International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN 2454-1850

Volume-4, Issue-3, March 2018

Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-3, March 2018, 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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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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Antifungal activity of banana rachis leachate on some fungi responsible for banana (*Musa acuminata* Colla) post-harvest diseases

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Abstract— *Post-harvest diseases are a major problem for banana yield. Despite treatments with chemical fungicides, a persistence of diseases is noticed. This study aims at proposing a biological control method against banana post-harvest diseases by using banana rachis leachate. The effect of leachate has been tested in vitro on mycelial growth, conidial germination and in vivo on pathogenic fungi virulence. All leachate concentrations (5, 15 and 20%) tested showed antifungal activity on the tested fungi. However, the 20% concentration was more effective with complete inhibition of mycelial growth and conidial germination of all fungi. No symptoms of crown rot and anthracnose were observed after treatment of bananas with leachate. However, with azoxystrobin, the prevalence of crown rot and anthracnose was 60% and 30%, respectively. Banana rachis leachate recorded highly significant reduction of banana finger rot prevalence compared to azoxystrobin. Banana rachis leachate have strong antifungal properties that may be useful to control banana post-harvest disease as a safe alternative option to chemical fungicides*

Keywords— banana; post-harvest diseases; banana rachis leachate, antifungal activity.

I. INTRODUCTION

Banana (*Musa acuminata* Colla.) plays a key role in the food security of more than 400 million people in developing countries (Arias *et al*., 2003). Banana is the fourth agricultural product worldwide after rice, wheat and maize (Lassoudière, 2007). Côte d'Ivoire is among the greatest African producer of banana dessert with 330 460 tons in 2016 (Faostat, 2016). The banana sector in Côte d'Ivoire provides employment to nearly 10 000 people (MINAGRI, 2015).

However, banana yield is facing many threats of biotic origin especially post-harvest diseases. These infections, such as crown rot, anthracnose, and finger rot, cause significant losses to producers (Dadzie and Orchard, 1997). These post-harvest diseases are caused by *Botryodiplodia theobromae*, *Colletotrichum musae*, *Fusarium* sp. and *Musicillium theobromae* (Lassois *et al*., 2010, Ewané *et al*., 2012, Abd-Alla *et al*., 2014). In Côte d'Ivoire, chemical fungicides with different active ingredients such as Azoxystrobin, Boscalid and Imazalil are used against banana post-harvest diseases. However, this control method has a high cost and the effectiveness of synthetic fungicides has been reduced by the frequent development of resistance by the pathogens. Currently, the search for natural products with novel uses, particularly related to pest management is very active. Recently, studies have focused on the use of composted organic matter as a biological control method (Oka and Yermiyahu 2002, Siddiqui 2004). Thus, the effectiveness of compost leachate in phytosanitary protection against several pathogenic fungi has been demonstrate (Weltzien 1992, Zhang *et al*., 1998). More recently, compost tea has being promoted as an effective tool to control rose powdery mildew (Ingham, 2005) as well as grape powdery mildew, leaf anthracnose and cherry brown rot (Rollins, 2004). The antifungal properties of leachate stemming from banana rachis composting were also demonstrated by Escobar *et al*. (2005) on *Mycosphaerella* spp, causal agent of Sigatoka. Moreover, DE Lapeyre *et al*. (2006) reported a significant control of *Mycosphaerella fijensis* by leachate stemming from plantain rachis composting.

This study aims at assessing the antifungal activity of banana rachis leachate on the fungi responsible for banana post-harvest diseases in Côte d'Ivoire.

II. MATERIAL AND METHOD

3.1 Fungal material

Pathogens fungi, *Botryodiplodia theobromae*, *Colletotrichum musae*, *Fusarium verticillioides*. and *Musicillium theobromae*, were obtained from the fungi collection of the plant pathology laboratory of the University Nangui Abrogoua, in Côte d'Ivoire and cultivated in potato dextrose agar (PDA) medium and incubated at 27 ± 1 °C.

3.2 Leachate preparation

The leachate was obtained from banana rachis previously disinfected with sodium hypochlorite diluted at 1% and then rinsed with distilled water. Banana rachis were crushed and mixed with distilled water (rachis:water ratio of 1:5) placed in a plastic container and stirred twice during a 10 day incubation at (27 ± 1 °C) according to Elad *et al* (1994) method slightly modified. After incubation, the leachate was collected and filtered with a 250 µm mesh screen (Znaidi, 2012).

3.3 Evaluation of banana rachis leachate activity on mycelial growth

Assessment of the antifungal effect of the leachate was carried out on *B. theobromae, C. musae, F. verticillioides*. and *M. theobromae*. For the preparation of culture media, dilutions of 10; 15 and 20% leachate were carried out in supercooling PDA medium. The positive control consisted of PDA media amended with azoxystrobin at a concentration of 1200 ppm. The negative control consisted of PDA medium. The culture media thus prepared were run into sterile Petri plate. A fungal disc (5 mm) cut from the periphery of a 7-day-old culture was placed in the center of each Petri plate. Five repetitions per dilution were made for each pathogenic fungus. The cultures were incubated at 27 ± 1 °C temperature. After 7 days of incubation, diameter of fungal growth was measured in each case, by averaging two diameter of fungal colony at right angle to one another and the percent inhibition of mycelial growth was calculated by using the formula (1) given by Harlapur *et al*. (2007). The sensitivity of each fungus was determined using the Kumar *et al*. (2007) sensitivity scale, I > 90 %: Highly sensitive (S +); 75 % < I < 90 %: Sensitive (S); 60 % < I< 75 %: Moderately resistant (R -); 40 % < I < 60 %: Resistant (R); I < 40 %: Highly resistant (R+).

$$I\ (\%) = \left(\frac{C - T}{C}\right) \times 100 \quad (1)$$

Where : I = inhibition rate ; C = diameter of the fungus colony on medium without fungicide ; T =diameter of the fungus colony in the presence of treatment

3.4 Evaluation of banana leachate activity on conidial germination

Agar plates amended with different concentrations (10; 15 and 25%) of leachate were inoculated with 0.2 ml of conidial suspension (10^6 conidia/ml) from pure culture (7 days old). Agar plate amended with azoxystrobin at the manufacturer's concentration (1200 ppm) served as positive control and negative control were agar plate without leachate. For each leachate concentration and controls, three Petri plate were prepared per fungus. All inoculating plates were incubated at 27 ± 1 °C temperature. Conidial germination was observed under a microscope 24 hours after incubation. Conidial germination was considered effective when the length of the germ tube was greater than the smallest conidia diameter according to Serghat *et al.* (2004) method. The count of germinated conidia was carried out on a total of 100 conidia. The inhibition rate of conidial germination was calculated according to formula (2).

$$.\ I\ (\%) = \frac{Gt - Ge}{Gt} \times 100 \quad (2)$$

Where : *I* = inhibition rate; *Gt* = number of germinated conidia without fungicide (control); *Ge* = number of germinated conidia in the trial

3.5 Effect of leachate on post-harvest disease prevalence

Banana hands of the Cavendish subgroup free of visual defects with uniform shape and weight were selected for the experiment. Fruits were disinfected with sodium hypochlorite diluted at 1% for 5 min, rinsed twice with distilled water and then dried with sterile blotting paper under a hood. A conidial suspension concentrated at 10^6 conidia/ml of each fungus was sprayed on entire surface of banana hand. Based on the results of *in-vitro* susceptibility test, only the most efficience concentration of banana rachis leachate was used in the subsequent *in-vivo* susceptibility assay. For the positive control, the inoculated bananas were treated with azoxystrobin at the manufacturer's concentration (1200 ppm). The negative control consisted of bananas inoculated with the conidial suspension without treatment. The incubation of bananas was done in sterile plastic tubs under laboratory conditions (27 ± 1 °C) and arranged in completely randomized design. Ten bananas were used per trial. After 21 days of incubation, the prevalence of each post-harvest disease on bananas was assessed using formula (3).

$$P(\%) = \frac{Ni}{Nt} \times 100 \quad (3)$$

Where : P = disease prevalence ; Ni = number of infected bananas ; Nt = total number of bananas

3.6 Statistical analyses

All experiments were conducted in a completely randomized design with three repetitions, for each treatment. The statistical analysis of the results was conducted by one-way analysis of variance (ANOVA 1) with the Statistica 7.1 software. Differences between means were determined by the least significant difference (LSD) test at P <0.05.

III. RESULTS

3.1 Effect of banana rachis leachate on mycelial growth

Banana rachis leachate inhibited the mycelial growth of each fungus responsible for banana post-harvest diseases (Fig 1). This antifungal activity varied significantly (P <0.05) depending on leachate concentrations in the culture medium. *C. musae* and *M. theobromae* were more sensitive to banana rachis leachate with total inhibition of mycelial growth at all concentrations. *B. theobromae* was sensitive to leachate at 15 and 20% concentrations with respective inhibition rates of 80 and 100%. However, a resistance of *B. theobromae* to the effect of azoxystrobin was noticed with an inhibition rate of 10% (Table 1). *F. verticillioides* strain also showed sensitivity to leachate at concentrations of 15 and 20% with successive inhibition rates of 75 and 100%. At 20% leachate concentration, the mycelial growth of all fungi was totally inhibited.

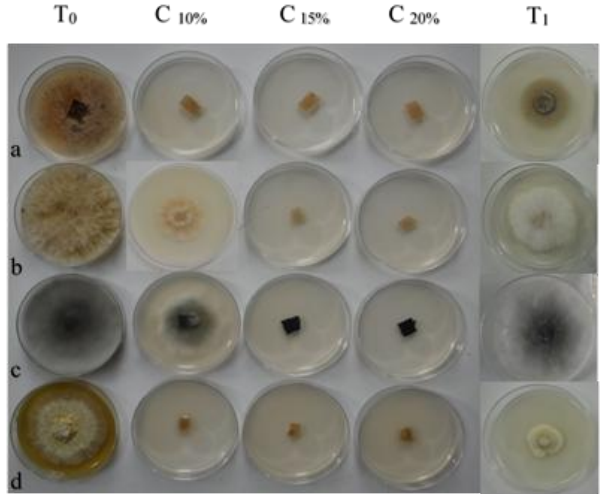

FIG 1: EFFECT OF LEACHATE AND AZOXYSTROBIN ON THE MYCELIAL GROWTH OF FOUR FUNGI RESPONSIBLE FOR BANANA POST-HARVEST DISEASES

a: Colletotrichum musae; b: Fusarium verticillioides; c: Botryodiplodia theobromae; d: Musicillium theobromae

T_0: Negative control (agar); T_1: Positive control (agar + azoxystrobin); C 10%; C 15%; C 20%: leachate concentrations

TABLE 1
INHIBITION RATE OF FUNGI MYCELIAL GROWTH

	Botryodiplodia. theobromae	*Colletotrichum musae*	*Fusarium verticillioides.*	*Musicillium. theobromae*
	Inhibition rates (%)			
Leachate (10 %)	30[c]	100[a]	20[d]	100[a]
Leachate (15 %)	80[b]	100[a]	75[b]	100[a]
Leachate (20 %)	100[a]	100[a]	100[a]	100[a]
azoxystrobin (1200 ppm)	10[d]	50[b]	60[c]	70[b]

The values bearing the same letters in the same column are identical according to the LSD test at 5% threshold.

3.2 Effect of banana rachis leachate on the conidial germination of fungal strains

The antifungal activity of banana rachis leachate on conidial germination varied significantly (P < 0.05) depending on fungal strains (Fig 2). Germination of *C. musae* and *M. theobromae* conidia was completely inhibited by leachate at all concentrations. In contrast, with azoxystrobin, conidial germination of *C. musae* and *M. theobromae* was inhibited at 40 and 60% respectively. *B. theobromae* conidia were sensitive to banana rachis leachate at all concentrations with conidial germination inhibition rates greater than 80% (Table 2). However, *B. theobromae* was moderately sensitive to azoxystrobin with an inhibition rate of 50%. *F. verticillioides*. was sensitive to all leachate concentrations with inhibition rates ranging

between 90 and 100%, however with azoxystrobin the conidial inhibition rate of this fungus was 25%. At 20% concentration, the leachate totally inhibited conidial germination of all fungal strains (Table 2).

TABLE 2
INHIBITION RATES OF CONIDIAL GERMINATION

	Botryodiplodia. theobromae	*Colletotrichum musae*	*Fusarium verticillioides.*	*Musicillium. theobromae*
	Inhibition rates (%)			
Leachate (10 %)	80^{c}	100^{a}	90^{c}	100^{a}
Leachate (15 %)	90^{b}	100^{a}	95^{b}	100^{a}
Leachate (20 %)	100^{a}	100^{a}	100^{a}	100^{a}
azoxystrobin (1200 ppm)	50^{d}	40^{b}	25^{d}	60^{b}

The values bearing the same letters in the same column are identical according to the LSD test at 5% threshold.

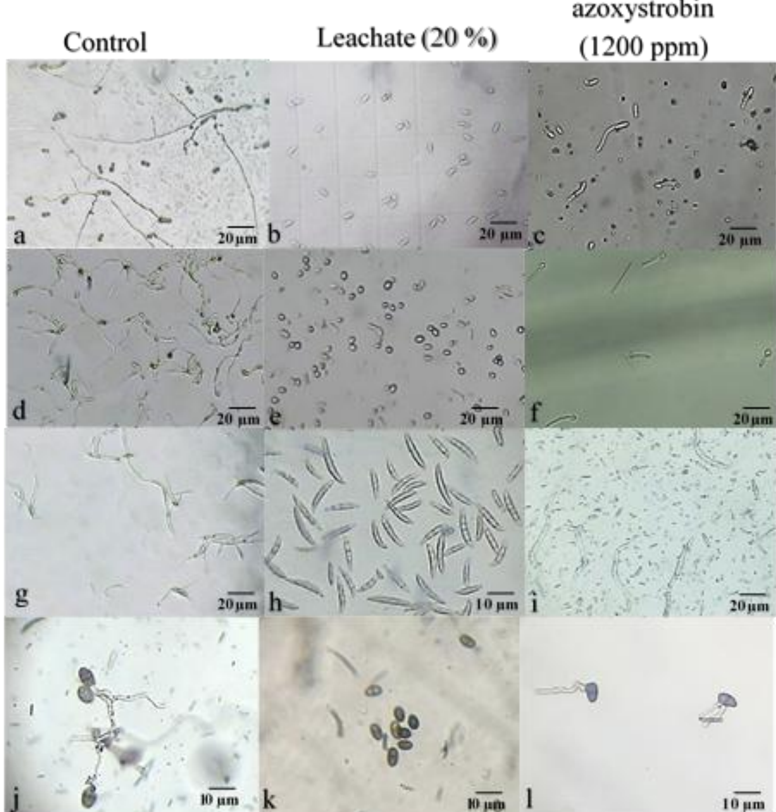

FIG 2: EFFECT OF LEACHATE AND AZOXYSTROBIN ON CONIDIAL GERMINATION OF THE FUNGI RESPONSIBLE FOR BANANA POST-HARVEST DISEASES
a, b, c: conidia of *Colletotrichum musae*; d, e, f: conidia of *Musicillium theobromae*; g, h, i: conidia of *Fusarium verticillioides*; j, k, l: conidia of *Botryodiplodia theobromae*

3.3 Effect of banana rachis leachate on post-harvest diseases

The treatment of bananas with banana rachis leachate showed a significant reduction ($P < 0.05$) in the prevalence of post-harvest diseases. The prevalence of crown rot was 10% for bananas treated with leachate, while for those treated with azoxystrobin, a prevalence of 60% was recorded. As for anthracnose, no symptom was observed on bananas treated with leachate, however a prevalence of 30% anthracnose was obtained with bananas treated with azoxystrobin (Fig 3). finger rot prevalence of bananas treated with leachate was significantly lower than that of bananas treated with azoxystrobin. Indeed bananas treated with leachate showed a prevalence of 5% to distal end rot while for those treated with azoxystrobin a prevalence of 50% was recorded.

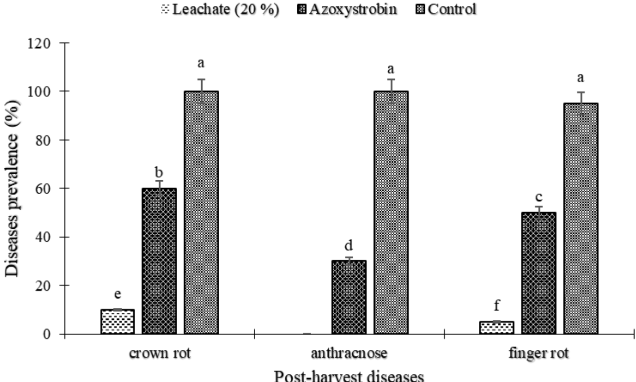

FIG 3: EFFECT OF LEACHATE AND AZOXYSTROBIN ON POST-HARVEST DISEASE PREVALENCE
Histograms bearing the same letters represent statistically identical prevalence according to the LSD test at 5% threshold

IV. DISCUSSION

Mycelium and conidia of the fungi responsible for post-harvest diseases were significantly affected by leachate, which demonstrate the ability of the extract to act on the different development stages of pathogenic fungi. Sikirou *et al* (2010) observed similar results on *Sclerotium rolfsi*, the agent responsible for tomato rust. Indeed banana rachis extract inhibited mycelial growth and germination of *S. rolfsi* sclerotia. The inhibitory activity of banana rachis leachate on conidial germination would help prevent the development of post-harvest diseases. Arauz (2000) and Ploetz (2003) have shown that post-harvest diseases occur as a result of fruit infections by fungal conidia. The application of leachate would stop penetration of the fungus into the host by inhibition of conidial germ tube emission.

Disease prevalence reduction after banana treatment with leachate at 20% concentration suggests that banana rachis leachate has the ability to control post-harvest diseases. Muñoz *et al* (2005) have indicated in their works a better activity of banana rachis leachate at a concentration of 25%. Other works conducted by Toribio (1989) cited by Messiaen *et al* (1991) mentioned that banana rachis extract induced antifungal activity on *S. rolfsi*. According to the same author, a one-tenth dilution of rachis extract showed excellent antifungal properties.

The antifungal activity of banana rachis leachate might be related to its fulvic acid composition. Fulvic acids contain a high concentration of potassium which tends to induce resistance to many diseases (Álvarez *et al*., 2002). Studies conducted by Weltzein (1992) and Yohalem *et al*. (1994) report that leachate has been used for many years in leaf sprinkling for the control of plant fungal diseases. Moreover, the study of Álvarez *et al*. (2002) showed that applications at 5% of fulvic acids stemming from banana leachate reduced the severity of powdery mildew in rose. Furthermore, Escobar *et al* (2005) reported that fulvic acids at 0.5% reduce the incidence of Black Sigatoka in banana tree.

