climate, altitude, and availability of land, low labor costs and other favorable conditions. In 2002, there were only five floriculture farms in the country; however, by 2008, this number rose to more than a hundred (EHPEA, 2014)

The study conducted by Kassa (2017) stated that Ethiopian floriculture industries currently produce several flower species, including roses, gypsophila, hypericum, limonium, carnations, and chrysanthemum. Currently, Ethiopia is benefiting from this development through creating employment opportunity for unemployed citizens. In addition, the floriculture industry has given the country's export sector an alternative export commodity to the traditional predominant export of coffee.

However, there are a number of challenges that must be resolved to continue the development of the sector with the present rapid pace. Among the challenges is high consumption of different chemicals by the sector which can damage the environment through its discharge. According to Tamiru (2007), the production of flowers uses more than 300 chemicals

such as pesticides and growth regulators, which can kill useful organisms in the soil and disturb the biodiversity surrounding the flower farms. It is known that soil pollution can lead to water pollution if toxic chemicals leach into groundwater, or if contaminated runoff reaches streams, lakes, or oceans (Bolo and Brachet, 2010; FAO, 2017). Phosphorus fertilizers are among the sources of heavy metal inputs; and superphosphate fertilizers contain, in addition to nutrient elements, trace metal impurities like cadmium (Cd) and lead (Pb). Malidareh and his colloeges (2014) showed that fertilizers might contain heavy metals that can cause serious problems in water and soil. Therefore, the objective of this study was to assess the status of environmental pollutants in five flower farms located in Central Ethiopia by analyzing physico-chemical parameters in wastewater and selected heavy metals in soils.

II. MATERIALS AND METHODS

2.1 Study area

The study area consists of five flower farms which were coded as Farm 1, Farm 2, Farm 3, Farm 4 and Farm 5 (Figure 1) for the sake of confidentiality. The flower farms were purposely selected based on the magnitude of the social complaint reported by Oromia Environment, Forest, and Climate Change Authority. Farm 1 and Farm 2 are found in Woliso and Bacho Woreda, respectively, of Southwest Shewa zone. Farm 3 and Farm 4 are both found in Walmera Woreda of West Shewa Zone and Farm 5 is located in Adami Tulu Jido Kombolcha Woreda of East Shewa zone.

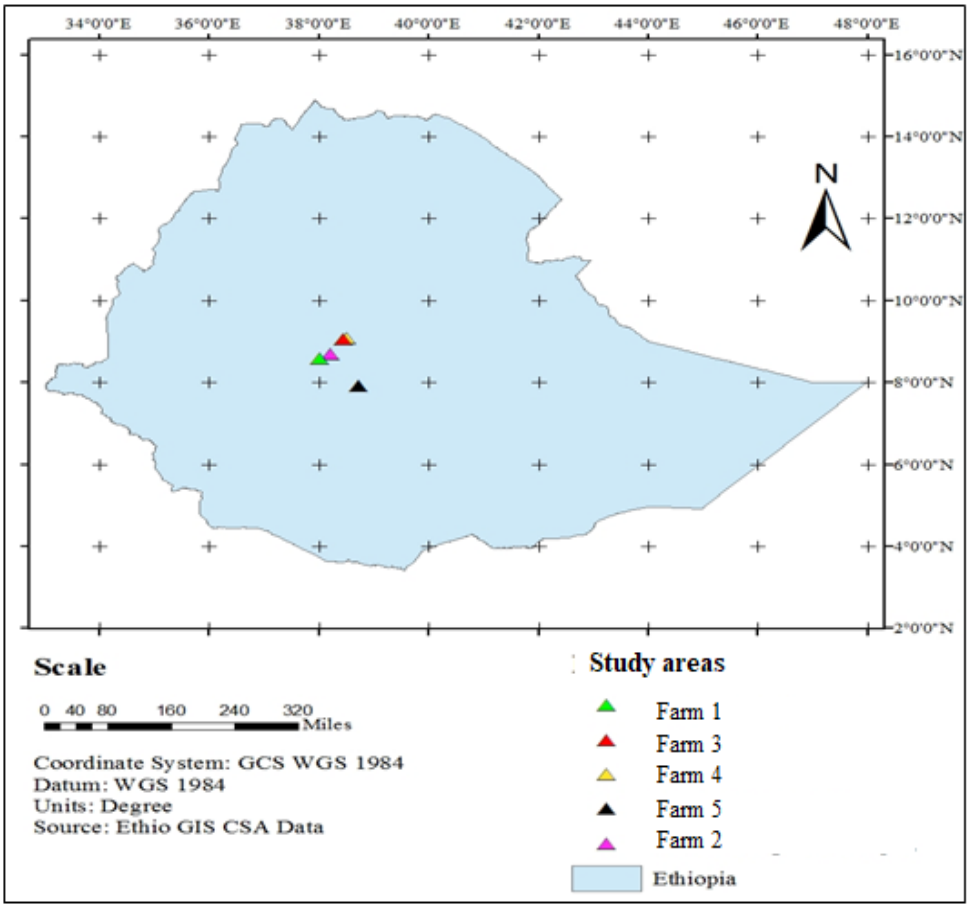

FIGURE 1: A map showing specific locations of the five flower farms

2.2 Sampling, sample handling, preparation and analysis

2.2.1 Wastewater sampling, handling, preparation and analysis

Wastewater samples were collected from five flower farms from April 1 to May 25, 2019. All samples were collected at the outlet in triplicate by using sealed plastic bottles which were thoroughly cleaned with detergent, rinsed with distilled water, soaked in 5% HNO_3^- for 24 hours and finally rinsed with distilled water. For each wastewater sample, pH, total dissolved solids (TDS) and electrical conductivity (EC) were measured at site using portable multi-meter (Jenway, model 3305). The remaining samples were labeled, preserved and transported to the laboratory in icebox. In the laboratory, the other parameters

were analyzed using standard methods (APHA, 2002) i.e. sulphate (SO_4^{2-}) by Turbidimetric, phosphate (PO_4^{3-}) by Vanadomolybdo phosphoric acid colorimetric, and chemical oxygen demand (COD) by the Open Reflux methods.