The antifungal properties of compost leachate would also be justified by its composition in several active ingredients other than fulvic acid. These compounds might be responsible for the effectiveness of compost leachate in plant protection against fungal diseases. The results obtained by Al zaemey *et al* 1993 describe the inhibitory effect of a variety of organic acids stemming from compost leachate on the growth of *C. musae in vitro*. These authors indicated that potassium, sodium benzoate and propionic acid are active ingredients present in these organic acids. The effectiveness of compost leachate has also been demonstrated by Welke *et al* (2004). Their works showed a significant reduction of gray mold of strawberry caused by *Botrytis cinerea* by leachate of different types of compost.

V. CONCLUSION

This study has showed the sensitivity of fungal agents responsible for post-harvest diseases to leachate stemming from the composting of banana rachis. Banana rachis leachate has antifungal compounds capable of controlling banana post-harvest diseases.

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Bacteriological Assessment of Lettuce Vended in Benin City Edo State, Nigeria

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***Abstract*— *The microbiological content of Lettuce (a vegetable), commonly vended in the Benin metropolis of Edo state were evaluated. Five vending locations were chosen for the study. Whole and soft rot samples were purchased and analysed for microbiological composition. Results showed high counts in soft rot samples in lettuce. Nutrient agar plated lettuce samples had bacterial counts in the range of 2.0x 103 to $4.7x10^7$. Pseudomonas species was the dominant species found in lettuce samples. Bacillus species was isolated from one location in the lettuce samples. Mac Conkey agar plated lettuce plated had bacterial counts in the range of 2.3×10^3 to $5.7x\ 10^7$. Enterobacter species, E. coli, and Klebsiella species were the dominant species isolated. Though, Proteus species was isolated from lettuce samples obtained from location five only. The study observes that consuming soft rot samples could pose a risk of introducing pathogens to the consumer due to their high microbial counts and could be detrimental to the health of the consumer.*

Keywords*— Bacteriological Assessment, Lettuce, Benin City, microbiological content.

I. INTRODUCTION

Food safety is of growing concern for consumer and professionals in the food industry worldwide. Food safety in ready to eat produce especially raw foods live fruits and vegetables has long been an object of study with many assessing the microbiological condition of raw fresh vegetables available in street markets as well as in self service and fast food restaurants (Angela *et al*., 2010).

Fresh vegetables are commonly found vended on the streets and in shops under both hygienic and unhygienic conditions. While many are less concerned with the processing and hygiene of these vegetables for consumption, they pose a direct risk of causing microbial food borne illness particularly when highly contaminated with microorganisms. Micronutrients, vitamins and fibre for humans can be easily metabolized from ingested vegetables which are known to be an extraordinary dietary source of nutrients, and are thus vital for health and well being. Well balanced diets, rich in vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006).

Normal microbial flora characteristic of living organisms are also found in fruits and vegetables which may be altered while transporting from farm to the table (Margaret *et al*., 2009). Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007; Ofor *et al*., 2009).

Vegetables may also be contaminated whilst growing in fields or during the stages of harvesting, processing, distribution, sale and use. The lack of effective antimicrobial treatments at any step from planting to consumption means that pathogens introduced at any point may be present on the final food product. Even when available antimicrobials are applied, they may bring about a change in the final product. Such changes may include a change in the taste, colour, or the quality of the product. Fresh vegetables may be washed or treated specifically to minimize microbial load (FDA, 2000). As much as possible vegetables should be purchased from known sources or from sources known to operate standard hygienic practices while the purchase of these food materials from streets and open markets should be avoided. This is because the common practice of cooking some vegetables particularly leaves half cooked does not allow for the total elimination of microbial pathogens, while other vegetables may be eaten fresh without cooking as in the case of salad, thus directly exposing the digestive system to the threat of these pathogens.

The objectives of this study therefore were to evaluate the bacteriologic assessment of lettuce from street vended locations in Benin city Edo state and to identify the bacteria genus present on locally obtained lettuce.

II. MATERIALS AND METHODS

2.1 Lettuce Samples

Lettuce a vegetable were used for this study representing a commonly consumed vegetable in Nigeria. A total of 100 samples of lettuce were purchased from 5 different vending locations in Benin metropolis in Edo State. The vegetable from each sampling location were purchased and transported to the laboratory in a cool box at ± 4^0c.

2.2 Preparation of Samples for Microbiological Analysis

Ten grams of lettuce were collected individually using a sterile scapel. These were separately added to 90ml of 0.1percent, peptone water and homogenized separately in a blender. One millilitre of each homogenate was transferred to separate test tubes containing 9ml peptone water to obtain a dilution of 10^{-1}. In a similar manner, 1ml each was transferred from this dilution to separate test tubes containing 9ml diluents and the process was repeated until a dilution of 10^{-9} was obtained for the lettuce samples.

2.3 Enumeration of Micro Organisms

0.1ml from each dilution of samples was transferred to plates of nutrient agar using the spread plate technique. Plates containing nutrient agar were incubated at 37^0C for 18-24hrs. Counts were made after incubation from plates having 30-300 colonies.

2.4 Identification of Bacterial Isolates

Bacterial colonies with characteristic edges, colours and sizes were isolated and purified by subculturing on nutrient agar plates and examined with a hand lens and each isolate subjected to biochemical test using the Bergey's manual of systematic bacteriology. The different tests carried out were used in identifying the isolates to their genus level.

III. RESULTS

Microbiological analyses of both whole and soft rot lettuce samples revealed that soft rot samples had the highest bacterial counts as compared to the whole samples. Soft rot samples had higher bacterial counts than whole samples as shown in table 7 and 11.The total viable count of soft rot lettuce samples were in the range of 2.1x 10^7 to 5.7x10^7cfu/g while whole samples had its total viable count as 2.0x10^3 to 6.4x10^3cfu/g.

Lettuce samples plated on nutrient agar revealed that *pseudomonasspecies* was the dominant organism found in both whole and soft rot samples obtained from locations 1 to 5. *Bacillusspecies* was isolated from soft rot samples obtained at location 5 only.A total number of six genera of microorganisms were isolated from lettuce samples which include *Pseudomonas spp* (23%), *Bacillus spp*(4%), *Enterobacterspp* (23%), *Klebsiellaspp* (23%), *Escherichia coli* (23%) and *Proteus spp* (4%).

Morphological characteristics of the test organisms revealed that the diameter of the colonies were in the range of 0.2-3.0mm.

TABLE 1
MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATE

Sample code	Organism	Colony Characteristics
L1.	Pseudomonas sp	Greenish colonies of 0.4mm in diameter, circular, raised, opaque, with entire edges.
L 2.	Escherichia coli	Pink, convex, opaque, smooth surface, entire edge, circular, 1-2mm in diameter
L 3.	Proteus sp	Milky, convex, opaque, smooth surface, mucoid, spreading 2-3mm in diameter.
L4.	Bacillus sp	Creamish colonies of 0.5mm in diameter, irregular, flat, opaque with curled edges.
L 5.	Klebsiellasp	Pink, convex, opaque, smooth surface, circular, entire edge, 1-2mm in diameter
L 6.	Enterobactersp	Colourless, flat, serrated edge circular, 1-2mm in diameter.

Key: L – Lettuce

TABLE 2
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 1

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-01	4.1 x 10^3	*Pseudomonas sp*	LSN1-01	3.9 x 10^7	*Pseudomonas sp*
LWN2-01	3.2 x 10^3	*Pseudomonas sp*	LSN2-01	3.7 x 10^7	*Pseudomonas sp*
LWN3-01	3.3 x 10^3	*Pseudomonas sp*	LSN3-01	4.2 x 10^7	*Pseudomonas sp*
LWN4-01	2.0 x 10^3	*Pseudomonas sp*	LSN4-01	4.4 x 10^7	*Pseudomonas sp*
LWN5-01	2.7 x 10^3	*Pseudomonas sp*	LSN5-01	4.5 x 10^7	*Pseudomonas sp*
LWN6-01	2.3 x 10^3	*Pseudomonas sp*	LSN6-01	4.7 x 10^7	*Pseudomonas sp*

TABLE 3
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 2

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-02	2.0 x 10^3	*Pseudomonas sp*	LSN1-02	3.7 x 10^7	*Pseudomonas sp*
LWN2-02	2.1 x 10^3	*Pseudomonas sp*	LSN2-02	3.5 x 10^7	*Pseudomonas sp*
LWN3-02	2.6 x 10^3	*Pseudomonas sp*	LSN3-02	3.7 x 10^7	*Pseudomonas sp*
LWN4-02	3.1 x 10^3	*Pseudomonas sp*	LSN4-02	3.6 x 10^7	*Pseudomonas sp*
LWN5-02	2.5 x 10^3	*Pseudomonas sp*	LSN5-02	3.9 x 10^7	*Pseudomonas sp*
LWN6-02	5.7 x 10^3	*Pseudomonas sp*	LSN6-02	2.1 x 10^7	*Pseudomonas sp*

TABLE 4
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 3

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-03	3.9 x 10^3	*Pseudomonas sp*	LSN1-03	3.1 x 10^7	*Pseudomonas sp*
LWN2-03	3.7 x 10^3	*Pseudomonas sp*	LSN2-03	2.9 x 10^7	*Pseudomonas sp*
LWN3-03	2.0 x 10^3	*Pseudomonas sp*	LSN3-03	3.7 x 10^7	*Pseudomonas sp*
LWN4-03	4.3 x 10^3	*Pseudomonas sp*	LSN4-03	3.3 x 10^7	*Pseudomonas sp*
LWN5-03	2.6 x 10^3	*Pseudomonas sp*	LSN5-03	3.5 x 10^7	*Pseudomonas sp*
LWN6-03	2.7 x 10^3	*Pseudomonas sp*	LSN6-03	4.1 x 10^7	*Pseudomonas sp*

TABLE 5
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 4

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-04	4.3 x 10^3	*Pseudomonas sp*	LSN1-04	2.1 x 10^7	*Pseudomonas sp*
LWN2-04	3.5 x 10^3	*Pseudomonas sp*	LSN2-04	3.2 x 10^7	*Pseudomonas sp*
LWN3-04	3.2 x 10^3	*Pseudomonas sp*	LSN3-04	3.1 x 10^7	*Pseudomonas sp*
LWN4-04	2.1 x 10^3	*Pseudomonas sp*	LSN4-04	3.3 x 10^7	*Pseudomonas sp*
LWN5-04	2.3 x 10^3	*Pseudomonas sp*	LSN5-04	3.2 x 10^7	*Pseudomonas sp*
LWN6-04	3.7 x 10^3	*Pseudomonas sp*	LSN6-04	3.1 x 10^7	*Pseudomonas sp*

TABLE 6
RESULTS FOR NUTRIENT AGAR PLATED LETTUCE SAMPLES FROM LOCATION 5

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWN1-05	2.1 x 10^3	*Pseudomonas sp*	LSN1-05	3.0 x 10^7	*Pseudomonas sp, Bacillus sp*
LWN2-05	2.3 x 10^3	*Pseudomonas sp*	LSN2-05	3.1 x 10^7	*Pseudomonas sp, Bacillus sp*
LWN3-05	2.9 x 10^3	*Pseudomonas sp*	LSN3-05	3.2 x 10^7	*Pseudomonas sp, Bacillus sp*
LWN4-05	3.5 x 10^3	*Pseudomonas sp*	LSN4-05	2.1 x 10^7	*Pseudomonas sp, Bacillus sp*
LWN5-05	2.7 x 10^3	*Pseudomonas sp*	LSN5-05	3.5 x 10^7	*Pseudomonas sp, Bacillus sp*
LWN6-05	3.4 x 10^3	*Pseudomonas sp*	LSN6-05	3.7 x 10^7	*Pseudomonas sp, Bacillus sp*

TABLE 7
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 1

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-01	2.4 x 10^3	*Escherichia coli*	LSM1-01	4.4 X 10^6	*Enterobacter sp, klebsiella sp, E. coli*
LWM2-01	2.3x 10^3	*Escherichia coli*	LSM2-01	5.4 X 10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM3-01	2.6x 10^3	*Escherichia coli*	LSM3-01	5.7 X 10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM4-01	3.1x 10^3	*Escherichia coli*	LSM4-01	4.3 X 10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM5-01	3.4x 10^3	*Escherichia coli*	LSM5-01	4.4 X 10^6	*Enterobacter sp, klebsiella sp, E. Coli*
LWM6-01	3.2x 10^3	*Escherichia coli*	LSM6-01	4.7 X 10^6	*Enterobacter sp, klebsiella sp, E. Coli*

TABLE 8
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 2

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-02	5.2 x 10^3	*Escherichia coli*	LSM1-02	5.4 X 10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM2-02	6.4x 10^3	*Escherichia coli*	LSM2-02	5.6 X 10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM3-02	2.2x 10^3	*Escherichia coli*	LSM3-02	5.4 X 10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM4-02	4.1x 10^3	*Escherichia coli*	LSM4-02	5.5 X 10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM5-02	3.4x 10^3	*Escherichia coli*	LSM5-02	5.3 X 10^7	*Enterobacter sp, klebsiella sp, E. Coli*
LWM6-02	4.2x 10^3	*Escherichia coli*	LSM6-02	5.2 X 10^7	*Enterobacter sp, klebsiella sp, E. Coli*

TABLE 9
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 3

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-03	3.7×10^3	*Escherichia coli*	LSM1-03	$4.5X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM2-03	2.3×10^3	*Escherichia coli*	LSM2-03	$4.4X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM3-03	3.5×10^3	*Escherichia coli*	LSM3-03	$5.2X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM4-03	2.4×10^3	*Escherichia coli*	LSM4-03	$5.3X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM5-03	3.4×10^3	*Escherichia coli*	LSM5-03	$5.4X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*
LWM6-03	2.4×10^3	*Escherichia coli*	LSM6-03	$4.9X\ 10^6$	*Enterobacter sp, klebsiella sp, E. Coli*

TABLE 10
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 4

Sample code	Whole samples	*Organism found*	Sample code	Soft rot samples	Organism found
LWM1-04	2.3×10^3	*Escherichia coli*	LSM1-04	$4.5X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM2-04	$2.6x\ 10^3$	*Escherichia coli*	LSM2-04	$4.2X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM3-04	$2.6x\ 10^3$	*Escherichia coli*	LSM3-04	$4.1X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM4-04	$2.4x\ 10^3$	*Escherichia coli*	LSM4-04	$4.0X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM5-04	$2.1x\ 10^3$	*Escherichia coli*	LSM5-04	$5.0X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*
LWM6-04	$2.5x\ 10^3$	*Escherichia coli*	LSM6-04	$5.1X\ 10^6$	*Enterobacter sp, klebsiella sp, E. coli*

TABLE 11
RESULTS FOR MAC CONKEY AGAR PLATED LETTUCE SAMPLES FROM LOCATION 5

Sample code	Whole samples	Organism found	Sample code	Soft rot samples	Organism found
LWM1-05	$4.2x\ 10^3$	*Escherichia coli*	LSM1-05	$4.1X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM2-05	$4.5x\ 10^3$	*Escherichia coli*	LSM2-05	$4.4X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM3-05	$6.4x\ 10^3$	*Escherichia coli*	LSM3-05	$4.3X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM4-05	$4.6x\ 10^3$	*Escherichia coli*	LSM4-05	$5.1X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM5-05	$5.3x\ 10^3$	*Escherichia coli*	LSM5-05	$5.7X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*
LWM6-05	$5.7x\ 10^3$	*Escherichia coli*	LSM6-05	$5.4X\ 10^7$	*Enterobacter sp, Proteus sp, E. coli, klebsiella sp*

KEY:
LSN 1-6 LETTUCE SOFT ROT SAMPLES PLATED ON NUTRIENT AGAR
LWN 1-6 LETTUCE WHOLE SAMPLES PLATED ON NUTRIENT AGAR
LWM 1-6 LETTUCE WHOLE SAMPLES PLATED ON MacConkey AGAR
LSM 1-6 LETTUCE SOFT ROT SAMPLES PLATED ON MacConkey AGAR
01 – 05 LOCATIONS FROM WHICH SAMPLES WERE PURCHASE

TABLE 12
BIOCHEMICAL CHARACTERIZATION OF BACTERIA ISOLATES FROM LETTUCE

Isolate code	Grams reaction	Cell morphology	Oxidase	Catalase	Citrate	Starch hydrolyses	Spore test	H_2S	MR	VP	Indole	Sucrose	Lactose	Motility	Maltose	Mannitol	Probable genera
	-	Rods	+	+	+	-	-	-	-	-	+	A/G	A/G	+	A	-	*Pseudomonas* sp
	-	Rods	-	+	-	-	-	-	+	-	+	A/G	-	+	-	A	*Escherischia coli*
	-	Rods	-	+	-	-	-	+	-	-	Neg	A/G	-	-	-	-	*Proteus* sp
	+	Rods	-	+	+	+	+	-	-	-	-	A/G	-	+	-	A	*Bacillus* sp
	-	Rods	-	+	+	-	-	-	-	+	-	A	-	-	A	A	*Klebsiellasp*
	-	Rods	-	+	+	-	+	-	+	+	-	A	A	+	-	A	*Enterobacter*sp

Note: +, Positive, -,Negative, A,acid production, G, gas production.

IV. DISCUSSION

There is an increasing consciousness of what people consume in the world today. This is because people tend to associate some food with health conditions after consumption or in later years of their life (Oriakpono, et *al*., 2011). This study evaluates the bacteriological quality of some vegetables sold in Benin metropolis, which were tagged locations 1,2,3,4 and 5 (representing the five market location).

Lettuce samples gotten from the five locations in Benin City had significant growth of microorganisms, but the microbial load of lettuce samples gotten from some locations where higher than the others, this may pose a threat to the health of regular consumers. Soft rot samples had higher bacterial counts than whole samples as shown in table 7 and 11.The total viable count of soft rot lettuce samples were in the range of 2.1x 10^7 to 5.7x10^7cfu/g while whole samples had its total viable count as 2.0x10^3 to 6.4x10^3cfu/g.

A total number of six genera of microorganisms were isolated from lettuce samples which include *Pseudomonas spp* (23%), *Bacillus spp*(4%), *Enterobacterspp* (23%), *Klebsiellaspp* (23%), *Escherichia coli* (23%) and *Proteus spp* (4%). The variation of microorganism isolated from lettuce may be due to the fact that lettuce is a creeping crop. The other possible reason for this variation may be due to harvesting, transportation, storage and during the vending process.

This study is in agreement with the work of (Brummel, 2006) which reports that soft rot is one of the significant spoilage diseases of vegetables. *Pseudomonas spp* have also been reported to cause spoilage of various vegetables like lettuce, spinach, tomato (Liao and Wells, 1987) which explains their high diversity. The soft rot group comprises several bacteria strains, of which *Pseudomonas spp* is a major soft rot causing bacteria (Toth *et al.*, 2001). *Pseudomonas spp* are unique among post harvest pathogens in that they are able to grow under refrigerated conditions and use a wide variety of compounds in samples as carbon which they utilize as energy sources. *Proteus* spp can cause serious disease condition on immune compromised patients causing infections of the respiratory tract (Jawetz *et al.*, 1982). *Bacillus* spp is a gram negative spore forming bacteria, it is a well known food borne pathogen causing two types of illness: the emetic and the diarrheal syndrome this is due to the production of enterotoxins that can withstand harsh conditions. There were considerable growths of *Bacillus* spp in lettuce samples obtained from location 5 as shown in table 6. This agrees with the result obtained by Valero and co-workers as they isolated *Bacillus* spp from vegetables in ready to eat sandwiches and salad (Valero *et al.*, 2002).

The vegetable (lettuce) have high water content or water activity this may encourage spoilage if not well preserved. The price of soft rot lettuce compared to whole samples is also a major factor encouraging the consumption of soft rot samples. This is because soft rot samples were found to be about half the price of whole samples in the market. Thus the people who purchase and consume the soft rot samples are at risk of the pathogen causing a disease.

V. CONCLUSION

Fresh vegetables are part of our daily diet. This study shows that there are a variety of organisms in both soft rot and whole samples of lettuce and these organisms may be introduced by various elements (wind, soil, water, insects, animals, human handling). They can become contaminated during growing, harvesting and transportation of the products. It is therefore necessary and important that both the farmer who harvests the vegetables into bags for transportation and the marketers take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables.

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A new biosorbent with controlled grain (I). Efficient elimination of cationic dyes from textile dyeing wastewater

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Abstract— *Environmental care is an increasing concern in our society, and therefore integrated, circular economy allowing to close the industrial cycle is an urging demand. This project employs a residue of the food industry to recover the wastewaters from the textile industry, allowing closing the loop in two different industrial processes. Orange peel is a very abundant residue in the food industry. By chemical modification of the orange peel, we aim to produce a biosorbent to be employed in the treatment of the textile industry wastewater containing cationic dyes. In this project, we evaluate the capacity of the treated orange peels as cationic interchanger with different dissolutions of copper (II). Finally, their capacities as biosorbents were evaluated with four cationic dyes, examining the influence of different parameters like: biosorbent concentration, contact time, temperature and pH of the medium. An adsorption between 51 and 92 % was reached with the first treatment and also the possibility of the biosorbent recovery.*

Keywords— ***basic dyes, biosorbent, biosorption, orange peel, wastewaters.***

I. INTRODUCTION

A trend in wastewater treatment of industrial processes is to perform primary treatments in the most troublesome waters. In this first part of the study the new way to obtain a biosorbent with controlled particle size is presented and also the results of batch tests for the elimination of dyes, as well as the reuse of biosorbent that have allowed the subsequent development of continuous process of decoloration of wastewater from dyeing of textile materials.

Dyes are widely used in dyeing textiles and food, so they constitute one of the greatest challenges in the treatment of industrial wastewater due to its visual impact and increase of the organic load and toxicity (Vieira 2000). The dye molecules distinguish three functional parts: the chromophore, which is responsible for giving the colour property to the dye molecule; the auxochrome, which provides affinity to the textile fibre and intensifies the colour, and the solubilizing groups (Zollinger2001).

These substances are persistent in wastewater and constituting a pollution problem . The dyes are found in the waste of the textile industries from their own production. Their main effect on the aquatic life is the limitation of photosynthetic activity as a result of the decrease in the light penetration and the toxicity affecting aquatic life due to the presence of aromatic and halogenated compounds and/or heavy metals(Robinson 2001).

The dyes currently used are mainly synthetic. Due to its diverse and complex reactive nature, the chemical stability of the dyes converts them in compounds difficult to treat with a general method. According to the conditions in which the dyeing process occurs, the dyes are classified into: acidic, cationic, direct, disperse, reactive, sulphur, vat and others (Aksu 2005).

The textile finishing industry is an industrial sector that consumes water, energy and auxiliary chemical products; therefore, the treatment of wastewater is important. These effluents have significant concentrations of dye, organic contaminants, heavy metals, surfactants and chlorinated compounds.

The treatment of textile effluents is carried out in two stages: homogenization, and physicochemical or biological treatment. Within this scheme, it is possible to selectively treat the dyeing wastewater, discolour, and incorporate them in the overall treatment system. Each method has its own technical and economic limitations and, usually, the use of a single process is not efficient enough to ensure the colour degradation and the mineralization of the compounds formed (Supaka 2004; Buitrón 2004).

Biological processes have been considered as effective alternatives to treat coloured effluents (Van der Zee 2005;Pandey 2007) but the elevated permanence times needed of some dyes and auxiliary products are now the major constraints for their application (Rai 2005).

There are many techniques used in dye removal, which include both physical and chemical processes, for example: ozonation, advanced oxidative processes, photochemical processes, membrane filtration, etc. (Robinson 2001)

The lines of research to obtain new low-cost adsorbents materials have focused, primarily, to produce activated carbon. Different activated carbons have been prepared from shell Walnut (Yalcin 2000; Bello 2002) rice husks, peach stones (Abdel-Nasser 2001), and from other waste materials. However, due to the high cost of the aforementioned substances, we have also considered low cost biosorbents as, for example, agro-industrial waste without any type of treatment. Namely, rice husks, cork and orange peels have been found to yield results such as sufficient retention of dyes. In fact, the valorisation of vegetable residues such as biosorbents, is gaining increasing significance in the environmental field (Brown 2000).

Adsorption is a transfer of matter that is being reintroduced as an alternative to dye removal. There are three kinds of adsorption according to the type of interaction given between the solute and the adsorbent. If the adsorption is done by an ion exchange mechanism, the ions of the substance of interest are concentrated in an area of the adsorbent material as a result of the electrostatic attraction between the two; this is called electrostatic adsorption. However, if the adsorbed molecule is not fixed in a specific place of the surface, but it is rather free to move into the interface, the adsorption is done due to the Van der Waals forces and it is called physisorption. Therefore, if the adsorbate has strong links in the active sites of the adsorbent, one can say that adsorption is of chemical nature. It may be highlighted that, in the physisorption, the adsorbed species preserve its chemical nature, while during the chemisorption the adsorbed species undergo a chemical transformation, giving place to different species (Appelo 2005). The systems based on physisorption can allow the reuse of the adsorbent, probably better than the systems based on chemisorption are able to.

The main parameters are: the specific surface of biosorbent, pH, temperature, the nature of the adsorbent, the nature and concentration of the adsorbate, the contact time and even the solute ionic force (Santos 2003). In the interaction between adsorbate and adsorbent, the factors that affect the process are: the adsorbate solubility (at lower solubility, best adsorption); molecular structure of the solute (as more branched best adsorption); molecular weight (large molecules show better adsorption); polarity (lower polarity has better adsorption and degree of saturation) (Fetter 2001).

The biosorption is an adsorption process that consists of the catchment of different chemical species by a biomass (living or dead), such as: algae, fungi, bacteria, shells of fruits, agricultural products and some types of biopolymers through physicochemical mechanisms as the adsorption or an ionic exchange (Chojnacka 2010).

The biosorption process involves a solid phase -biomass- (sorbent or adsorbent) and a liquid phase (solvent) that contains the dissolved species (adsorbate), which is to be retained by the solid. To carry out this process affinity should exist between the adsorbent and the adsorbate, so that those are transported toward the solid, where they are retained by different mechanisms. This operation continues until a balance between the dissolved adsorbate and the adsorbent is established and bound to the solid. This process continues until a steady state of concentration is reached. The use of dead biomass has advantages compared to the use of living biomass, since it is not necessary to add nutrients to dead biomass. Additionally, the adsorbent is immune to the toxicity or to the adverse conditions of the operation so the processes are not governed by biological limitations anymore (McKay 1986).

The cellular walls of biosorbent materials contain polysaccharides, proteins and lipids, and, therefore, functional groups with capacity to bind heavy metals and cationic molecules in their surface. The main functional groups present here are the amino, carboxylic, hidroxilic, phosphate and thiol groups that differ in their affinity and specificity of joining different metal ions (Ghimire 2003).

The orange peel (Citrus sinensis) is obtained as a byproduct of orange juice manufacturing, and is eliminated as scrap. However, the orange peel and other citrus fruits have been widely used in the elimination of heavy metals and textile dyes (Annadurai 2002; Arami 2005; Pavan 2006;Pérez 2007;Popuri 2007;Hameed 2008; Li2008; Gupta 2009; Lu 2009; Arjona 2016).

The bioadsorption in orange peels is because they contain pectin in their composition. Pectin is a natural high molecular compound widely-existing in cell wall and middle lamella structure of all higher plants (Qiu, Tian,Qiao, & Deng, 2009). Pectin is usually considered as a complex polysaccharide which consists of α-1,4-linkedD-galacuronic acid, which is partly methyl esterified, and the side chain contains various neutral sugars, such as L-rhamnose,L-arabinose, and D-galactose (Mohnen, 2008; Xie, Li, &Guo, 2008). Pectin properties include gelatification, thickening and stabilization, giving it widespread use in food, medical, chemical, textile and other industrial fields (Sato et al., 2011).

FIGURE 1: PECTIN A POLYMER OF α-GALACTURONIC ACID WITH A VARIABLE NUMBER OF METHYL ESTER GROUPS

When the proportion of methoxy groups is low and, therefore, the proportion of COO-groups available is high, the links that are established between the molecules can be made through divalent cations (Ca 2+, Cu2 +, etc. . .).

The main objective of this study is to develop and optimize the treatment of orange peel to obtain a reusable biosorbent, which will allow the removal of heavy metals and cationic dyes from wastewater.

II. MATERIAL AND METHOD

2.1 The process of the biosorbent preparation

The first stage is the collection and cleaning of the orange peels. It is important to select peels of oranges in good state, without fungi, worms or parts in decomposition. Then the edible part (endocarp) is separated, so that the shell (flavelo and albedo) is free from pulp residues. The process continues by rubbing the peel surface with detergent to remove the waxes, which had been added to improve the appearance of the fruit in the commercial circuit.

The second stage is drying till reaching a constant weight. The process continues with the crushing and screening, selecting a particle size between 500 µm and 1000 µm. All those operations are presented in figure 2:

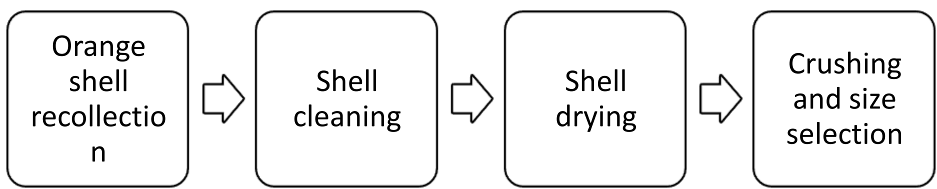

FIGURE 2 PHYSICAL RECONDITIONING PROCESS OF THE ORANGE SHELL

The selected particle size fraction was subjected to a clarification treatment with tetrahydrofuran (THF) in a Soxhlet equipment, to extract the lipid fraction, the essential oils and the bioflavonoids.

In this research, the orange peel was subjected to a first treatment in acid medium to clean the surface and extract secondary products, followed by a second treatment in an alkaline medium with calcium hydroxide to reticulate the Ca^{2+} in the surface of the orange peel. The removal of metals in dissolution by the Ca^{2+} reticulated pectin occurs basically due to a phenomenon of ion exchange between the Ca^{2+} and metal ions in solution until equilibrium is reached.

The procedure was:

The orange peel was clarified with THF in the Soxhlet equipment. Experimentally, it proceeded as follows:

- 500 mL of distilled water, 50 g of orange peel and 5 g of citric acid were added to a vessel and were treated for 45 minutes with ultrasonic equipment, and was filtered.
- 500 mL of water distilled was added to the solid fraction, (orange peel subject to the process of extraction), and was treated again for 45 minutes with ultrasonic equipment to eliminate the remains of the acid added in the previous process and was filtered again.
- In the resulting solid fraction, 500 ml of distilled water and 2.5 g of calcium hydroxide were added and it was treated again for 45 minutes with the ultrasonic.

The solid fraction was separated and treated with 500 mL of distilled water for 45 minutes with ultrasound. This last operation was repeated two times more with the objective of eliminating the excess of alkali in dissolution and eliminate

possible particles that could have stayed in suspension (fine) which could constitute an interference in the spectrophotometric determination of the dyes when they were subjected to the action of the cationic exchanger

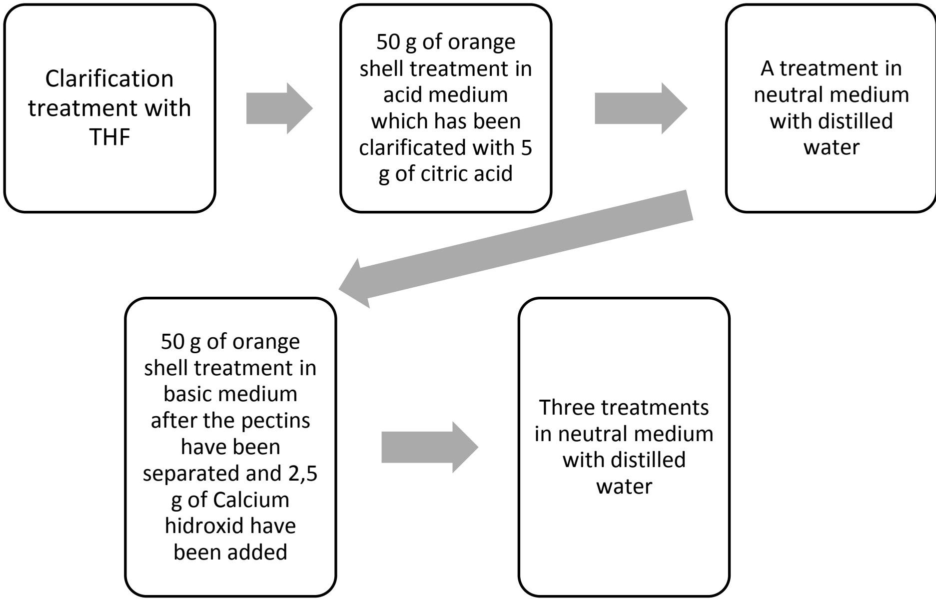

FIGURE 3: CHEMICAL CONDITIONING PROCESS OF THE ORANGE PEEL

The equipment used in the treatment of orange peel was Ultrasonic LC 30 H from Elmasonic with frequency set of 37 KHz.

2.2 Procedure for controlling the efficiency of preparation of the biosorbent

The verification of the characteristics of the biosorbent obtained was made with a series of synthetic solutions of Cu (II). Experimentally, it proceeded as follows:

- 0.5 g of biosorbent and 25 mL of a known Cu (II) solution in a test tube equipped with a screw cap were added and a controlled stirring was followed for 10 minutes.
- A 5 ml aliquot of the Cu (II) solution was extracted and 5 mL of 5% potassium iodide solution was added and shaken manually, in order to check the formation of iodine.
- Then 5 ml of dichloromethane were added and it was shaken again manually. A pink coloration of the organic layer confirmed the formation of iodine.

In the case that the organic phase of dichloromethane remained colourless, it is interpreted as that the biosorbent (cation exchanger) has adsorbed the totality of the Cu (II). In the case that a light pink colour appears it means as that the cation exchanger had not completely absorbed the Cu (II) and enough iodine has been formed to detect it in the dichloromethane phase, according to the reaction: $5I^{-} + 2Cu^{2+} \rightarrow Cu_2I_2 + I^{3-}$

Under the described conditions, 0.5 grams of the described biosorbentwere completely removed, through an ion exchange mechanism, 200 ppm (mg/L) of dissolution of Cu (II) prepared from $CuSO_4 \cdot 5H_2O$.

Dissolution of 500 ppm of Cu (II) was used to show that it had overcome the adsorption capacity of the quantity used in the biosorbent. Under those conditions a light pink coloration appeared during the organic phase

The following table shows the results obtained by the adsorption of synthetic aqueous solutions of Cu (II) (25 mL at different concentrations) with 5.0 g of the biosorbent. This test was measured by the atomic absorption at 324.8 nm.

TABLE 1
EFFICIENCY OF THE BIOSORBENT IN THE REMOVAL OF CU (II)

Initial Cu (II) concentration (ppm)	Final Cu (II) concentration (ppm)	Cu (II) retained (%)
5.00	1.25	**75.00**
10.00	1.15	**88.50**
30.00	2.69	**91.03**
100.00	3.64	**96.36**

The results show that the biosorbent obtained from orange peel allows the exchange of cations in an aqueous solution. It is also observed that as the initial concentration of the Cu (II) solution increases, the efficiency increases.

The removal of Copper (II) by the calcium pectinate is mainly due to an ion exchange process between the Ca (II) and the ions that are in the solution (Jang 1990). In this way, the Cu (II) displaces the Ca (II) initially attached to the polygalacturonicchain until reaching equilibrium concentrations in both phases.

In order to explain this biosorption process, the following model has been proposed: Initially, a fast mechanism of Cu(II) ion migration towards the surface of the bioadsorbent and in the second, slower stage, the migration of the Cu(II) ion to the active site displacing the Ca (II) ion.

FIGURE 4 CU (II) BIOSORPTION MODEL

2.3 Decolouration of textile wastewaters containing cationic dyes

Four cationic dyes were used in this research: C.I. Basic Blue 3; C.I. Basic Yellow 21; C.I. Basic Red 18 and C.I. Basic Green 4. The removal achieved was between 50% and 90%, using a batch shaken process at controlled pH 4.26 and a contact time of 45 minutes at 25 ºC with a solution of 30 ppm (0.03 g/L) of the dye. Table 2 presents the molecular and the structural formula of the dyes and their molar mass.