2.2.2 Soil sampling, handling, preparation and analysis

First, ten soil samples were collected from the top 30 cm depth of each farm using auger, spade and spoon, by applying random sampling technique. Then, ten soil sub samples of each flower farm were mixed, homogenized, placed into clean polyethylene bags in triplicate, labeled and transported to JIJE Analytical testing service laboratory P.L.C. in an icebox to avoid cross contamination and change of composition by weather conditions. In the laboratory, the samples were air dried and sieved to pass through 2 mm sieve, digested by microwave system and finally Pb, Co and Zn were analyzed by Flame Atomic Absorption Spectrophotometer (FAAS).

2.3 Statistical analysis

The data were analyzed by using Microsoft Excel 2010. Descriptive statistics were used to analyze the data obtained from physico-chemical analysis of wastewater and heavy metal concentrations of the soil samples.

III. RESULTS AND DISCUSSION

3.1 Physicochemical parameters for the wastewater

The mean pH value for each flower farm is shown in Table 1. The results indicated that the mean pH values of the four farms (Farm 1, Farm 2, Farm 4 and Farm 5) were slightly acidic and below minimum pH 6 which is allowed for wastewater effluent by Ethiopian Environmental Protection Authority (EPA, 2003). This may be associated to the addition of nitric acid and sulfuric acid to decrease the high pH of dripping water that arises from the use of fertilizers by the flower farms as stated by Tamiru and Leta (2017).

Wastewater with high amount of dissolved inorganic substances in ionized form could originate from fertilizers and pesticides used in flower farms. The mean EC values of wastewater were in the range 1,489.7- 17,546.6 μScm^{-1} (Table 1). The results obtained in this study were all higher than the optimum EC value of 1000 µS/cm for wastewater discharge set by EPA in 2003. High values of EC shows high inorganic ions in the wastewater (Aniyikaiye et al., 2019; Benit & Roslin, 2015). On other hand, the measured mean value of TDS in the wastewater samples of the flower farms varied from 1117.5 to 13160 mg/L. The TDS values of the selected farms were within the limit of provisional standard 3000 mg/L set by EPA except farm 4. The measured mean COD values of the studied flower farms' wastewater varied from 11.2 to 339.2 mg/L). The COD concentration was above permissible limit for Farm 3 and Farm 4 which could be attributed to excessive organic and inorganic chemical use in the flower farms. The results are presented in Table 1.

In this study the measured values of sulphate concentration are below provisional standard set by EPA (200 mg/L) except at Farm 4 (716.0 mg/L) which is above the limit. Similarly, the maximum phosphate concentration was detected at Farm 4 which was by far above EPA recommended value of 10 mg/L. The obtained maximum concentration of phosphate recorded might be due to the flower farm's high application of phosphate based fertilizers like ammonium phosphate. The result was shown in Table 1.

TABLE 1
PHYSICOCHEMICAL PARAMETERS IN WASTEWATER SAMPLES

Flower farms	Mean values of parameters					
	pH	EC(μScm^{-1})	TDS(mg/L)	COD(mg/L)	SO_4^{2-}(mg/L)	PO_4^{3-}(mg/L)
Farm 1	5.7	3116.9	2337.7	12.8	40	2.1
Farm 2	5.8	3120	2340.3	11.2	66	11.5
Farm 3	6.5	2683.6	2012.7	339.2	125.4	7.5
Farm 4	5.9	17546.6	13160	320	716	309
Farm 5	5.4	1489.7	1117.5	16	35.1	1
*EPA permissible limit	6-9	<1000	3000	250	200	10

Ethiopian Environment protection Authority (2003).

3.2 Heavy metal concentration of Soil samples

In this study, except for Farm 2 (12.4 mg/kg), the concentration of lead was below detection limit for all analyzed soil samples. The obtained result was within the EPA recommended value of 40 mg/kg (EPA, 2003). The mean concentrations of cobalt in soil samples taken from the flower farms were between 2.8-46.6 mg/kg (Table 2). The results revealed that the concentration of zinc was highest among the heavy metals analyzed from all the sample sites. The obtained values were below provisional standard of 500 mg/L set by EPA (2003). The low concentration level detected for soil analysis might be attributed to the washing of the soil by runoff, dispersion by air, and infiltration below soil sampling depth.

TABLE 2
HEAVY METAL CONCENTRATION IN SOIL SAMPLES

Flower farms	Mean value of heavy metals in mg/kg		
	Pb	Co	Zn
Farm 1	12.4	37.1	105.2
Farm 2	ND	22.2	106.4
Farm 3	ND	46.6	111.1
Farm 4	ND	23.8	91.6
Farm 5	ND	2.8	54.4
EPA standard	40	-	500

ND= Not detected

IV. LIMITATION OF THE STUDY

Wastewater discharges and soil samples were taken only once and analyzed for few main physicochemical and heavy metal parameters. Hence, it cannot be generalized for broader flower farm pollution status. The parameters that were found to be above the standard limits could have been justified in a better way had there been additional information on the use of fertilizer and other chemicals, wastewater treatment employed and efficiency.