TABLE 2
CATIONIC DYES USED

Name	Molecular formula	Molecular weight (g/mol)	Structural formula
C. I. Basic Blue 3	C20H26ClN3O	359.9	
C. I. Basic Yellow 21	C22H25ClN2	352.9	
C. I. Basic Red 18	C19H25Cl2N5O2	426.3	
C. I. Basic Green 4	C23H25ClN2	364.9	

2.4 Recovery of the biosorbent (cationic exchanger)

The treatment to which orange peel has been submitted has two purposes. The first is extract partially the content of pectin and the second isthe saponification the ester (methyl) groups, so that Ca (II) ions facilitate the formation of three-dimensional structures with the chemically modified orange peel (biosorbent).

In contact with concentrated solutions of other cations, the movement of the calcium cation concerned is achieved demonstrating behaviour of ionic exchange. However, in the case of copper ionic exchangeit is strongly retained in the three-dimensional structure, in a virtually irreversible way, which promotes the movement of calcium ions in the solution that is in contact with the biosorbent but not the displacement of Cu (II).

In the case of cationic dyes, which are very voluminous molecules, the biosorption mechanism does not occur by ion exchange. Electrostatic attractions and / or hydrogen bridges produce the surface adsorption of these molecules.

The biosorbent can be reused when treated with a polar solvent such as ethyl alcohol, since cationic dyes are dissolved in this medium.

After that, the biosorbent is treated with calcium chloride to enhance the three-dimensional structure that acts as a biosorbent. In this way, the cationic exchanger can be reused to adsorb new cationic dyes(basic dyes).

III. RESULTS AND DISCUSSION

The influence of pH, temperature, biosorbent concentration, contact time of the biosorbent with cationic dye, the influence of moisture on the biosorbent, and the possibility of eliminating dye have been verified by successive treatments. In addition, the biosorbent regeneration process and the efficiency of the recovery have also been verified.

3.1 Influence of the pH

The influence of the pH on the interaction between the biosorbent (cationic exchanger) and the four cationic dyes (C.I. Basic Blue 3; C.I. Basic Yellow 21; C.I. Basic Red 18 and C.I. Basic Green 4) were determined. Dissolutions of 30 ppm (mg/L) of the four dyes were prepared at different pH values: 8.2; 5.2 and 4.0 and determined how much dye was left in the dissolution when treating 25 ml aliquots with 0.5 g biosorbent for 30 minutes. The results are shown in Table 3.

TABLE 3
COMPARISON BETWEEN THE BIOSORPTION AT PH 8.2; 5.2 AND 4.0

	Biosorption (%)			Biosorption variation between max. and min. value (%)
	pH = 8.2	pH = 5.2	pH = 4.0	
C.I. Basic Red 18	91.33	91.00	89.00	2.33
C.I. Basic Yellow 21	51.67	52.33	54.67	3.00
C.I. Basic Blue 3	64.33	65.67	67.00	2.67
C.I. Basic Green 4	90.33	92.00	88.67	3.33

It was found that pH does not have a significant influence on the process of removal of the dyes, since the variation between the different dyes is less than 4 %. Therefore, due to the low influence of pH, it was decided to work in the conditions in which usually the textile industry works for the dyeing processes with cationic dyes: a regulatory solution of pH of acetic acid and sodium acetate with the concentration of 1 M (pH ≈ 4.26).

3.2 Influence of the temperature

The biosorbent efficiency was compared at 25 ° C and 65 ° C. The textile industry has its usual thermal recovery processes of the baths of dye, and therefore, the temperature of the bath that arrives to the columns of adsorption is an ambient temperature of around 25°C.

In the pH conditions described in the previous section (pH ≈ 4.26, with a pH buffer solution of acetic acid and sodium acetate), the efficiency of the biosorbent has been compared at 25 ºC and 65 ºC. The results are shown in the table 4:

TABLE 4
COMPARISON BETWEEN THE ADSORPTION AT 25 AND 65 °C

	Biosorption (%)		Variation (%)
	25 °C	65 °C	
C.I. Basic Red 18	81.0	80.5	0.5
C.I. Basic Blue 3	39.3	42.6	3.3
C.I. Basic Yellow 21	24.5	24.9	0.4
C.I. Basic Green 4	54.7	62.3	7.6

In the case of the C.I. Basic Red 18 the difference in the % of adsorption between the two temperatures is 0.5 %. In the case of the C.I. Basic Blue 3 the difference is 3.3 %. For the C.I. Basic Yellow 21, the variation is 0.4 %. Finally, for the C.I. Basic Green 4, the variation is 7.6 %.

Due to these results, it was decided to continue working at 25 ° C as this favors its transfer to the industrial sector of dye treatment of wastewater.

3.3 Influence of the biosorbent concentration

At the pH and temperature predetermined values, the following process was to establish the optimum concentration of biosorbent and the contact time. Therefore, solutions of 30 ppm of each of the four cationic dyes were prepared in a pH ≈ 4.26 (with a pH buffer solution of acetic acid and sodium acetate), then different quantities of biosorbent were added to 25 ml aliquots of each dissolution. The results are shown in Figure 5.

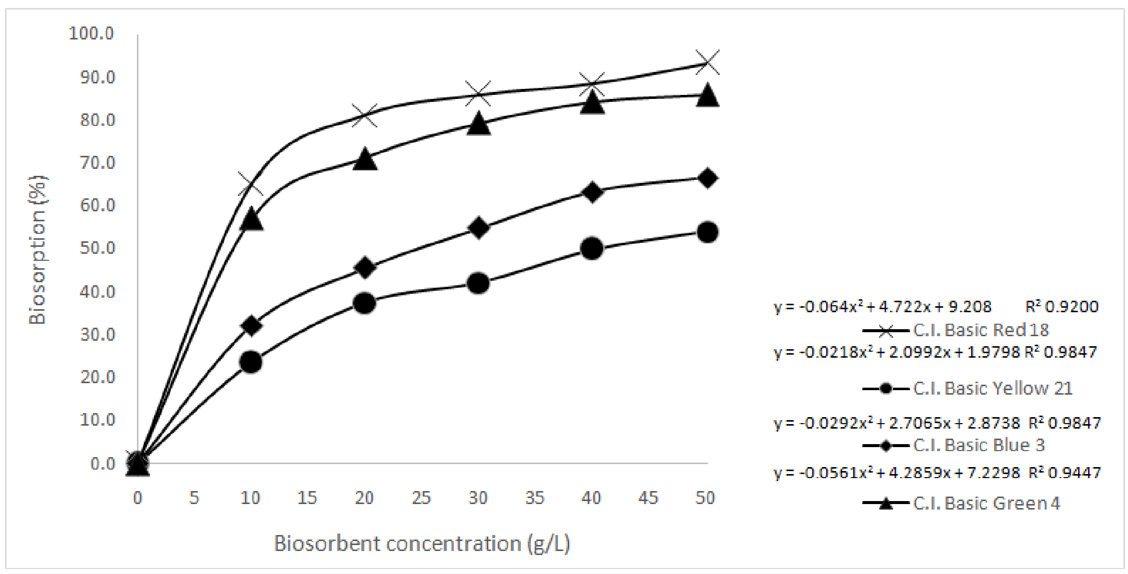

FIGURE 5. DYE BIOSORPTION DEPENDING ON THE BIOSORBENT CONCENTRATION

Fixed the values of the pH ≈ 4.26 and the temperature (25 ºC), the influence of the concentration of biosorbent in the elimination of cationic dyes of the wastewater of the textile industry was confirmed.

According to the results, it was considered that the optimal biosorbent mass for 25 ml of the different dissolutions of 30 ppm (mg / L) was 1.0 g, which is equivalent to say 0.04g biosorbent / ml dissolution or 40 g / L, since from this value the biosorption percentage did not increase significantly.

At low concentrations of biosorbent, between 10 and 20 g/L, a dye adsorption between 65 and 81 % takes place for the C.I. Basic Red 18, between 23 and 37 % for the C.I. Basic Yellow 21, between 32 and 45 % for the C.I. Basic Blue 3 and between 57 and 71 % for the C.I. Basic Green 4.

The increase of the concentration of the biosorbent between 20 and 50 g/L results in smaller increase in the adsorption of dyes.

With 40 g / L of adsorbent, a removal of 88.4% is achieved for C.I Basic Red 18, 49.8% for C.I. Basic Yellow 21, 63.3% for C.I. Basic Blue 3 and 84.2% for C.I. Basic Green 4.

In view of these results it is recommended that the industrial conditions of biosorbent concentration are of 40 g / L.

3.4 Contact time influence between biosorbent and the residual dyeing bath

At the optimalbiosorbentconcentration we proceeded to make a series of experiments in which 25 mL aliquots of dye was added 1 g of biosorbent (40 g/L). All dyes were prepared to a initial concentration of 30 ppm (mg/L), atpH 4.26 and 25 ° c. In these conditions, the time of contact between the biosorbent and dye was in the range between 6 and 84 minutes.The results are presented in Figure 6:

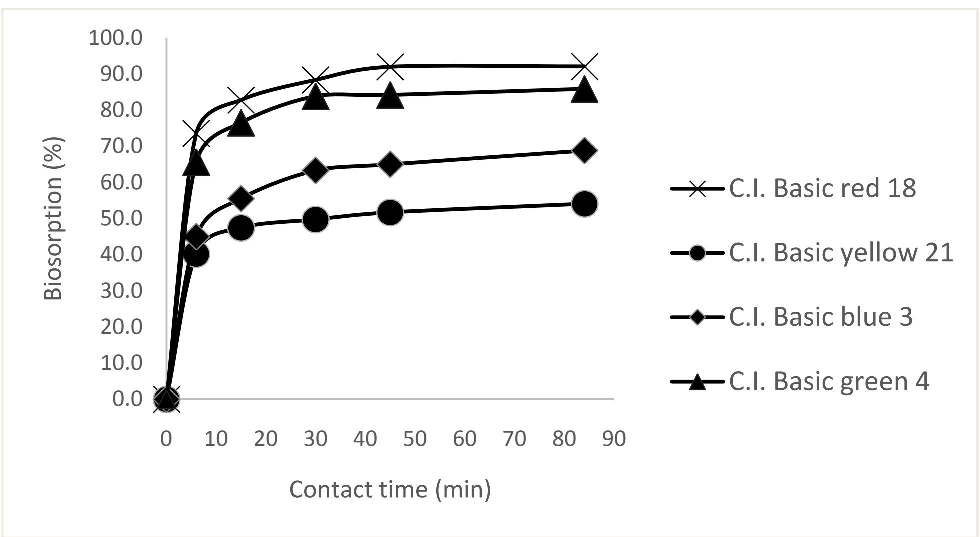

FIGURE 6: RESIDUAL CONCENTRATION OF THE FOUR CATIONIC DYES DEPENDING ON THE CONTACT TIME

There is an asymptotic behaviour in the adsorption of dye from the 45 min of contact time.

At short contact times, from 6 to 15 min, between 73.6% and 82.8% is eliminated for C.I. Basic Red 18, between 40.2% and 47.5% for C.I. Basic Yellow 21, between 45.0% and 55.5% for C.I. Basic Blue 3 and between 65.6% and 76.5% for C.I. Basic Green 4. In the table 6, the initial rates of dye biosorption (at 6 minutes) in the table 5

TABLE 5
INITIAL BIOSORPTION RATES (AT 6 MIN)

	Initial biosorption rates (% biosorption / min)
C.I. Basic Red 18	12.26
C.I. Basic Yellow 21	6.70
C.I. Basic Blue 3	7.50
C.I. Basic Green 4	10.93

When comparing the biosorption at 15 and 45 minutes it was observed that the retention of cationic dyes increased from 82.8% to 92.0% by C.I Basic Red 18, from 47.5% to 51.7% by C.I. Basic Yellow 21, from 55.5% to 65.0% by C.I. Basic Blue 3 and 76.5% to 84.2% by C.I. Basic Green 4.. According to our results, the optimal contact time between the residual dyeing bath and the new biosorbent is 45 min

It was found that dyes that have a low initial rate also have a lower adsorption in the recommended conditions. This fact shows that there is a relationship between the chemical structure of the dye, the efficiency of the process and the initial rates.

In the recommended conditions of concentration of biosorbent (40 g/L) and contact time (45 min), the biosorption of the four cationic dyes by effect of the biosorbent are shown in table 6.

TABLE 6
THE FOUR CATIONIC DYES BIOSORPTION AT RECOMMENDED CONDITIONS

	Biosorption (%)
C.I. Basic Red 18	92.0
C.I. Basic Yellow 21	51.7
C.I. Basic Blue 3	65.0
C.I. Basic Green 4	84.2

In all cases this supposes an important elimination of dye from the wastewater.

3.5 Biosorbent reuse: adsorption at low concentrations

The efficiency of removal of cationic dyes was determined after two, three or four successive treatments with fresh biosorbent, until a residual concentration of 2 ppm or less is reached. The residual bath of the first treatment is subjected to a second biosorption treatment with 40 g/L of fresh biosorbent, and then, if necessary, to a third or fourth treatment with the same concentration of biosorbent. In all cases, the cationic dye solution is fixed at pH 4.26 and 25 °C.

The results of each treatment efficiency are shown in table 7:

TABLE 7
EFFICIENCY OF THE SUCCESSIVE TREATMENTS AT LOW CONCENTRATIONS

	Efficiency of 1st treatment (%)	**Efficiency of 2nd treatment (%)**	**Efficiency of 3rd treatment (%)**	**Efficiency of 4th treatment (%)**	**Final efficiency (%)**
C.I. Basic Red 18	92.0	100.0	-	-	100.0
	C_i = 30.0 ppm	C_{i1} = 2.4 ppm			C_f = 0.0 ppm
C.I. Basic Yellow 21	51,7	51,0	58,8	38,8	94.0
	C_i = 30.0 ppm	C_{i1} = 14.5 ppm	C_{i2} = 7.1 ppm	C_{i3} = 2.9 ppm	C_f = 1.8 ppm
C.I. Basic Blue 3	65,0	62,7	68,5	-	96.0
	C_i = 30.0 ppm	C_{i1} = 10.5 ppm	C_{i2} = 1.2 ppm		C_f = 1.2 ppm
C.I. Basic Green 4	84,2	82,4	-	-	97.3
	C_i = 30.0 ppm	C_{i1} = 4.7 ppm			C_f = 0.8 ppm

Successive biosorbent treatments always increase the efficiency of removal of the cationic dyes from wastewater.

With two successive treatments it is possible to eliminate 100% of C.I. Basic Red 18 of the sample. For C.I. Basic Yellow 21, four treatments are required to remove 94% of dye. In the case of C.I. Basic Blue 3, it is possible to eliminate 96% of the dye with three treatments. Finally, with two treatments, 97% of C.I. Basic Green 4 is removed.

3.6 Influence of pre hydration of the biosorbent

The efficiency of the biosorption process of cationic dyes from biosorbent powder or previously hydration biosorbent in distilled water for 24 h at 25 °C has been tested. 25 mL of cationic dye solution at pH 4.26 and 25 °C and 30 ppm were added with 20 g/L of biosorbent (0.5 g of biosorbent). The results are shown in figure 7:

FIGURE 7. COMPARISON BETWEEN THE ADSORTION OF THE FOUR CATIONIC DYES WITH MOIST AND DRIED BIOSORBENT

It is therefore concluded that the hydration biosorbent tends to have a higher efficiency than the dried biosorbent.This base the subsequent development of a continuous process of treatment with the biosorbent used in this work.

3.7 Influence of higher dyestuffs concentrations in the wastewater

An initial dyeing bath of 2 % owf of dyestuffs, and bath relation 1:40, if the bath exhaustion is 80 %, approximately 100 ppm (mg/L) of the dye may remain in the bath. Therefore, the biosorption of dye solutions at concentrations of 60, 90, 120 ppm and even 240 ppm has been studied. This study has been carried out at the same conditions (25 mL of solution at pH 4.26 and 1.0 g of treated citric derivate biosorbent) with higher or, at least, similar efficiencylevels previously obtained.

3.8 Recovery of the cationic exchanger

Finally, the recovery of the biosorbent was studied. For 1.0 g of used biosorbent two successive extractions of 25 mLof ethyl alcohol were added and stirred in a mechanical shaker for 45 minutes.

Then the regeneration of the biosorbent with calcium chloride ($CaCl_2$) followed in order to cross-link the calcium ion in the cellulosic wall. For this process a solution of 0.2 M of $CaCl_2$ was prepared and 0.5 g of cationic exchangerwas treated with 25 mL of the solution (with a concentration of 20 g/L). Finally, a last treatment with distilled water was undertaken so as to eliminate the possible excess of calcium chloride. The biosorption of dye yields were compared with regenerated biosorbent and recovered biosorbent, without the calcium chloride treatment. The tests were done with solutions of 25 mL at pH 4.26 and 25 ºC with a dye concentration of 30 ppm and with 0.5 g of biosorbent (20 g/L). The results shown in Figure 8 indicate that the biosorbent increased the efficiency when it was not cross-linked.

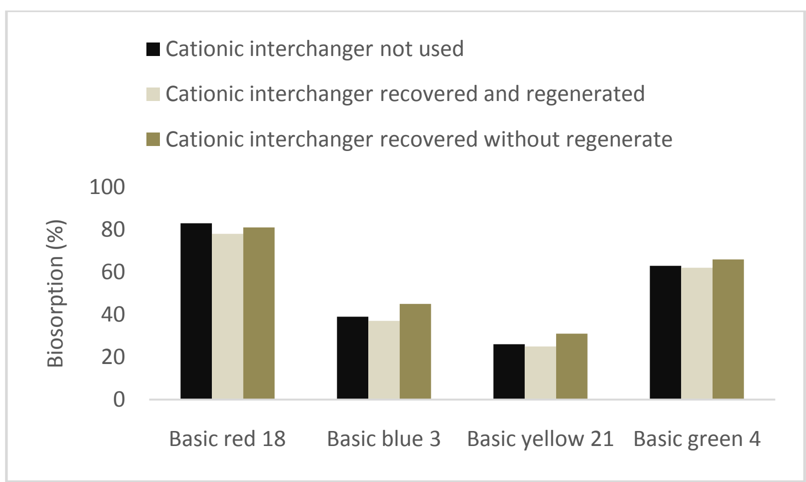

FIGURE 8. EFFICIENCY COMPARISON BETWEEN REGENERATED BIOSORBENT AND BIOSORBENT WITHOUT REGENERATION

Our results confirm the work of (Sivakumar 2010), that this is not anionic exchange mechanism, but that electrostatic attractions and/or hydrogen bonds adsorb the dyes. In figure 9 is shown how (Sivakumar 2010) reported a four-stage adsorption mechanism for removal of C.I. Acid Blue 92, C.I. Basic Red 29, C.I. Reactive Red 4 and C.I. Direct Blue 53 by precursor wood.

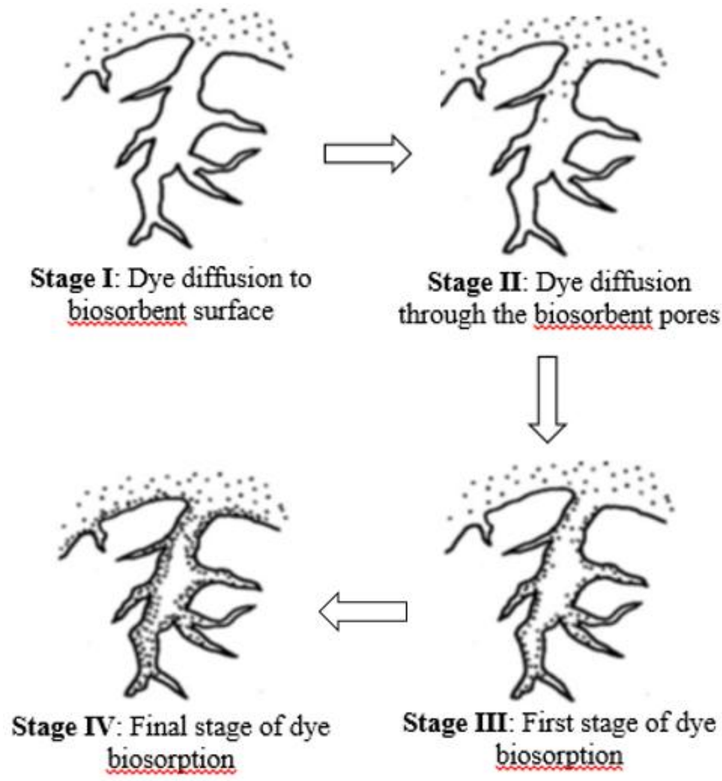

FIGURE 9. PROPOSED FOUR-STAGE MECHANISM OF THE DYE ADSORPTION (SIVAKUMAR 2010)

In Figure 10, the proposed mechanism for the C.I. Basic Green 4 is shown.

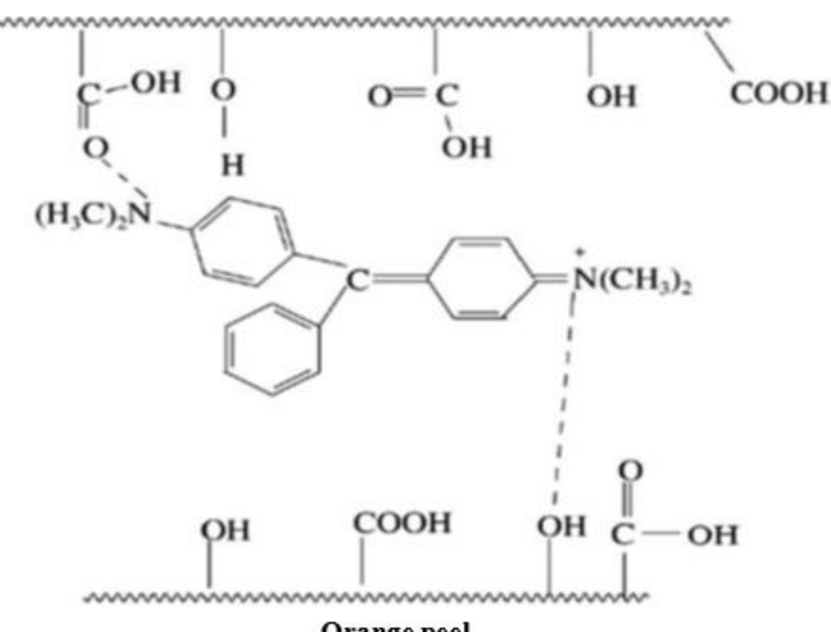

FIGURE 10. PROPOSED MECHANISM FOR THE BIOSORPTION OF THE C.I. BASIC GREEN 4

In the view of those results with ethyl alcohol we chose the recovery of the adsorbed dye, and the biosorbent was used for four more biosorption cycles. Figure 10 shows the results:

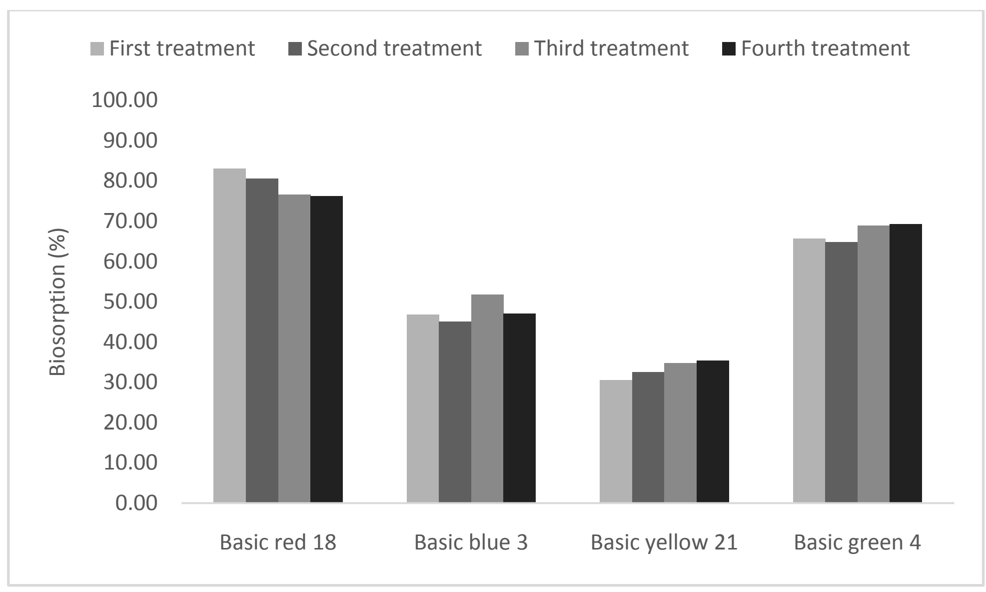

FIGURE 11. ADSORBED PERCENTAGE OF THE DYES IN SUCCESSIVE TREATMENTS

With the bioadsorbent developed in this work, it has been shown that the elimination of cationic dyes of the textile waste water is not produced by ion exchange but its efficacy is based on bonds by Hydrogen Bridge and by forces of London.

IV. CONCLUSION

The removal of heavy metals by treated orange peel is basically done through an ionic exchange phenomenon between Ca (II) and the metal ions in solution until the equilibrium is reached. That way, Ca (II), linked to the polygalacturonic chains, is displaced by the metal ion.

The saponification process (desmetoxilation) and the pectins reticulation sensible to the Ca(II) ion are usually ,done in two steps. At the first step, the desmetoxilation with NaOH is done, while in the second step the reticulation by $CaCl_2$ takes place. In this project, both steps were simultaneously done using $Ca(OH)_2$.

The testing of this method has been rendered effective through a removal of Cu (II), using 0.5 grams of the biosorbent, through an ion exchange mechanism, almost 100 ppm of dissolution of Cu (II). It is accepted that this removal is done mainly because of an ionic exchanger process, in a way that Ca(II) is initially linked to the pectin chains and displaced by Cu (II) until reaching the equilibrium conditions. Furthermore, the biosorbent presents a high selectivity to the Cu (II) even in presence of other cations (Hang 1970).

In the light of these results, it is possible to assert that the removal of the cationic dyes used is not explained by a simple mechanism of ionic exchange, leaving the establishment of a better-adjusted mechanism according to experimental data for a later study.

The physical-chemical treatment developed in this work in the orange peel shell has achieved a final stable product (biosorbent), which can be reused several times.

Its application in the removal of cationic textile dyes has been tested yielding positive results at laboratory level. Nevertheless, the experimental results discard that, with this type of organic molecules, this biosorbent works as a cationic exchanger.

It has been demonstrated that 40 g/l of the biosorbent is effective in the removal of 30 ppm of cationic dyes, for 45 minutes of stirring, obtaining yields ranging from 52 to 92%.

It is possible to reach the total removal of the biosorbed dyes if the process is successively repeated, which suggests the developing of a new removal procedure as a continuous process.

The removal of cationic dyes has been studied in a solution with higher dyestuff concentrations (between 60 and 240 ppm) at the same conditions with higher or, at least, similar efficiency. This means that the dilution of the wastewater is not necessary to treat with this biosorbent.

It has been demonstrated that it is possible to recover the biosorbent and to reuse it with a constant efficiency for at least four cycles.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of European Commission under H2020 through TECLO Project.

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Community composition and species diversity of fruit-eating-insects of *Gymnacranthera paniculata, Macaranga aleuritoides* and *Mastixiodendron pachyclado* in a Papua New Guinea Primary Forest

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***Abstract*— *Community composition and species diversity of fruit-eating-insects were studied in a primary forest at Wanang, Madang, Papua New Guinea (PNG) using fruits regularly sampled and insects attacking them reared, preserved and identified. Sampling was done in different areas of the forest including low and high abundance of the host trees. Fruits of three predominant host trees, G. paniculata (Myristicaceae), M. aleuritoides (Euphorbiaceae) and M. pachyclados (Rubiaceae) were regularly collected and insects associated with them studied. The emergence from the fruits were 13 insect families and 16 species from G. paniculata, 17 insect families and 21 species from M. aleuritoides and 17 insect families and 25 species from M. pachyclados. Diversity assessment showed M. pachyclados was more diverse (H=2.0258) followed by G. paniculata (H=2.007). M. aleuritoides was the least diverse (H=1.443). A high percentage of scavengers and wood eaters were found in G. paniculata and M. aleuritoides. In M. pachyclados, more seed eaters, chewers and parasitoids were found instead. These results have implications for management of the community composition and diversity of the fruit-eating insects of the three host three species.*

***Keywords*— Community composition, species diversity, fruit-eating-insects, Papua New Guinea.**

I. INTRODUCTION

Frugivorous insect biodiversity has not been documented in New Guinea Forests in regard to their communities and composition on *G. paniculata*, *M. aleuritoides* and *M. pachyclados*. These trees are predominant in Wanang Conservation Area and throughout the New Guinea forests, and have economic importance to the local people. A tropical tree species supports a number of species of insect herbivores which are often large and unknown [1, 2]. [3] reared Dacine fruit flies (Tephritidae: Dacinae) from a sample size of more than 100 fruits weighing more than 1 kg of fruits from plant species while other insect taxa from this guild and seed predators in species of Lepidoptera, Coleoptera and Diptera were not studied [3]. A diversity of frugivorous insects exist in tropical forests were quantitatively reared. With 57 frugivorous weevil species representing 10,485 individuals from 326 woody plant species in lowland rain forest in PNG [4]. These frugivorous insects can be partitioned into two feeding guilds; mesocarp feeders (flesh feeders) and those feeding on endocarp (seed predators).

Plants possess chemical and mechanical defences in seeds versus mesocarp that adheres to these specific feeding guilds [4]. Seeds are often shield by high concentrations of secondary compounds [5, 6, 7, 8], thus contribute to narrow attack by group of specialized predators that possesses detoxifying counteract mechanisms unlike against generalists which lack such protection [9]. Scolytine beetles attack palms [10] while *Revena rubiginosa* Boheman (curculionidae) predates on seeds of single-stemmed palm *Syagrus romanzoffiana* Cham (Arecaceae) [11, 12]. [13] recorded about 60% of fruit attack by seed predators in which weevils, katydids, and moth larvae were predominant on understory palm *Calyptrogyne ghiesbreghtiana* [14]. Most seed-beetles are oval shape [15] and have dietary specialization [16].

Parasitoids also strive with insect since they depend on them as hosts to complete their life cycles. [17] and [18] studied seven species of Braconid parasitoids under sub-family Opiinae: *Doryctobracon areolatus* Szépligeti, *Utetes anastrephae* Viereck, and *Opius* sp. Muesebeck, and Alysiinae: *Asobara anastrephae* Muesebeck, *Phaenocarpa pericarpa* Foerster, *Idiasta delicata* Papp, and *Asobara* sp. Nees. [19] stated that *Braconid* wasps were frequently reared from *Cydia* sp. larvae (Tortricidae: Grapholitini) from understorey palm (*Calyptrogyne ghiesbreghtiana* Linden) in Costa Rica where fruits containing *Cydia* have oviposition punctures of parasitoid and explains high parasitism due to few adults reared. Twenty-two

Braconid species belonging to Agathidinae, Braconinae, Helconinae, Homolobinae and Rogadinae subfamilies were recorded from Brazil, Chile, Costa Rica, Guatemala, Mexico and Panama [20, 21]. In this study, the community composition and species diversity of host-specific fruit eating insects of *G. paniculata*, *M. aleuritoides* and *M. pachyclado* were studied.

II. MATERIALS AND METHODS

2.1 Field Sampling

The study was conducted in a primary forest at Wanang Conservation Area (5^o13 ′S, 145^o04 ′E, 100 m.a.s.l), Madang, PNG [22, 23]. The map of the conservation area is shown in Fig. 1. [24] described the vegetation of the site as mixed evergreen rain forest on Latosol with a humid climate, and of mean annual rainfall of 3600 mm, having a mild dry season from July to September, and mean annual temperature of 26°C [25]. Fruits were sampled systematically following rows from an existing 50 ha forest plant plot in the Conservation Area and a few were sampled outside of the plot. Three locally abundant tree species were selected for the study: *G. paniculata*, *M. aleuritoides* and *M. pachyclados*. Sampling was done in different areas of the forest including both low and high abundance of the host trees. Fresh fallen fruits of each tree species was collected from the ground then were separately placed into plastic bags, given a unique tree number code and brought to the onsite laboratory. A fruit from each tree was sliced in half and photographed, together with unsliced fruits along with their respective tree number code.

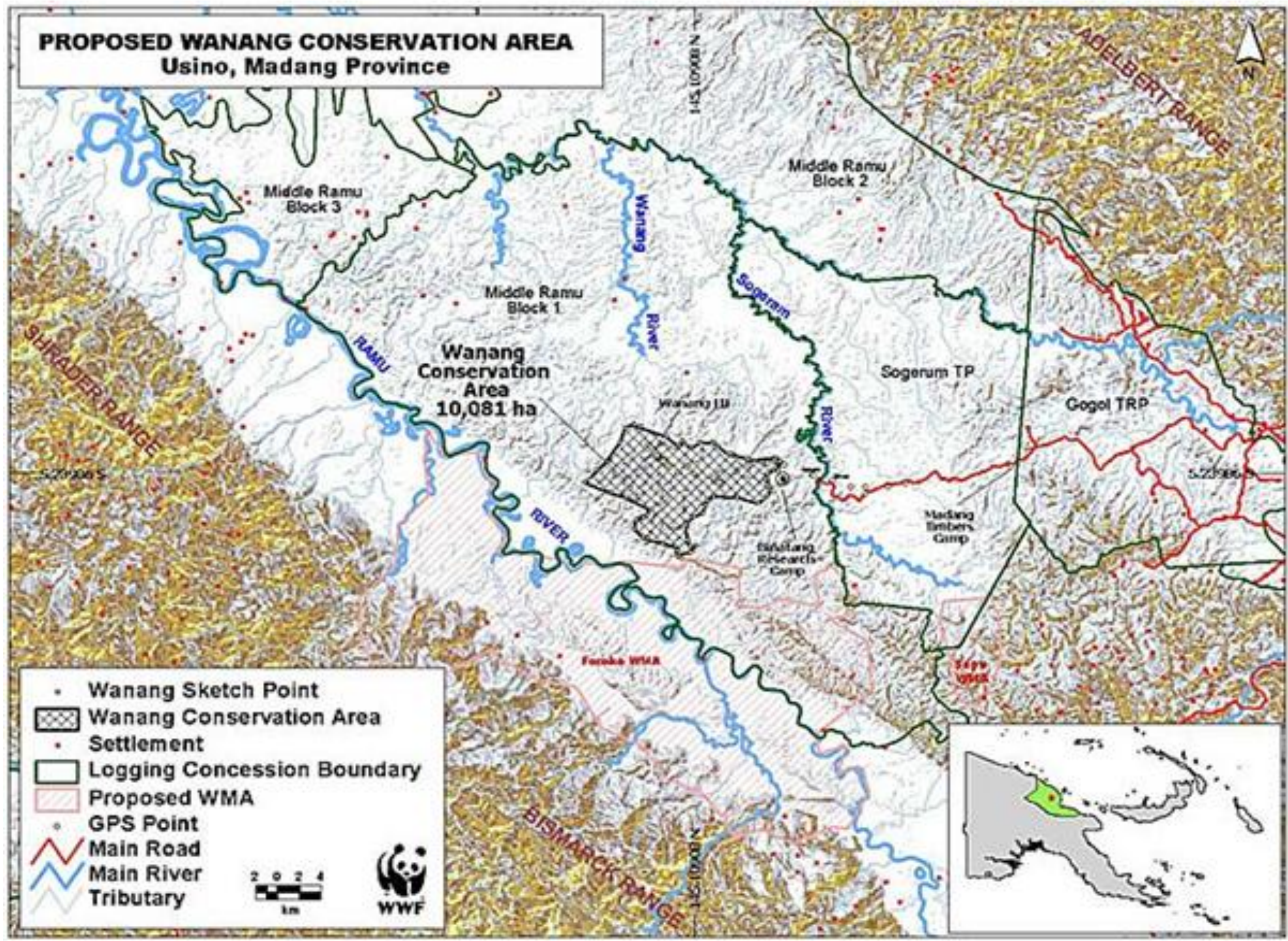

FIGURE 1. THE LOCALITY OF STUDY SITE, WANANG CONSERVATION AREA, MADANG, PNG

The fruits were then separated into plastic rearing containers (lunch boxes) and weighed on an electronic balance. Fruits from each tree represented by 3 fruit samples were sliced and measured (fruit and seed length, width, and fruit height) were measured. The rearing containers were closely monitored on a daily basis for insect emergence. Once insects emerged, they were collected by opening the side of the plastic led and collected with a medium sized plastic test tube and preserved in 99% ethanol.