V. CONCLUSIONS AND RECOMMENDATIONS

This study revealed that wastewater discharged from most study flower farms has contaminant concentration not in accordance with permissible level. Among the physicochemical parameters investigated: pH (for Farm 1, 2, 4, and 5), electrical conductivity (for Farm 1 to 5), chemical oxygen demand (for Farm 3 and 4), and phosphate (for Farm 4) not comply with the levels recommended for wastewater discharge set by EPA. Conclusively, the wastewater quality discharged from flower farms is not at a level it cannot cause harmful effect on the environment.

It is inevitable that some amount of pesticides from flower farms can reach our primary concern i.e. human beings and cause undesired impacts. Hence, the flower farms should shift to organic farming which relies on natural methods to control pests and diseases such as crop rotations, composting, encouraging the natural predators of common pests, and developing healthy flowers that have a natural resistance to pests and diseases, so that the related risk can be reduced. And also there is a need to ensure that wastewater is properly treated before discharge into the environment. Adding to this, the author's recommend that further holistic investigation should be carried out on socio-economic and soil pollution status of the floriculture industry in Ethiopia.

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Cellulosimicrobium funkei: A Novel Bacterium in Potassium Solubilization from Soil in Bangalore

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Abstract— *Potassium (K) is a very essential element needed by plants for healthy growth and good yield. Most soils have abundance of potassium underneath in rock as insoluble forms that are unavailable for plant use. This research was carried out to join in the search to unearth microorganisms from the rhizosphere soil that are able to act on the mineral containing substances, solubilizing them to release the needed soluble form of the potassium for plant use. An isolate, which was characterized and identified to be Cellulosimicrobium funkei, showed significant solubilization on feldspar (a potassium containing compound) supplemented media. It is novel for potassium solubilization. The amount of potassium released by the isolate in comparison to reference cultures varied but favourably compared with the reference cultures. In glucose amended broth, solubilization was: Cellulosimicrobium funkei 7.04mg/l, Enterobacter hormaechei 7.15 mg/l and 6.91mg/l for Aspergillus terreus. Urea supplemented broth: Cellulosimicrobium funkei 5.45mg/l, Enterobacter hormaechei 5.38mg/l and Aspergillus terreus 6.33mg/l. KCl supplemented broth: Cellulosimicrobium funkei 10.23mg/l, Enterobacter hormaechei 8.05mg/l and Aspergillus terreus 9.11mg/l. For temperature, the cultures solubilized best at these respective temperatures: Cellulosimicrobium funkei 27^oC, Enterobacter hormaechei 35^oC and Aspergillus terreus 30^oC. P^H was 7.5 for Cellulosimicrobium funkei, 8 for Enterobacter hormaechei and for 7.5 for Aspergillus terreus. When they were now cultured using the combination of the above parameters Cellulosimicrobium funkei, Enterobacter hormaechei and Aspergillus terreus gave a maximum yield of 7.24mg/l, 7.03mg/l and 6.81mg/l of solubilized potassium respectively. This means that the isolate Cellulosimicrobium funkei yielded more solubilized potassium from feldspar than the reference cultures and could therefore be a better potassium solubilizer.*

Keywords— Aspergillus terreus, Cellulosimicrobium funkei, Enterobacter hormaechei, Potassium, Soil, Solubilizing.

I. INTRODUCTION

Potassium (K) exists in several forms in the soil, including mineral potassium, non-exchangeable potassium and exchangeable potassium and dissolved or solution potassium (K^+ ions). Plants can only directly take up solution potassium (Shanware, 2014).

Soils commonly hold over 20000 ppm of total potassium, plants can use only the exchangeable potassium on the surface of the soil particles and that dissolved in the soil water which often amounts to less than 100 ppm and comprise only 0.1 to 2% of the total potassium (George & Michael, 2002). The rest is held up in insoluble minerals such as feldspar and mica. This is further compounded by the imbalance in fertilizer application where the ratio of potassium to other minerals like phosphorus and nitrogen is very small.

Potassium (K) is a major essential macronutrient for plant growth. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals. Potassium (K), one of the seventeen chemical elements required for plant growth and reproduction, is often referred to as "the regulator" since it is involved with over 60 different enzyme systems in plants. Besides its potential to resist drought and disease (Cakmak, 2005; Billore, et al., 2009), it helps in the production of starch, controls root growth and regulates the stomata movement in plant cells and also contributes to quality.

Organic matter after decomposition produces acids like citric acid, formic acid, malic acid, oxalic acid. These organic acids produced, enhance the dissolution of potassium compounds by supplying protons and by complexing Ca^{2+} ions .Previous work has shown organic compounds produced by micro-organisms such as acetate, citrate and oxalate can increase mineral dissolution in soil (Sheng, 2003). Solubilization of potassium occurs by complex formation between organic acids and metal ions such as Fe^{2+}, Al^{3+} and Ca^{2+} (Styriakova, 2003).

In Indian soil, the soluble potassium form is present in approximately 2% and the insoluble form is present in the range of 98% in form of minerals like biotite, feldspar, mica, muscovite and vermiculite (Goldstein, 1994).

This presents an apparent need to search for alternative sources of potassium for plant uptake and use as well as maintaining its availability in the soil for a sustained use. Soil microbes have been reported to play a key role in the natural potassium cycle and therefore, potassium solubilizing microorganisms present in the soil could provide an alternative technology to make potassium available for uptake by plants (Rogers et. al., 1998).