2.2 Insect Sorting and Identification

All wet specimens were taken to the New Guinea Binatang Research Center (NGBRC) for identification. Identification was done using the aid of reference text books [26, 27], online insect databases (www.buglife.com), and insect database and reference collections of NGBRC. Insect specimens were initially sorted into morpho-species and given codes based on their distinct morphological features. Identification was done to genus level and coleopterans and lepidopterans to species level respectively. The resulting data on species abundance and richness of frugivorous insects were recorded and sorted using Microsoft Excel 2010.

2.3 Data Analysis

The Shannon-Wiener diversity index was used to measure the diversity of insects associated with each of the three host trees. Species diversity differs from species richness since diversity encompasses both the numbers of species present and the dominance or evenness of species in relation to one another [28].

$$\text{Shannon Index (H)} = -\sum_{i=1}^{s} p_i \ln p_i$$

p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N). **ln** is the natural log, $\sum$ is the sum of the calculations, and ***s*** is the number of species.

Menhinick's index was used to measure species richness [29], and served as a measure of the number of species found in a sample and species evenness, measure the relative abundance of different species making up the richness on each tree.

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

where **s** equals the number of different species represented in a sample, and **N** equals the total number of individual in the sample.

Species evenness is also an important component of diversity indices [30, 31, 32] and expresses how evenly each species is distribution among different habitat (host trees).

$$\text{Species evenness (E)} = H/H_{max}$$

where H_{max} = **ln** (N) and H = Shannon Index value. N is the number of species (species richness)

Feeding guilds are based on type of feeding mode and the fruit parts fed on as per [4, 9].

III. RESULTS

A total of 91 fruit samples weighing 14.5 kg were collected from three host trees. A total of 28 samples collected from *G. paniculata* weighing 2.07 kg reared 184 individual insects, 33 samples from *M. aleuritoides* weighing 5.07 kg reared 1938 individual insects, and 30 samples from *M. pachyclados* weighing 3.77 kg reared 289 individual insects. The remaining samples weighing 3.63 kg did not rear any frugivorous insects. A total of 2, 414 individual insects emerged from a total fruit sample weighing 10.90 kg. Thirteen (13) insect families and 16 species emerged from *G. paniculata*, seventeen (17) insect families and 21 species emerged from *M. aleuritoides* while seventeen (17) insect families and 25 species emerged from *M. pachyclados*.

The species diversity, richness, evenness and similarity of the frugivorous insects reared from each tree species are shown in Table 1. All dipterans and hymenopterans, and few lepidopterans and hemipterans were sorted into morpho-species and given codes based on their distinct morphological features.

The feeding guilds of the insects reared are shown in Figure 2. There were more scavengers in *G. paniculata* than in *M. aleuritoides* than in *M. pachyclados*. In addition, there were more wood eaters in *M. aleuritoides* than in *G. paniculata* and the least was in *M. pachyclados.* Seed predator and chewer were abundant in *M. pachyclados,* compared to the other two tree species.

TABLE 1
ABUNDANCE AND COMPOSITION OF INSECTS

Insect Taxa	Number of individual insects per Tree		
	G. paniculata	*M. aleuritoides*	*M. pachyclados*
Anisopodidae (Diptera)	1 (0.543478261)	1 (0.051519835)	1 (0.346020761)
Agonoxenidae (Lepidoptera)	0 (0)	2 (0.10303967)	0 (0)
Araecerus sp .1 (Anthribidae: Coleoptera)	0 (0)	0 (0)	10 (3.460207612)
Araecerus sp.2 (Anthribidae: Coleoptera)	0 (0)	0 (0)	9 (3.114186851)
Araecerus sp.3 (Anthribidae: Coleoptera)	0 (0)	0 (0)	3 (1.038062284)
Araecerus sp.4 (Anthribidae: Coleoptera)	0 (0)	0 (0)	1 (0.346020761)
Araecerus sp.5 (Anthribidae: Coleoptera)	0 (0)	0 (0)	2 (0.692041522)
Baris sp. (Curculionidae: Coleoptera)	0 (0)	0 (0)	44 (15.22491349)
Blastobasis sp. (Blastobasidae: Lepidoptera)	6 (3.260869565)	0 (0)	1 (0.346020761)
Braconidae (Hymenoptera)	0 (0)	6 (0.309119011)	73 (25.25951557)
Cillaeus sp. (Nitidulidae: Coleoptera)	6 (3.260869565)	7 (0.360638846)	0 (0)
Coccotrypes dactyliperda (Scolytinae: Coleoptera)	29 (15.76086957)	1112 (57.29005667)	16 (5.53633218
Conotrachelus sp. (Curculionidae: Coleoptera)	0 (0)	0 (0)	6 (2.076124567)
Drosophilidae (Diptera)	74 (40.2173913)	147 (7.573415765)	13 (4.498269896)
Eucoilidae (Hymenoptera)	0 (0)	0 (0)	1 (0.346020761)
Eulophidae (Hymenoptera)	0 (0)	0 (0)	1 (0.346020761)
Formicidae (Hymenoptera)	0 (0)	1 (0.051519835)	0 (0)
Haplonyx sp. (Curculionidae: Coleoptera)	0 (0)	13 (0.669757857)	1 (0.346020761)
Ichneumonoidea (Hymenoptera)	0 (0)	0 (0)	1 (0.346020761)
Lonchaeidae (Diptera)	8 (4.347826087)	0 (0)	0 (0)
Lygaeidae (Hemiptera)	1 (0.543478261)	1 (0.051519835)	0 (0)
Mimemodes sp. (Coccinellidae: Coleoptera)	1 (0.543478261)	0 (0)	0 (0)
Muscidae (Diptera)	0 (0)	2 (0.10303967)	5 (1.730103806)
Mussidia pectinicornella (Pyralidae: Lepidoptera)	0 (0)	0 (0)	72 (24.91349481)
Mycetophilidae (Diptera)	1 (0.543478261)	0 (0)	0 (0)
New moth family (Lepidoptera)	2 (1.086956522)	0 (0)	5 (1.730103806)
Periscelididae (Diptera)	5 (2.717391304)	332 (17.10458527)	2 (0.692041522)
Phenolia sp.1 (Nitidulidae: Coleoptera)	0 (0)	2 (0.10303967)	0 (0)
Phenolia sp.2 (Nitidulidae: Coleoptera)	5 (2.717391304)	1 (0.051519835)	4 (1.384083045)
Phoridae (Diptera)	0 (0)	1 (0.051519835)	1 (0.346020761)
Psychodidae (Diptera)	20 (10.86956522)	156 (8.037094281)	0 (0)
Spaerosoma sp. (Coccinellidae: Coleoptera)	0 (0)	49 (2.524471922)	0 (0)
Thiotricha sp. (Gelechiidae: Lepidoptera)	0 (0)	6 (0.309119011)	0 (0)
Tipulidae (Diptera)	15 (8.152173913)	5 (0.257599176)	14 (4.844290657)
Torymidae (Hymenoptera)	0 (0)	3 (0.154559505)	0 (0)
Xyleborinus saxeseni (Scolytinae: Coleoptera)	2 (1.086956522)	70 (3.60638846)	1 (0.346020761)
Xyleborus metacuneolus (Scolytinae: Coleoptera)	8 (4.347826087)	24 (1.236476043)	2 (0.692041522)
Total	**184 (100)**	**1941 (100)**	**289 (100)**

Note: a) G. paniculata: Diversity index (H) = 2.006637167, Richness index (D) = 1.179535649, Evenness index (E) = 0.723741373
b). M. aleuritoides: Diversity index (H) = 1.442591566, Richness index (D) = 0.477026393, Evenness index (E) = 0.473831806
c). M. pachyclados: Diversity index (H) = 2.258377854, Richness index (D) = 1.470588235, Evenness index (E) = 0.701604528

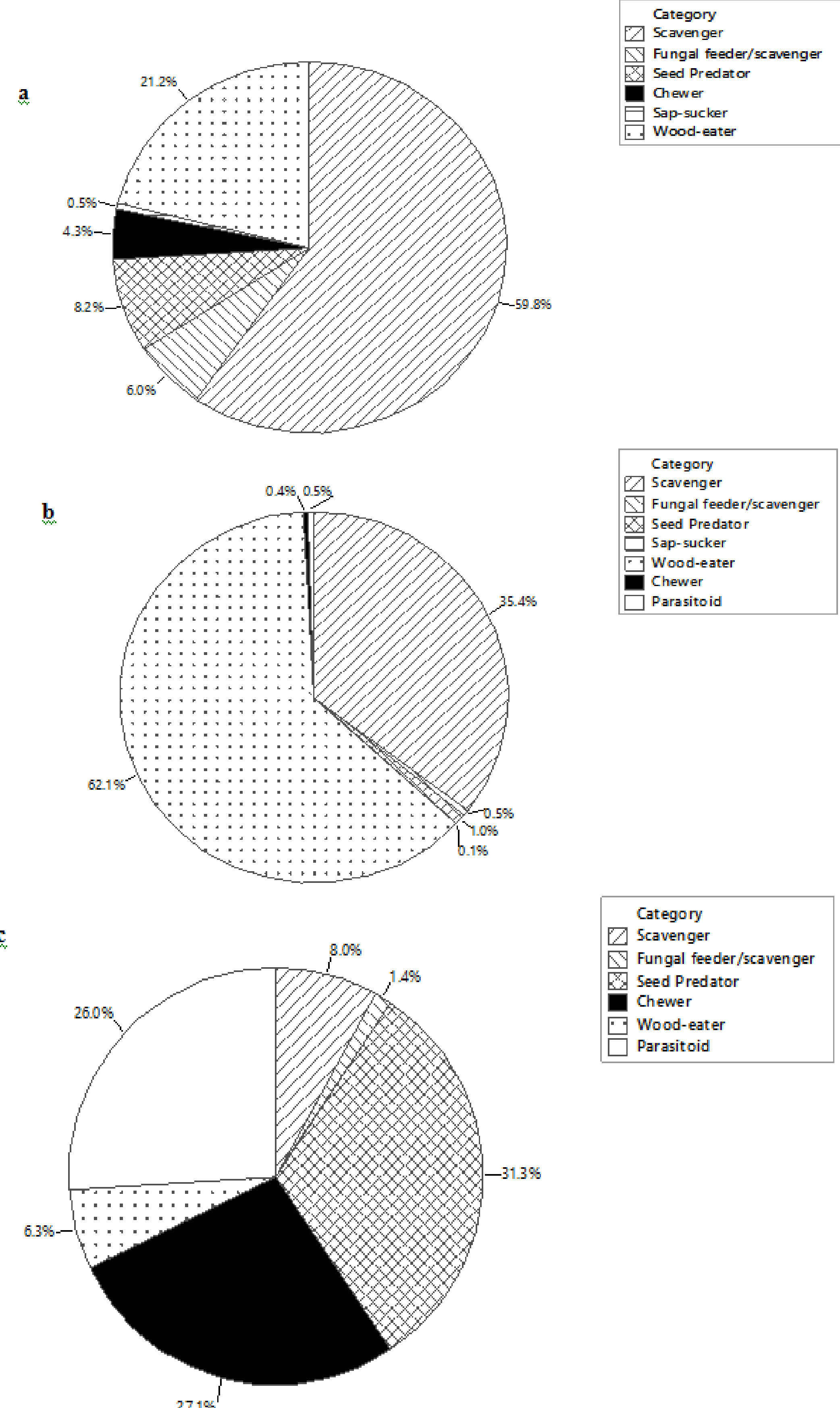

FIGURE 2. FEEDING GUILDS OF INSECTS ON (a) *G. paniculata*, (b) *M. aleuritoides* and (c) *M. pachyclados*

IV. DISCUSSION

Fruit size and morphology can influence chances of weevil attack to fruits [4]. Only fruits with sufficient mesocarp (fleshiness) with large seeds concurrently host both seed- and flesh-eaters where weevils preferably avoid small fruits due to lack of substantial resources for larval development. However, weevils also avoid fruits of larger than average size-fruits that have very thick and hard endocarp which is recognized as a significant barrier to seed predation [33]. Fruits of the tree species had generally thin mesocarp thickness 2.5 mm (*G. paniculata*), 1.8 mm (*M. aleuritoides*) and 1.8 mm (*M. pachyclados*) with corresponding seed (endocarp) thickness of 5 mm, 4 mm and 4.3 mm, respectively.

The tree species had different fruit-eating insect diversity (H), species richness (D) and species evenness (E). The insect diversity associated with fruits was highest in *M. pachyclados* (25 insect species, H= 2.0258) due to larger seed size and soft endocarp (seed) for ease of penetration by frugivorous insect larvae. *G. paniculata* (H= 2.007) with second highest insect diversity was attributed to its soft mesocarp and semi-soft endocarp (seed) that permits penetration by insect larvae. *M. aleuritoides* (H= 1.443) was least diverse probably due to chemical and mechanical defenses mechanisms of the mesocarp [5, 6, 8] and attacked only by specialists, e.g. seed predators which possess detoxifying mechanisms [9]. Even though *M. aleuritoides* had the highest species number (21 insect species) than *G. paniculata* (16 insect species, E= 0.724), this insect was not well distributed on *M. aleuritoides* (E= 0.474).

As Table 1 shows, *Coccotrypes dactyliperda* (Scolytinae) had very high abundance (1112 individuals) while other insect species had abundance quite lower than that (abundance values not close together). Since *C. dactyliperda* numerically dominates the other 20 insect species, *M. aleuritoides* is considered less diverse than *G. paniculata*. Evenness indices ranges from 0 when most individuals belong to a few species, while close to 1, when species are nearly equally abundant, and often used to standardize abundance [34, 35]. It is obvious that insect species are not nearly equally abundant on *M. aleuritoides* (E= 0.474).

The feeding guilds based on the food resources provided or associated with fruits were shown in Figure 2. Drosophilidae infests small fruits and cherries of a variety of wild, ornamental, and uncultivated hosts during both ripening or overripe stages, and even attacks damaged fallen fruits [36] of *G. paniculata* therefore they can be regarded as both frugivores and scavengers. Both Drosophilidae and Tephritid flies feed on sugar either from split fruit, floral nectar, extrafloral nectar, sap, yeast or insect honeydew [37]. Tephritid flies were not reared in this study mainly due to very thin fruit mesocarp of *G. paniculata* (2.5 mm), *M. aleuritoides* (1.8 mm) and *M. pachyclados* (1.8 mm). Female tephritid flies usually deposit their eggs 2-4 mm under the aril and needs sufficient mesocarp (>4 mm) to provide enough food substrate for the developing larvae [38].

All the fruits studied did not support the oviposition requirement and proceeding larval development of tephritid flies. An experiment performed by [36] showed that tephritid fly (*Bactrocera invadens* Drew) preferred to oviposit in mango variety with thicker mesocarp than those with lesser mesocarp. Nitidulidae (*Phenolia* & *Cillaeus* sp.) are considered both scavengers feeding on decaying matter and as fungal-feeders or mycetophagous [9; 40]. *Coccotrypes dactyliperda* (Scolytinae: Coleoptera) predominates *M. aleuritoides* and emerged via seeds. *C. dactyliperda* are commonly known as bark (wood) beetles and the reason they dominate *M. aleuritoides* is probably due to the woody nature of seeds. Periscelididae, Psychodidae and Drosophilidae were abundant on *M. aleuritoides* due to their scavenging ability [41] when the fruit rotted.

Braconid wasps were abundant on *M. pachyclados* [33]. *Mussidia pectinicornella* (Pyralidae: Lepidoptera) and *Baris* sp. (Curculionidae: Coleoptera) were prevalent on *M. pachyclados* and might have served as host for Braconid wasps. Parasitism of host larvae by female *Braconid* wasps may have occurred prior to rearing. Singleton insect species in any of the three fruit trees having abundance of only one individual may indicate the fruit as alternate host where a suitable host seems rare or absent. Singleton species such as parasitic wasps (Eucoilidae & Eulophidae) as observed only on *M. pachyclados* might be due to low host-larvae number associated with the fruits. There is a need to investigate Eucoilid and Eulophid parasitic wasps and their host as there is few or no detail information on these wasps, particularly in PNG forest.

V. CONCLUSION

The diversity of fruit-eating insects in PNG forests and their roles in maintaining high plant diversity in the tropical forests are not often documented. The resulted of this study showed that the diversity of frugivorous insects differ between tree species. The highest insect diversity was recorded on *M. pachyclados* (H=2.0258), followed by *G. paniculata* (H=2.007) and *M. aleuritoides* (H=1.443). Feeding guild assessment showed there were more scavengers and wood eaters in *G. paniculata*

and *M. aleuritoides* than in *M. pachyclados*. Comparatively, there were more seed eaters, chewers and parasitoids in *M. pachyclados*. Communities of frugivorous insects feed on fruits of tropical forest trees and their feeding guilds pertain to distribution and thus diversity on plants. Therefore, the results of the feeding communities of fruit-eating insects on the tress thus contribute to better understanding of the ecology of tropical forests, particularly the dynamics of tree species, and the potential role of frugivorous insects. The findings of this study have implication for management of the fruit-eating-insects of primary forests of PNG.

ACKNOWLEDGEMENTS

We are grateful to the management and helpful staff of New Guinea Binatang Research Center in Madang, PNG for their valuable assistance in field research. The first author extends his appreciation to Professor Y. Basset for providing research materials and resources at Wanang, Madang, PNG

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Bravecto (fluralaner) chewable tablets have been thoroughly evaluated in multiple countries and are approved as a safe and effective flea, tick and mite treatment for dogs

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***Abstract* — *Bravecto (fluralaner) is thoroughly tested to international safety standards for veterinary drugs, meeting approval requirements for over 70 countries. This valuable antiparasite (fleas, ticks and mites) treatment contributes to the health of millions of dogs and promotes dog health worldwide by protecting them against dangerous parasite infestations that are known to lead to pathogen transmission, blood loss, local irritation, and skin allergies. In 2017, the European Medicines Agency (EMA) completed an in depth targeted review of all reported adverse events (ADE) related to various potential disorders and confirmed the positive benefit-risk profile of Bravecto. Official records that monitor adverse events are often available online and these reports can be easily misunderstood by people unfamiliar with the procedures and how to interpret monitoring information. For example, many people do not know that the FDA advises "For any given ADE report, there is no certainty that the reported drug caused the adverse event." This means that the cause of a problem reported to this agency has not been determined, and this is typical of drug use reports. Multiple communications from individual dog and cat owners provided photographs showing how their pet has dramatically improved with the help of fluralaner treatment.*

Keywords*— *cat, dog, fluralaner, pharmacovigilance, safety.