This research was therefore embarked upon to further search out such microorganisms from rhizosphere soil with capabilities of dissolving the insoluble forms of potassium compound to release the soluble potassium for plant use, healthy growth and increased yield.

II. MATERIALS AND METHODS

2.1 Sample Collection

Rhizosphere soil samples from University of Agricultural Science and Lalbagh Garden, both in Bangalore were collected. Samples were collected from six different sites at each location. The collected samples were pooled together to make the composite sample (Parmar and Sindhu, 2013).

2.1.1 Adaptation and Enrichment

The soil samples collected were mixed with insoluble potassium (Feldspar) and incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05% yeast extract and 0.5% feldspar and incubated at 37^0 C on 120 rpm for 1 week (Parmar and Sindhu, 2013).

Enriched samples were inoculated after serial dilution up to 10^{-6} on Aleksandrov agar medium constituted as 1% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPO_4$ and 0.5% potassium aluminium silicate, agar 3 % at pH 6.5 (Sugumaran and Janartham, 2007) and incubated at 37^0C for 1 week. Colony exhibiting clear zone of potassium solubilization was selected as potassium solubilizer from the 10^{-5} dilution containing plate. Secondary Screening was carried out on the basis of study of zone of activity of the isolate by using Khandeparkar's selection ratio.

Ratio = D/d = Diameter of zone of clearance / Diameter of growth

2.2 Characterization of Potassium Solubilizing Bacterial Isolate

Bacterial isolate was characterized using different cultural, microscopical and biochemical characteristics (Osman, 2009).

Colony morphology like shape, margin and elevation of the isolate were observed. Gram staining was also done and the slide observed under oil immersion lens (magnification X100) of the microscope.

Standard biochemical tests were then carried out as follows:

2.2.1 Catalase Test

48hrs old bacterial culture was placed on a clean glass slide, drops of 3% H_2O_2 was added and mixed with a tooth pick. Observation of bubble formation indicated the positive test for catalase test (Kumar et al., 2012)

2.2.2 Indole Production

Trypton broth was prepared and inoculated with bacterial culture. This was incubated at 37^oC for 48 hours. 0.5ml of Kovac's reagent was then added to the culture. After 2 minutes observation for appearance of a red colour band at the junction of medium and reagent was made for indole production (Chand et al., 2014).

2.2.3 Methyl red and Voges-Proskauer Test

Three tubes of MRVP broth were taken. Two of the tubes were inoculated with the bacterial culture and one as control (uninoculated). The three tubes were incubated at 35^{o}C for 48 hours. 5 drops of methyl indicator was added into only one of the culture tube. The change in colour was observed for methyl red test (Chand et al., 2014).

To the second culture tube and the control were added 10 drops of VP1 and 2-3 drops of VP2 reagents. The tubes were gently shaken, the cap/plugs were removed and the tubes left for 15-30 minutes to complete the reaction. The colour was then observed for Voges-Proskauer test (Chand et al., 2014).

2.2.4 Starch Hydrolysis

Starch agar plates were prepared and streaked with culture. The culture was allowed to grow at 37^{0}C for 48 hours. Iodine solution was then poured in the culture plate. Colour change around the streaked culture was observed for starch hydrolysis (Chand et al., 2014).

2.2.5 Bacterial Identification- 16 S rRNA Sequencing DNA Extraction

Lysis/homogenization: Cells were grown in monolayer and lysed by suspending 1-3 colonies aseptically and mixed with 450 µl of "B Cube" lysis buffer in a 2 ml micro centrifuge tube and lysed by repeated pipetting. 4 µl of RNAse A and 250 µl of "B Cube" neutralization buffer were added. The content was vortexed and the tubes incubated for 30 minutes at 65˚C in water bath. To minimize shearing the DNA molecules, DNA solutions were mixed by inversion. The tubes were centrifuged for 20 minutes at 14,000 rpm at 10 ˚C. Following centrifugation, the resulting viscous supernatant was transferred into a fresh 2 ml micro centrifuge tube without disturbing the pellet. 600 µl of "B Cube" binding buffer was added to the content and mixed thoroughly by pipetting and the content incubated at room temperature for 5 minutes. 600 µl of the contents was transferred to a spin column placed in 2 ml collection tube. This was centrifuged for 2 minutes at 14,000 rpm and discarded flow-through. The spin column was reassembled and the collection tube then transferred the remaining 600 µl of the lysate. It was then centrifuged for 2 minutes at 14,000 rpm and discarded flow-through. 500 µL "B Cube" washing buffer I was added to the spin column. Centrifuged at 14,000 rpm for 2 minutes and discarded flow-through. The spin column was reassembled and 500 µl "B Cube" washing buffer II added and Centrifuged at 14,000 rpm for 2 mins and discarded flow-through. The spin column was transferred to a sterile 1.5-ml micro centrifuge tube. 100 µl of "B Cube" Elution buffer was added at the middle of spin column. Care was taken to avoid touch with the filter. The tubes were incubated for 5 minutes at room temperature and Centrifuged at 6000 rpm for 1 min. 16. The above mentioned step 14 and 15 were repeated for complete elution. The buffer in the micro centrifuge tube contained the DNA. 17. DNA concentrations were measured by running aliquots on 1% agarose gel. 18. The DNA samples were stored at -20°C until further use.

Reference bacterium *Enterobacter hormaechei* (MTTC Code: 10240) known for solubilizing potassium, were sourced from Microbial Type Collection and Gene Bank (MTTC) of Institute of Microbial Technology, Chandigarh, India. The culture was used as reference culture to compare with the test culture.

The second organism (a fungus) used as reference culture was isolated and identified as *Aspergillus terreus* 28S ribosomal RNA gene, Sequence ID: gb|KF800672.1with accession number as: NCBI is KX775949.

2.3 Solubilization Activity and Optimization Conditions for Efficient K Solubilization

The isolated K solubilizing bacterium and the reference bacteria were tested for their K solubilizing activity under varying conditions of carbon, nitrogen, potassium, temperature and pH sources used.

A loopful of 48 hour old grown bacterial culture was inoculated into 25ml Aleksandrov medium broth in 50ml capacity flask containing either of different sugars: fructose, galactose, glucose and xylose with added flask for control. All the inoculated flasks plus the control were incubated at 28±2^{o}C for 10 days. Same was done for nitrogen sources (beef extract, $NaNO_3$, peptone and urea), potassium sources (KCl and K_2SO_4), varying temperatures (25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C) and varying pH (6.5, 7.0, 7.5and 8.0) (Parmar and Sindhu, 2013).

2.3.1 Quantitative Estimation of Potassium Release

Different concentrations of KCl solution, ranging from 0 – 100 ppm, were used for preparation of standard curve. Sodium cobaltinitrite solution (5ml) was added slowly to each test tube containing varied concentrations of potassium and volume made up to 10ml by adding distilled water. The reaction mixture was incubated at 37^{o}C for 45 minutes to precipitate the potassium and centrifuged at 13,000 rpm for 5 minutes to permit the precipitate to settle down in the tube. The supernatant

was decanted, precipitate collected and washed twice with distilled water and once with absolute ethanol. After washing, 10ml of conc. HCl was added to the precipitate and incubated at 37^{O}C for 15 - 20 minutes to develop the green colour and absorbance was measured at 600nm using the colorimeter.

Following the same procedure and conditions, potassium was estimated in 5ml of culture supernatant, with reference to the standard curve generated. Estimation for each parameter was carried out thrice to obtain the average which was now referenced to the standard curve to obtain the estimate of solubilisation (Rajawat, M. V. S. et al., 2014).

III. RESULTS AND DISCUSSION

3.1 Isolation and Solubilization Activity of Bacteria

A total of 20 different types of colonies were able to grow on Aleksandrov agar. Among these isolated colonies, one bacterial colony was found to make a clearance zone indicating k-solubilization on Aleksandrov agar.

3.2 Morphological and Biochemical Characters of Isolated Strain

The isolate was medium, round, creamy to brownish. It was gram positive rod (fig. 1), starch hydrolysis, indole production, voges-proskauer, methyl red and catalase positive. It was identified to be *Cellulosimicrobium funkei*. The accession number from GenBank is: SUB2919821 Seq1 MF590168. This organism is novel in this regard as it has never been reported for potassium solubilization.

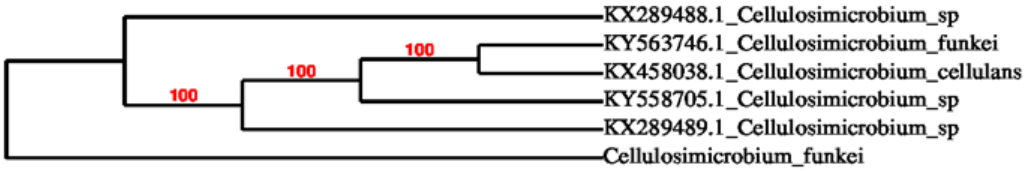
16 S rRNA Sequencing was used

27F 5' AGAGTTTGATCMTGGCTCAG 3'

1492R 5' AGAGTTTGATCMTGGCTCAG 3'

Organism: *Cellulosimicrobium funkei*

Gene Sequence of *Cellulosimicrobium funkei*



LAYOUT 1: Phylogenic tree of *Cellulosimicrobium funkei*

TABLE 1
K SOLUBILIZATION ZONE FORMATION BY ISOLATE *CELLULOSIMICROBIUM FUNKEI*

Isolate	Diameter of Zone of clearance (D) in mm	Diameter of growth of Colony (d) in mm	D/d Ratio
Cellulosimicrobium funkei	12	07	1.71

Khandeparkar's ratio: D/d. D = Diameter of zone of clearance, d = Diameter of growth of isolate

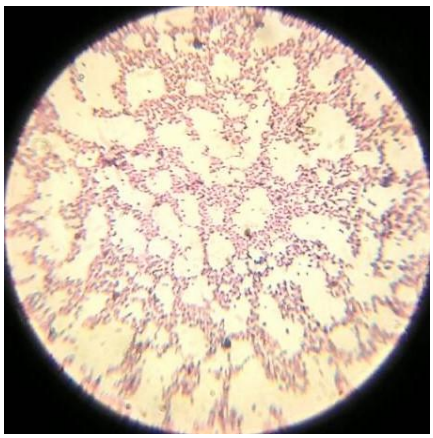


FIGURE 1: Microscopic View of *Cellulosimicrobium funkei* (2A) (Magnification X100)

3.3 Potassium (K) Solubilization Activity

The isolate (*Cellulosimicrobium funkei*) solubilized potassium better than the reference organisms in the four carbon sources of fructose, galactose, glucose and xylose. It solubilized best with glucose (7.04mg/l) while the reference organisms were 7.15mg/l and 6.91mg/l for *Enterobacter hormaechei* and *Aspergillus terreus* respectively as seen in Table 2 and fig. 2. This compares well with the work of Parma and Sindhu (2013) that potassium solubilization by two bacterial isolates was more in glucose supplemented medium than galactose, xylose or arabinose. Etesami, H. et al (2017) also found that the best carbon source for solubilisation of potassium was found to be glucose.