I. INTRODUCTION

Fluralaner is the active ingredient in the Bravecto Chew (MSD Animal Health, Giralda Farms, NJ, USA), a treatment that offers a highly effective way to control external arthropod parasites affecting dogs, including fleas, ticks and mites, for up to 12 weeks following a single dose. This treatment is approved in over 70 countries based on careful review of a comprehensive dossier consisting of multiple field and laboratory studies proving the safety and efficacy of fluralaner. In addition, there are more than fifty peer-reviewed publications available in the scientific literature that provide expert scientific evidence of the safety and efficacy of fluralaner [1-50].

All approved medicines go through an intensive ongoing safety monitoring service to evaluate any potential problems that may become apparent with experience but that did not show up even under intensive pre-approval testing. This monitoring evaluation is called pharmacovigilance and the process follows strict rules that allow experts in the area to detect evidence of any kind of "safety signal". This signal may indicate a previously unrecognized problem with a medicine and be a sign that users should be informed of additional information regarding the profile of the product.

Many regulatory authorities provide access to the public to the reported pharmacovigilance data on approved veterinary products, such as fluralaner. Unfortunately, this information is potentially misinterpreted by some readers, who are uninformed as to how these reports are prepared and assume that every report is an indication that the medicine caused the problem. The careful reader, on the other hand, quickly realizes that this is not the case. In addition to pharmacovigilance, medicines may also be evaluated in other safety studies performed after launch. For example, studies may be conducted because the active ingredient in a veterinary medicine is being reviewed for other uses in other animal species.

Fluralaner belongs to a class of flea and tick treatment drugs called the isoxazolines that distribute systemically in the dog after administration, so that the medicine spreads through the bloodstream to all areas of the skin. These medicines kill the flea or tick when it tries to bite and is then exposed to the active ingredient. Multiple studies on both fleas and ticks have now shown that this approach to controlling these parasites can prevent the damaging effects associated with these bites, including prevention of allergies to biting parasite saliva and reducing the risk of parasite borne disease transmission. The isoxazolines were superior to a topically administered treatment for killing ticks attached to dogs at the time of treatment [28].

II. MATERIALS AND METHODS

All peer reviewed scientific literature and on-line published scientific sources regarding the safety and efficacy of fluralaner were identified and reviewed [1-56]. There are over fifty relevant publications providing in depth review of the mechanism of action, the safety of treating dogs and cats and effectiveness against multiple parasitic infestations. These papers are indexed in multiple scientific databases and are often freely available online to all readers. In addition there are online reports available from government agencies that document the safety of fluralaner, including some unique studies not available for any other isoxazoline approved for treatment of dogs that were reviewed in preparation of this report [57].

III. RESULTS AND DISCUSSION

A unique and comprehensive body of scientific work supports the use of fluralaner against external parasites affecting dogs and cats. Careful and knowledgeable review of the scientific literature confirms the well-documented safety and efficacy profile of fluralaner for dog and cat treatment. Government agency intensive reviews of all adverse event reports from everywhere around the world find that the risk-benefit profile for use of this treatment is positive.

Recent investigations into the safety of fluralaner use in dogs present new evidence for the safety profile of fluralaner following administration [57]. Fluralaner was administered to dogs daily at up to 4 mg/kg for one year (52 weeks) without report of a serious adverse event. The same reference also reports no adverse events in dogs receiving very high fluralaner doses (up to 750 mg/kg) daily for 28 consecutive days [57].

Additional evidence and testimonials from pet owners, while anecdotal, further confirm the often dramatic effect that fluralaner treatment can produce in parasite-infested dogs (Figures 1-7). The illustrations below were provided by pet owners who documented the dramatic improvement seen in their formerly parasite-affected animals following fluralaner treatment. In at least one case, the owner was so desperate for an effective treatment and concerned about the discomfort their dog was in that they were considering euthanasia.

FIGURE 1. Head on and lateral view of a dog with a severe skin parasitic infestation just before treatment with Bravecto (fluralaner).

FIGURE 2. The same dog as in fig. 1 photographed 8 weeks later. (Photo credit Emma O'Brien, used with permission)

FIGURE 3. Another untreated dog with a severe skin parasitic disease

FIGURE 4. The same dog as in fig. 3 photographed 8 weeks after fluralaner treatment

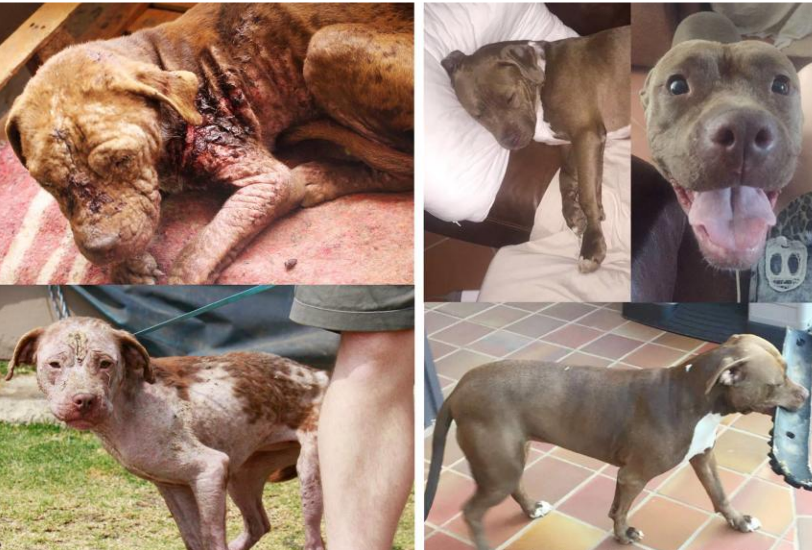

FIGURE 5. A puppy presented with severe parasitic skin disease (on the left) and the same puppy 8 weeks later following treatment with fluralaner (on the right).

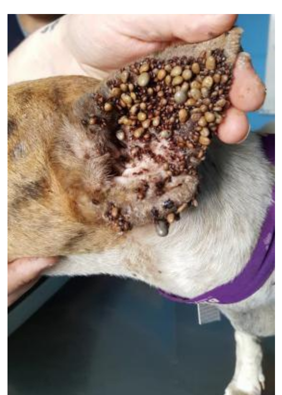

FIGURE 6. Heavy tick infestation in the ear of a dog before treatment with fluralaner.

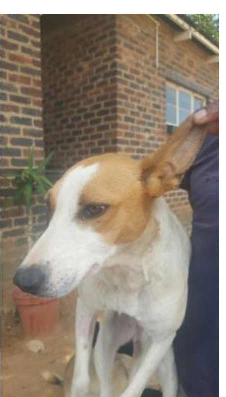

FIGURE 7. The same dog as in Fig. 6 one week following fluralaner treatment

One frequent source of information regarding potential adverse events associated with treatments is the US FDA adverse drug event database. The US FDA provides clear statements for this database to help readers correctly interpret the report numbers, but these guidelines may be ignored or misunderstood by individuals who review information from the database and then present these numbers in their published work. It is helpful to review the recommendations the US FDA makes to help readers understand these reports and to consider their meaning:

- "For any given ADE report, there is no certainty that the reported drug caused the adverse event. The adverse event may have been related to an underlying disease, using other drugs at the same time, or other non-drug related causes. The clinical detail listing does not include information about underlying diseases, other drugs used at the same time, other non-drug related causes, or the final outcome of the reaction.
- The accuracy of information regarding the ADE is dependent on the quality of information received from the reporting veterinarian or animal owner.
- Accumulated ADE reports should not be used to calculate incidence rates or estimates of drug risk, because there is no accurate way to determine how many animals were given the drug, which is needed as the denominator in calculations of incidence and relative risk.
- It is inappropriate to make use of adverse event data to compare the safety of different products. For example, if a drug is widely used to treat certain conditions, there may be more ADEs for that drug than another product that is not used as often. This would not mean that the first drug was more unsafe than the second.

- The number of reports simply represents the number of ADEs received for a particular drug and should not be used for any type of comparison purposes.
- Underreporting occurs with most adverse event reporting systems. The frequency of reporting for a given drug product varies over time, and may be greater when the drug is newly marketed, or when media publicity occurs.
- Information on how the drugs were used (for indications on the product label or in an extra label manner) is not provided in the clinical detail listing."

These cautionary statements make it clear that the ADE database information needs to be interpreted by people who understand how summarized reports can be used to look for evidence of safety signals. These reports should not be presented as evidence of lack of drug safety and this would be an incorrect conclusion without more background details. Those who have the expertise to assess such reports – namely the global regulatory agencies – have concluded that the benefit-risk profile of Bravecto remains favorable.

IV. CONCLUSION

The scientific literature contains convincing data showing that Bravecto (fluralaner) offers a unique combination of long lasting efficacy and safety for dogs and cats and provides multiple benefits for pet owners of pets by helping them to prevent dangerous parasite skin infestations with fleas, ticks and mites.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge New Beginnings Rescue Centre, Community Led Animal Welfare and Emma O'Brien for photographs and the permission to use these. Thank you also to the many thousands of veterinarians and millions of pet parents who strive every day to improve the health of their dogs and cats based on careful science and thoughtful understanding and who are not swayed by unsupported internet rumors.

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Faunistic Analysis of Soil Mites in Coffee Plantation

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Abstract — *The soil-litter system is the natural habitat for a wide variety of organisms, microorganisms and invertebrates, with differences in size and metabolism, which are responsible for numerous functions. The soil mesofauna is composed of animals of body diameter between 100 µm and 2 mm, consisting of the groups Araneida, Acari, Collembola, Hymenoptera, Diptera, Protura, Diplura, Symphyla, Enchytraeidae (Oligochaeta), Isoptera, Chilopoda, Diplopoda and Mollusca. These animals, extremely dependent on humidity, move in the pores of the soil and at the interface between the litter and the soil. The edaphic fauna, besides having a great functional diversity, presents a rich diversity of species. As a result, these organisms affect the physical, chemical and, consequently, the biological factors of the soil. Therefore, the edaphic fauna and its activities are of extreme importance so that the soil is fertile and can vigorously support the vegetation found there, being spontaneous or cultivated. The composition, distribution and density of the edaphic acarofauna varies according to the soil depth, mites size, location and the season of the year. Edaphic mites are generally found in greater quantities in the organic matter layer than in the soil mineral. The subclass Acari is divided in seven orders being the Mesostigmata, Trombidiformes, Endeostigmata and Sarcoptiformes those that frequently occur in the soil. In the order Sarcoptiformes the suborder Oribatida (formerly Cryptostigmata) is one of the more numerous groups of soil arthropods, both in number of species and specimens. Considering the above facts, it was the objective of this work to know the acarofauna of the soil in a coffee plantation and rank the taxa in a decreasing way, by the use of faunistic analysis. The soil samples were taken in coffee plantation in the Experimental Station of EPAMIG, in São Sebastião do Paraíso, MG, Brazil, in two periods, end of dry and end of rainy season of the year 2013, and the extraction of edaphic mites of the soil mesofauna was done at the Laboratory of Acarology of EPAMIG Sul/EcoCentro, in Lavras, as well as other activities related to the study. The result show that edaphic mites of the cohort Astigmatina and suborder Oribatid are dominant in both periods studied, and can be worked to be an indicative of soil quality.*

***Keywords —* Agricultural acarology, Coffea arabica, Edaphic mites, Soil mites, Soil mesofauna.**

I. INTRODUCTION

The edaphic fauna reflects the environmental conditions, and are the characteristics of habitat such as climate, soil type, amount of accumulated litter, amount of organic matter, type of soil management, among others, which determine the groups of the soil fauna that will be present and in what quantities [1].

The global knowledge of the richness of groups of soil organisms provides an indication of the ecological complexity of soil communities [2] [3].

The soil-litter system is the natural habitat for a wide variety of organisms, microorganisms and invertebrates, with differences in size and metabolism, which are responsible for numerous functions. The diversity of soil fauna is related to the great variety of resources and microhabitats that the soil-litter system offers, a mixture of highly compartmentalized aquatic and aerial phases, generating a mosaic of microclimatic conditions and thus favoring, therefore, a large number of associated functional groups [4] [5].

Soil biota can be classified as: (1) **Microfauna -** composed of protozoa, nematodes and rotifers, whose diameter varies from 4 to 100 µm, which act indirectly in the nutrient cycle, regulating bacterial and fungal populations; (2) **Mesofauna -** which is composed of animals of body diameter between 100 µm and 2 mm, consisting of the groups Araneida, Acari, Collembola,

Hymenoptera, Diptera, Protura, Diplura, Symphyla, Oligochaeta, Isoptera, Chilopoda, Diplopoda and Mollusca, and can be included small specimens from the Coleoptera order. These animals, extremely dependent on humidity, move in the pores of the soil and at the interface between the litter and the soil. Among the trophic levels of this group, it stands out its significant contribution in the regulation of the microbial population; (3) **Macrofauna -** composed of animals that present body diameter between 2 and 20 mm and can belong to almost all orders found also in the mesofauna, except Acari, Collembola, Protura and Diplura and including Annelida and Coleoptera. They are animals of great mobility and that play an important role in the transportation of materials, both for making nests and burrows, and for building galleries that reach varying depths in the soil. Its main functions are the fragmentation of the vegetal residue and its redistribution, the predation of other invertebrates and the direct contribution in the structuring of the soil [6] [7].

Because they are sensitive and react to changes induced by natural phenomena to the soil and their vegetal cover, as well as by anthropic activities, the populations and the diversity of the edaphic fauna can be used as bioindicators of the use of the soil or its fertility, giving a notion of its current state and changes induced by internal and external forces (biotic and abiotic) over time. Such disturbances alter the distribution of soil fauna as they alter the availability of food resources, modifying the intra and interspecific ecological interactions. As changes in the environment, is cited for example, epigeous species, i.e., those that are restricted to inhabit the topsoil, which are normally associated with the layer of litter, and therefore disappear with deforestation or larger soil disturbances such as the use of plow and chemical products [8].

The edaphic fauna, besides having a great functional diversity, presents a rich diversity of species. As a result, these organisms affect the physical, chemical and, consequently, the biological factors of the soil. Therefore, the edaphic fauna and its activities are of extreme importance so that the soil is fertile and can vigorously support the vegetation found there, being spontaneous or cultivated [9].

The edaphic mesofauna study has been directed to the evaluation of the influence of agricultural practices on its taxonomic units as a whole, particularly to numerically more representative groups such as mites and springtails [10].

The study of these organisms initially consists of their capture, identification and quantification of the components of the community in question. The literature found research that use different methods, adopted at the discretion of the researchers, taking into account mainly the study objectives and the practical procedures. The most commonly used method is the extraction of soil samples and subsequent removal of the organisms by use of collection funnel [11].

One of the used types of biological indicator of soil quality is the population monitoring of the edaphic mesofauna. Therefore, the determination of the mesofauna is a biological indicator of the quality of the organic residues in order to contribute to the evaluation of a soil management system [12].

Mites are members of the Arthropoda phylum, which comprise a vast array of terrestrial and marine invertebrates that share the features of jointed legs and a chitinous exoskeleton. The mites belong to the large and diversified subphylum Chelicerata, where the largest group is of the Arachnida class. The arachnids are terrestrial chelicerates, including the Acari subclass, which includes mites and ticks, and which differs from the other arachnids by the absence of apparent segmentation [13].

The mites have as much diversity of food and way of feeding as of localities where they live. Regarding food habits, the variation occurs even within each family, ranging from parasitic species of vertebrates and invertebrates to phytophagous and predatory species [13].

The composition, distribution and density of the edaphic acarofauna varies according to the soil depth, mites size, location and the season of the year [14 [15]. Edaphic mites are generally found in greater quantities in the organic matter layer than in the soil mineral fraction [16].

The subclass Acari is divided in seven orders being the Mesostigmata, Trombidiformes, Endeostigmata and Sarcoptiformes those that frequently occur in the soil. In the order Sarcoptiformes the suborder Oribatida (formerly Cryptostigmata) is one of the more numerous groups of soil arthropods, both in number of species and specimens [17] [18] [13].

Considering the aforementioned facts, it was the objective of this work to know the acarofauna of the soil in a coffee and forest plantation, as well as to classify by the use of faunistic analysis, in a decreasing way of occurrence, the found taxa.

II. MATERIAL AND METHODS

2.1 Study site

The study was accomplished at the Experimental Station of the *Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG Sul*, in the municipality of *São Sebastião do Paraíso,* MG, Brazil, in an already existing coffee plantation (*Coffea arabica* L.) cultivar Paraíso with six years' age, in the Dystroferric Red Latosol (Oxisol) soil type, in the spacing of 4.0 m between the lines and 0.70 m between the plants, and in a neighboring forest in the same soil type.

2.2 Experimental design

The experimental design was in randomized block with eight treatments, being seven in the coffee plantation and the eighth treatment in the forest, with three replications. The forest was an area located very close to the coffee plantation, with the same type of soil, and constituted of a subperenifolia tropical native forest, having been used as a reference treatment of the type of soil before the agricultural exploitation of the farm.

The samplings were made in 48 experimental plots, 42 in plots of coffee plantation and 6 in the forest, in each sampling period that corresponded to the end of dry season and end of rainy season in the region of the study.

The coffee experimental plots consisted of four lines, 50 plants/line, and the useful part of the plot being composed of the two central lines totaling 80 plants,40 plants/line, and the remaining other lines served as border.

2.3 Soil sampling

Two soil samplings, one at the end of the rainy season (June) and the other at the end of the dry season of the year in the study region (October of 2013), were used to quantify the edaphic mites in the soil mesofauna.

The soil samples were extracted by means of a cylindrical probe made of stainless steel with 50 mm internal diameter and 53 mm high (100 cm^3), known as a cylinder for collecting not deformed soil samples (*BRAVIFER - Indústria de Equipamentos e Assessoria Agronômica Ltda. ME).*

At each sampling time, two soil samples were extracted, one at 5 m from the beginning and the other at 5 m from the end of each plot and every time in the central position between the lines of coffee plants. In the forest, the samples were taken also at each sampling time and with the same cylindrical probe, 50 m from the border.

2.4 Extraction and identification of edaphic mites

The edaphic mites' extractions of the soil samples were performed by means of Berlese-Tüllgren funnel extractor [19] [11]. In the funnel, the samples were subjected to light and heat for seven days [20] to create a temperature and humidity gradient, making the environment unfavorable for the organisms present, forcing them down until they fall into a collector vial containing 70% alcohol.