TABLE 2
SOLUBILIZATION BY CULTURES IN GLUCOSE FORTIFIED MEDIUM

Amount of Glucose Added (g)	K – Solubilized (mg/l)		
	***Cellulosimicrobium funkei* (2A)**	***Enterobacter hormaechei* (RF)**	***Aspergillus terreus* (2B)**
Control	0.12	0.42	0.22
1	0.23	1.31	2.02
2	2.51	3.21	2.10
3	2.40	3.60	3.81
4	3.15	4.16	3.60
5	4.22	5.47	4.51
6	5.30	6.89	6.28
7	7.04	7.15	6.91
8	6.91	6.25	6.80
9	6.52	6.27	6.32
10	4.66	5.78	4.31

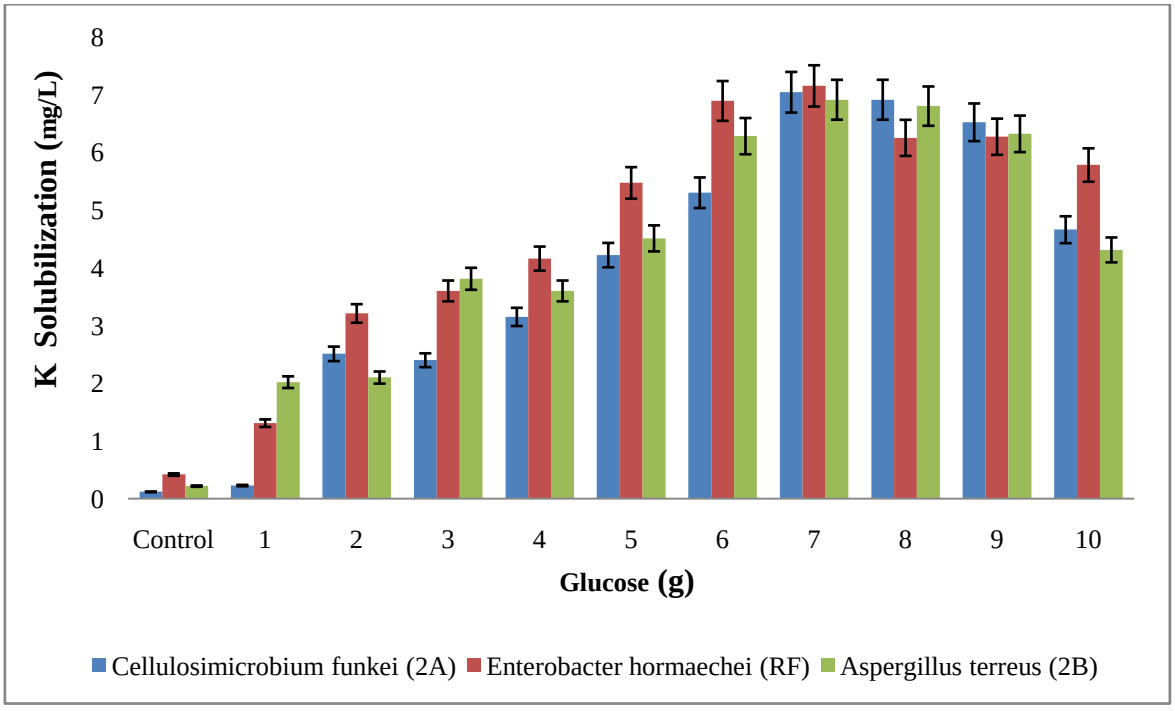


FIGURE 2: Graph showing Solubilization by cultures in glucose fortified medium

Solubilization by the *Cellulosimicrobium funkei* was best in urea culture as nitrogen source (5.45mg/l) and favourably compared with the reference organisms: *Enterobacter hormaechei* 5.38mg/l and *Aspergillus terreus* 6.33mg/l in the entire nitrogen sources as presented in table 3 and fig 3.

TABLE 3
SOLUBILIZATION BY CULTURES IN UREA FORTIFIED MEDIUM

Amount of Urea Added (g)	K – Solubilized (mg/l)		
	Cellulosimicrobium funkei (2A)	*Enterobacter hormaechei* (RF)	*Aspergillus terreus* (2B)
Control	0.86	1.01	0.02
1	2.22	2.41	1.57
2	3.21	2.56	2.71
3	4.92	3.87	2.77
4	5.00	4.85	3.94
5	5.21	4.93	5.03
6	5.45	5.38	5.26
7	5.41	4.92	6.33
8	4.82	4.83	2.78
9	3.80	4.51	2.50
10	3.52	2.60	2.13

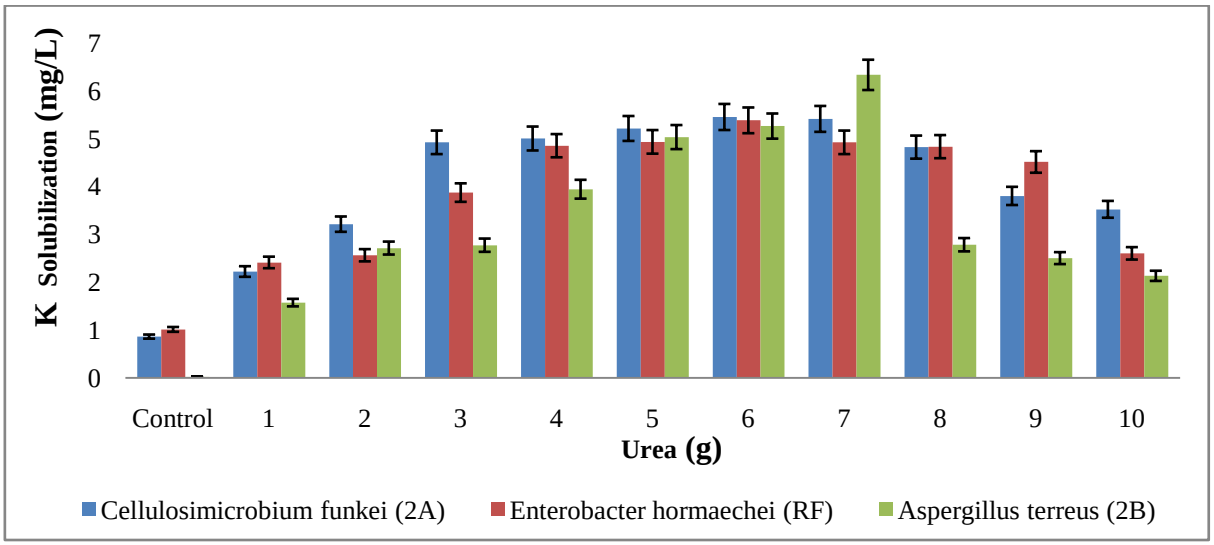


FIGURE 3: Graph showing Solubilization by cultures in Urea fortified medium

The three organisms performed best with the potassium chloride (KCl) medium as potassium source but *Cellulosimicrobium funkei* surpassed the reference organisms with solubilization of 10.23mg/l as against 8.05mg/l for *Enterobacter hormaechei* and 9.11mg/l for *Aspergillus terreus* respectively as shown in Table 4 and fig. 4.

TABLE 4
SOLUBILIZATION BY CULTURES IN KCL FORTIFIED MEDIUM

Amount of KCl Added (g)	K – Solubilized (mg/l)		
	Cellulosimicrobium funkei (2A)	*Enterobacter hormaechei* (RF)	*Aspergillus terreus* (2B)
Control	0.04	1.22	1.10
1	1.81	2.07	2.51
2	2.66	3.15	3.88
3	5.24	4.22	5.25
4	5.41	5.31	6.11
5	6.08	5.89	8.01
6	8.11	6.53	8.11
7	8.62	6.73	8.13
8	10.23	8.05	9.11
9	9.31	6.28	8.37
10	7.22	5.34	7.89

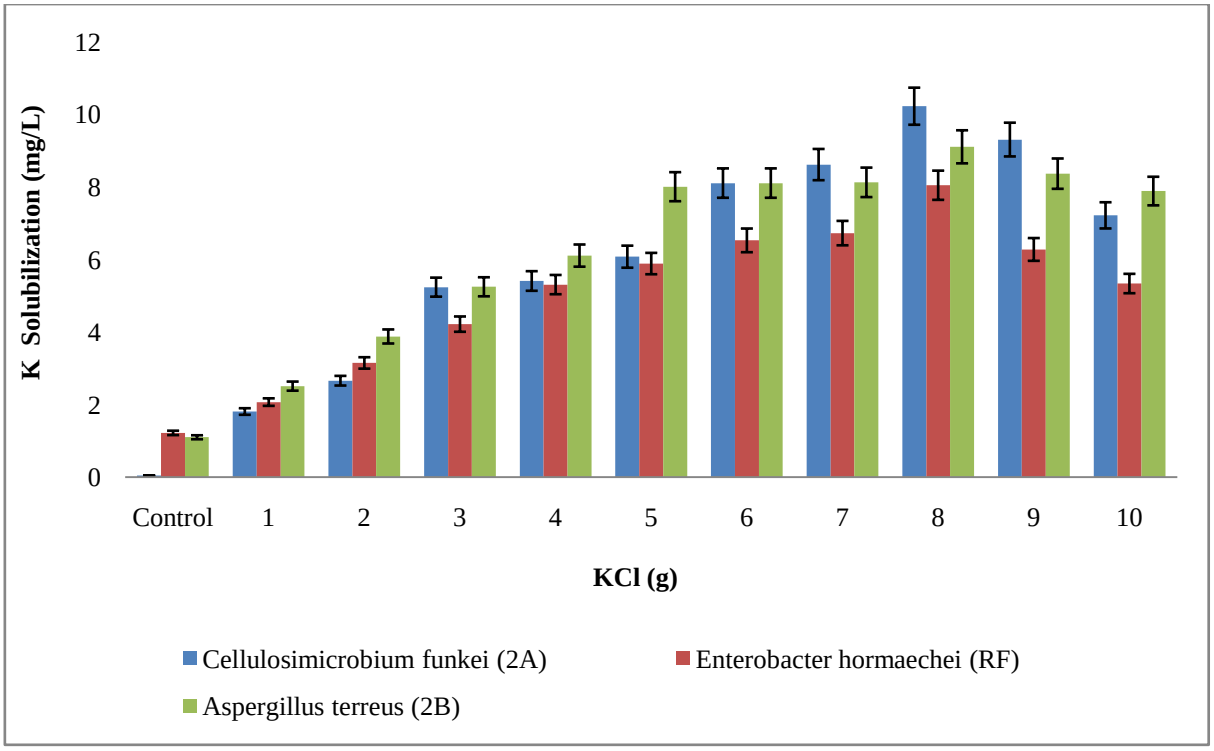


FIGURE 4: Graph showing Solubilization by cultures in KCl fortified medium

It was noticed that the organisms gave best yield at different temperatures. 27°C, 35°C and 30°C for *Cellulosimicrobium funkei, Enterobacter hormaechei* and *Aspergillus terreus* respectively as shown in fig. 5. The temperature (27°C) at which the *Cellulosimicrobium funkei* solubilized best compares favourably with the finding of Prajapati and Modi (2012) which showed *Bacillus* solubilizing insoluble potassium well in aleksandrov medium at a temperature range of 25°C to 35°C. Etesami, H. et al (2017) also reported a better solubilization of potassium in glucose as carbon source at 35°C.

Cellulosimicrobium funkei solubilized best at pH range of 7.5, *Enterobacter hormaechei* at 8 and *Aspergillus terreus* at 7.5 fig. 6. Again, the performance of the isolate (*Cellulosimicrobium funkei*) at pH 7.5 is same with the finding of Prajapati and Modi (2012) with *Bacillus spp*.

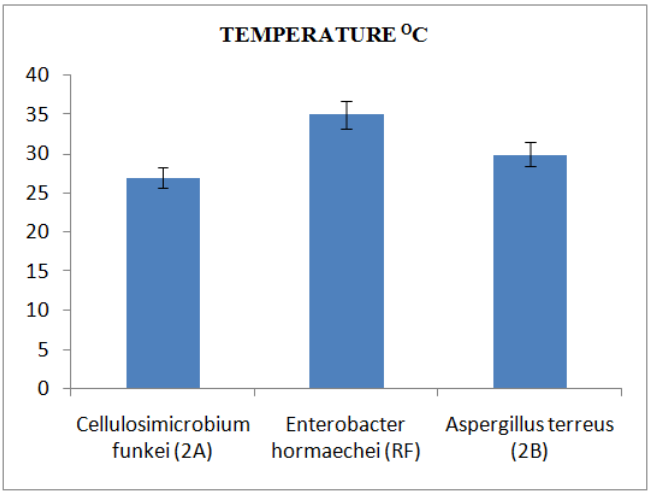


FIGURE 5: Graph showing Solubilization by cultures at their respective temperatures

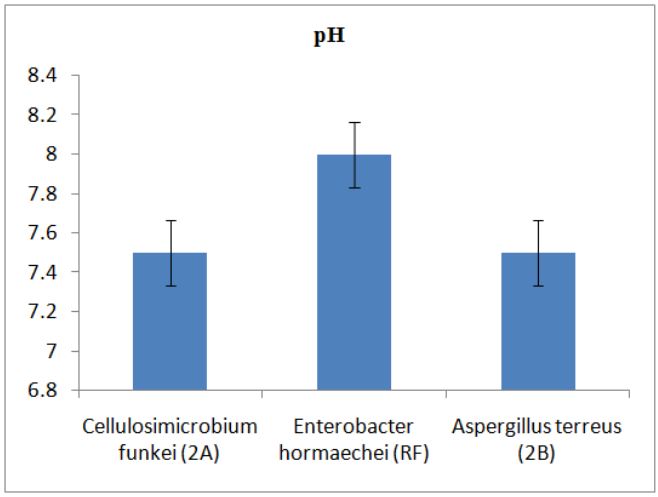


FIGURE 6: Graph showing Solubilization by cultures at their respective pH

When the three microorganisms were cultured under a combined condition of all parameters at the values earlier shown they yielded solubilized potassium at the respective amount of 7.24mg/l, 7.03mg/l and 6.81mg/l by *Cellulosimicrobium funkei*, *Enterobacter hormaechei*, and *Aspergillus terreus*. This is shown on Table 5.

TABLE 5
SUMMARY OF SOLUBILIZATION BY THE CULTURES

Culture	Glucose (g)	UREA (g)	KCl (g)	Temperature ^{O}C	P^{H}	K – Solubilized (mg/l)
Cellulosimicrobium funkei (2A)	8	6	8	27	7.5	7.24
Enterobacter hormaechei (RF)	8	6	8	35	8	7.03
Aspergillus terreus (2B)	8	7	8	30	7.5	6.81

IV. CONCLUSION

In this study, an isolate showed zone of potassium solublization in aleksandrov medium using feldspar as the insoluble potassium source. Morphological and biochemical tests on the isolate from the rhizosphere soil that showed solubilization activity pointed to its identity as *Cellulosimicrobium funkei* a novel organism in potassium solubilization. Optimization tests with varying concentrations of carbon, nitrogen, potassium sources, temperature and pH showed encouraging solubilization ability by the isolate and it compared favourably and in some instances better than the reference organisms.

This agrees with some other researches carried out like Parmar and Sindhu (2013) who reported some bacterial species like *Enterobacter hormaechei, Paenibacillus glucanolyticus , Bacillus edaphicus* and *Bacillus circulans* as potassium solubilizers. Just as Etesami, H. etal (2017) also reported that KSB are usually present in all soils but that their number, diversity and ability for K solubilisation vary depending on upon the soil and climatic conditions. They mentioned some bacteria like *Paenibacillus spp*. and *Bacillus circulans* as having capacity to solubilize potassium.

Therefore, more studies on *Cellulosimicrobium funkei* is needful and worthwhile to further ascertain its solubization capabilities on potassium compounds and others. This could add to the number of candidates for the production of biofertilizers to enrich our potassium starved soils for the better health, growth and production of plants. This will consequently add to more abundance of healthy food for the growing human populace.

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