After being extracted, the mites were counted and removed from the alcohol of the collector vial with the use of a fine paint-brush and with the assistance of a binocular stereomicroscope at 40x magnification, and after were mounted on a microscopy glass slide in Hoyer's medium and after being covered with a glass coverslip were identified with the use of a phase-contrast binocular microscope.

The mites' extraction from soil samples and their taxonomic identification were conducted in the Laboratory of Agricultural Acarology of EPAMIG Sul/Research Center in Ecological Management of Pests and Plant Diseases - EcoCenter, in the city of Lavras, Minas Gerais, Brazil.

The proposal presented by Lindquist, Krantz and Walter was used [13], with the elevation of suborder Endeostigmata to order, as suggested by Pepato and Klimov [21]. In addition, in order to better visualize the results, the order Sarcoptiformes was split in two groups, with the cohort Astigmatina [22] presented separately from the other species that belong to the suborder Oribatida.

2.5 Faunistic analysis

The obtained data were analyzed using the ANAFAU software, developed in the Department of Entomology, Phytopathology e. Agricultural Zoology of the Luiz de Quiroz College of Agriculture/University of São Paulo, Brazil, recommended for great

diversity of species [23] which allows to know the indexes of frequency, abundance, constancy and dominance of each taxon found [24].

III. RESULTS AND DISCUSSION

3.1 Soil samples from the end of rainy season

In the faunal analysis performed with soil mites collected at the end of the rainy season of 2013, the families Nanorchestidae (*Spelenorchestes* sp.), Alycidae (*Bimichaelia* sp.), Eupodidae (*Eupodes* sp.1, sp.2), Rhodacaridae (*Multidentorhodacarus* sp.), Tarsonemidae (*Tarsonemus* sp.), mites of the suborder Oribatida and hypopus phase (cohort Astigmatina, Family Acaridae, genus *Ryzopglyphus* sp.) [22] (OCONNOR, 2009) were the ones that presented species or morphospecies considered to be dominants (D), since they presented the maximum rates of faunistic classification, very abundant (ma), very frequent (MF) and constant (W) during the period in which the study was performed (Table 1).

The suborder Oribatida was the one that presented the largest number of species, 20 species and morphospecies in four families in the period. Of these, among others, the families Brachichthoniidae, Licnodamaeidae and the species *Galumna flabellifera*, *Berlezetes brasilizetoides*, *Sherolibates* sp., *Arcoppia* aff. *dechambrierorum* and *Suctobelbella* sp. presented as dominant (D), very abundant (ma), very frequent (MF) and constant (W) in the end of rainy period (Table 1). In most soils, oribatid mites are numerically dominat and the most diverse among microarthropods, especially among soil invertebrates [25].

TABLE 1
FAUNISTIC ANALYSIS FOR TAXA OF EDAPHIC MITES COLLECTED BETWEEN THE LINES OF THE COFFEE PLANTATION AND FOREST AT THE END OF THE RAINY SEASON OF 2013. SÃO SEBASTIÃO DO PARAÍSO, MG.

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Galumna flabellifera (Oribatida, Galumnidae)	138	4	D	ma	MF	W
Rizoglyphus sp. (Astigmatina, Acaridae) (Hypopus)	134	6	D	ma	MF	W
Scheloribates sp. (Oribatida, Scheloribatidae)	115	4	D	ma	MF	W
Oribatida, Licnodamaeidae	105	3	D	ma	MF	Y
Suctobelbella sp. (Oribatida, Suctobelbidae)	96	7	D	ma	MF	W
Eupodes sp.1 (Prostigmata, Eupodidae)	95	7	D	ma	MF	W
Arcoppia aff. dechambrierorum (Oribatida, Oppiidae)	81	5	D	ma	MF	W
Spelenorchestes sp. (Endeostigmata: Nanorchestidae)	78	5	D	ma	MF	W
Multidentorhodacarus sp.1 (Mesostigmata, Rhodacaridae)	67	6	D	ma	F	W
Oribatida, Brachichthoniidae	54	6	D	ma	MF	W
Tarsonemus sp. (Prostigmata, Heterostigmatina, Tarsonemidae)	48	6	D	ma	MF	W
Oribatida (immature) (sp.9) (Suborder)	46	5	D	ma	MF	W
Multidentorhodacarus sp.2 (Mesostigmata, Rhodacaridae)	39	5	D	ma	MF	W
Eremulus crispus (Oribatida, Eremulidae)	38	3	D	ma	MF	Y
Bimichaelia sp. (Endeostigmata, Alycidae)	37	5	D	ma	MF	W
Oribatida (immature) (sp.23) (Suborder)	37	8	D	ma	MF	W
Berlezetes brasilozetoides (Oribatida, Microzetidae)	36	6	D	ma	MF	W
Eupodes sp.2 (Prostigmata, Eupodidae)	32	5	D	ma	MF	W
Microdispidae (Prostigmata, Heterostigmata)	27	4	D	a	MF	W
Oplitis sp. (Mesostigmata, Uropodina, Oplitidae)	25	3	D	c	F	W
Epilohmannia pallida americana (Oribatida, Epilohmanniidae)	25	6	D	c	F	W
Oribatida (immature) (sp.47) (Suborder)	24	5	D	c	F	W
Prostigmata, Heterostigmata, Pygmephoridae	20	3	D	c	F	Y

Protogamasellus mica (Mesostigmata, Ascidae)	18	5	D	c	F	W
Cultroribula zicsii (Oribatida, Astegistidae)	18	2	D	c	F	Y
Protogamasellus sigillophorus (Mesostigmata, Ascidae)	17	4	D	c	F	W
Asca sp.1 (Mesostigmata, Ascidae)	16	3	D	c	F	W
Lamellobates molecula (Oribatida, Austrachipteriidae)	16	2	D	c	F	Y
Oribatida, Phthiracaridae	16	4	D	c	F	W
Rhizoglyphus sp. (Astigmatina, Acaridae)	15	4	D	c	F	W
Ramusella (*Insculptoppia*) sp. (Oribatida, Oppiidae)	14	1	D	c	F	Z
Scutacarus sp. (Prostigmata, Heterostigmata, Scutacaridae)	14	3	D	c	F	Y
Mesostigmata, Gamasina, Uropodina (sp.2) (Cohort)	13	4	D	c	F	W

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Prostigmata, Bdelloidea, Cunaxidae (sp.1)	13	5	D	c	F	W
Mesostigmata, Gamasina, Ologamasidae, (sp.2)	12	3	D	d	PF	W
Rhodacarellus sp. (Mesostigmata, Rhodacaridae)	12	4	D	d	PF	W
Torpacarus ommitens paraguayensis (Oribatida, Lohmanniidae)	12	1	D	d	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.4) (Cohort)	11	3	D	d	PF	Y
Oribatida (immature) (sp.25) (Suborder)	11	3	D	d	PF	Y
Mesostigmata, Gamasina, Ologamasidae (sp.1)	9	1	D	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.3) (Cohort)	9	1	D	r	PF	Z
Striatoppia sp. (Oribatida, Oppiidae)	9	4	D	r	PF	W
Nanorchestes sp. (Endeostigmata, Nanorchestidae)	8	4	D	r	PF	W
Eohypochthonius sp. (Oribatida, Hypochthoniidae)	8	1	D	r	PF	Z
Oribatida (immature) (sp.3) (Suborder)	8	8	D	r	PF	W
Oribatida (immature) (sp.14) (Suborder)	8	3	D	r	PF	W
Pseudoparasitus sp. (Mesostigmata, Laelapidae)	6	1	D	r	PF	Z
Prostigmata, Heterostigmata, Scutacaridae (sp.1)	5	3	ND	r	PF	Y
Gaeolaelaps sp.1 (Mesostigmata, Laelapidae)	4	2	ND	r	PF	W
Eremobelba zicsii (Oribatida, Eremobelbidae)	4	1	ND	r	PF	Z
Oppiella nova (Oribatida, Oppiidae)	4	1	ND	r	PF	Z
Tectocepheus velatus (Oribatida, Tectocepheidae)	4	2	ND	r	PF	Y
Stigmaeus sp. (Prostigmata, Eleutherengona, Stigmaeidae)	4	2	ND	r	PF	Y
Prostigmata, Tydeidae	4	2	ND	r	PF	Y
Alycus sp. (Endeostigmata, Alycidae)	3	1	ND	r	PF	Z
Proctolaelaps paulista (Mesostigmata, Ascidae)	3	2	ND	r	PF	W
Mesostigmata, Gamasina, Uropodina (sp.1) (Cohort)	3	1	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.7) (Cohort)	3	2	ND	r	PF	Y
Oribatida (immature) (sp.29) (Suborder)	3	1	ND	r	PF	Z
Lohmanniidae, Oribatida	3	3	ND	r	PF	Y

Prostigmata, Tydeoidea, Ereynetidae	3	2	ND	r	PF	Y
Prostigmata, Eupodina, Rhagidiidae (sp.1)	3	2	ND	r	PF	Y
Mesostigmata, Gamasida, Eviphidae	2	1	ND	r	PF	Z
Proprioseiopsis sp.2 (Mesostigmata, Gamasina, Phytoseiidae)	2	1	ND	r	PF	Z
Rhodacarus sp. (Mesostigmata, Rhodacaridae)	2	2	ND	r	PF	Y
Mesostigmata, Trachytidae	2	2	ND	r	PF	Y
Tyrophagus sp. (Astigmatina, Acaridae)	2	2	ND	r	PF	Y
Rysotritia peruensis (Oribatida, Euphthiracaridae)	2	1	ND	r	PF	Z

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Oribatida (immature) (sp.22) (Suborder)	2	1	ND	r	PF	Z
Oribatida (immature) (sp.8) (Suborder)	2	2	ND	r	PF	Y
Quadroppia circumita (Oribatida, Quadroppiidae)	2	1	ND	r	PF	Z
Bdella sp.1 (Prostigmata, Eupodina, Bdellidae)	2	1	ND	r	PF	Z
Prostigmata, Bdelloidea, Cunaxidae (sp.3)	2	1	ND	r	PF	Z
Rhagidia sp.2 (Prostigmata, Eupodina, Rhagidiidae)	2	1	ND	r	PF	Z
Rhaphignatus sp. (Prostigmata, Raphignathidae)	2	1	ND	r	PF	Z
Mesostigmata, Gamasina, Ameroseiidae	1	1	ND	r	PF	Z
Asca sp.2 (Mesostigmata, Ascidae)	1	1	ND	r	PF	Z
Protogamasellus sp.2 (Mesostigmata, Ascidae)	1	1	ND	r	PF	Z
Cosmolaelaps sp.1 (Mesostigmata, Laelapidae)	1	1	ND	r	PF	Z
Typhlodromus sp.1 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Podocinum sp. (Mesostigmata, Podocinidae)	1	1	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.9) (Cohort)	1	1	ND	r	PF	Z
Adelphacarus sp. (Oribatida, Aphelacaridae, Adelphacaridae syn.)	1	1	ND	r	PF	Z
Fosseremus quadripertitus (Oribatida, Damaeolidae)	1	1	ND	r	PF	Z
Brachioppia sp. (Oribatida, Oppiidae)	1	1	ND	r	PF	Z
Bdella sp.2 (Prostigmata, Eupodina, Bdellidae)	1	1	ND	r	PF	Z
Mexecheles sp. (Prostigmata, Eleutherengona, Cheyletidae)	1	1	ND	r	PF	Z
Cryptognathus sp. (Prostigmata, Cryptognathidae)	1	1	ND	r	PF	Z
Mesostigmata, Digamaselidae	1	1	ND	r	PF	Z
Prostigmata, Erythraeidae	1	1	ND	r	PF	Z
Prostigmata, Erythraeidae (sp.) (adult)	1	1	ND	r	PF	Z
Astigmatina, Pyemotidae	1	1	ND	r	PF	Z
Rhagidia sp.3 (Prostigmata, Eupodina, Rhagidiidae)	1	1	ND	r	PF	Z
Prostigmata, Heterostigmata, Scutacaridae (sp2)	1	1	ND	r	PF	Z
Total	1.788					

[1] Dominance: D - dominant, ND - non - dominant. Laroca and Meilke method [26], Moraes et al. [23].
[2] Abundance: ma - very abundant, a - abundant, c - common, d - dispersed, r - rare.
[3] Frequency: PF - little frequent, MF - very frequent, F - frequent.
[4] Constancy: W - constant, Y - accessory, Z - accidental.

3.2 Soil samples from the end of dry season

By the end of the dry season of 2013, the families Acaridae (*Rhyzoglyphus* sp.), Oplitidae (*Oplitis* sp.) and Eupodidae (*Eupodes* sp.1), the suborder Oribatida, the cohort Uropodina (sp.2) and the hypopus phase (cohort Astigmatina, Family Acaridae, genus *Ryzopglyphus* sp.) were the most favored. The oribatid were represented by 28 species and morphospecies and five families. The oribatid *Scherolibates* spp., *Galumna flabellifera*, *Arcoppia aff. dechambrierorum*, *Epilohmannia pallida americana*, *Eremulus crispus*, *Berlezetes brasilozetoides*, *Suctobelbella* sp. and the family Licnodomaeidae were the most representative, being dominant (D), very abundant (ma), very frequent (MF) and constant (W) during the evaluation of the end of dry season (Table 2).

TABLE 2
FAUNISTIC ANALYSIS FOR TAXA OF EDAPHIC MITES COLLECTED BETWEEN THE LINES OF THE COFFEE PLANTATION AND FOREST AT THE END OF THE DRY SEASON OF 2013. SÃO SEBASTIÃO DO PARAÍSO, MG.

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Rizoglyphus sp. (Astigmatina, Acaridae) (Hypopus)	492	17	D	ma	MF	W
Scheloribates sp. (Oribatida, Scheloribatidae)	164	7	D	ma	MF	W
Suctobelbella sp. (Oribatida, Suctobelbidae)	154	7	D	ma	MF	W
Oplitis sp. (Mesostigmata, Uropodina, Oplitidae)	153	16	D	ma	MF	W
Galumna flabellifera (Oribatida, Galumnidae)	133	7	D	ma	MF	W
Oribatida, Licnodamaeidae	118	7	D	ma	MF	W
Rizoglyphus sp. (Astigmatina, Acaridae)	91	6	D	ma	MF	W
Eupodes sp.1 (Prostigmata, Eupodidae)	73	7	D	ma	MF	W
Arcoppia aff. dechambrierorum (Oribatida, Oppiidae)	60	7	D	ma	MF	W
Epilohmannia pallida americana (Oribatida, Epilohmanniidae)	52	8	D	ma	MF	W
Eremulus crispus (Oribatida, Eremulidae)	40	6	D	ma	MF	W
Berlezetes brasilozetoides (Oribatida, Microzetidae)	34	6	D	ma	MF	W
Mesostigmata, Gamasina, Uropodina (sp.2) (Cohort)	32	4	D	ma	MF	W
Prostigmata, Heterostigmata, Pygmephoridae	27	2	D	c	F	Y
Cultroribula zicsii (Oribatida, Astegistidae)	25	5	D	c	F	W
Spelenorchestes sp. (Endeostigmata: Nanorchestidae)	22	6	D	c	F	W
Protogamasellus mica (Mesostigmata, Ascidae)	20	5	D	c	F	W
Tarsonemus sp. (Prostigmata, Heterostigmatina, Tarsonemidae)	20	4	D	c	F	W
Oribatida (immature) sp.47 (Suborder)	19	5	D	c	F	W
Oribatida, Phthiracaridae	18	5	D	c	F	W
Protogamasellus sigillophorus (Mesostigmata, Ascidae)	16	3	D	c	F	Y
Oribatida (immature) (sp. 9) (Suborder)	16	7	D	c	F	W
Ramusella (*Insculptoppia*) sp. (Oribatida, Oppiidae)	16	4	D	c	F	W
Multidentorhodacarus sp.1 (Mesostigmata, Rhodacaridae)	15	5	D	c	F	W
Mesostigmata, Gamasina, Uropodina (sp.3) (Cohort)	15	4	D	c	F	W
Prostigmata, Bdelloidea, Cunaxidae (sp.1)	15	6	D	c	F	W
Gaeolaelaps sp.1 (Mesostigmata, Laelapidae)	13	4	D	c	F	W
Proctolaelaps paulista (Mesostigmata, Ascidae)	13	4	D	c	F	W
Mesostigmata, Gamasina, Uropodina (sp.4) (Cohort)	13	3	D	c	F	W
Mesostigmata, Gamasina, Uropodina (sp.5) (Cohort)	13	4	D	c	F	W
Oribatida (immature) (sp. 23) (Suborder)	13	6	D	c	F	W

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Mesostigmata, Gamasina, Ologamasidae (sp.1)	12	4	D	c	F	W
Rostrozetes foveolatus (Oribatida, Haplozetidae)	12	2	D	c	F	Y
Tectocepheus velatus (Oribatida, Tectocepheidae)	12	3	D	c	F	Y
Winterschmidtiidae, Astigmata	12	2	D	c	F	Y
Striatoppia sp. (Oribatida, Oppiidae)	11	5	D	c	F	W
Bimichaelia sp. (Endeostigmata, Alycidae)	9	4	D	c	F	W
Multidentorhodacarus sp.2 (Mesostigmata, Rhodacaridae)	9	2	D	c	F	Y
Oribatida (immature) (sp.25) (Suborder)	9	4	D	c	F	W
Nothrus aff. monticola (Oribatida, Nothridae)	9	1	D	c	F	Z
Xylobates capucinus (Oribatida, Haplozetidae)	9	2	D	c	F	W
Mesostigmata, Gamasina, Uropodina (sp.1) (Cohort)	8	4	D	d	PF	W
Nanorchestes sp. (Endeostigmata, Nanorchestidae)	7	4	D	d	PF	W
Rhodacarellus sp. (Mesostigmata, Rhodacaridae)	7	2	D	d	PF	Y
Lamellobates molecula (Oribatida, Austrachipteriidae)	7	4	D	d	PF	W
Oribatida, Brachichthoniidae	7	3	D	d	PF	W
Malacoangelia sp. (Oribatida, Hypochthoniidae)	7	1	D	d	PF	Z
Graptoppia sp. (Oribatida, Oppiidae)	7	1	D	d	PF	Z
Neosuctobelba transitoria (Oribatida, Suctobelbidae)	7	3	D	d	PF	W
Cosmolaelaps sp.3 (Mesostigmata, Laelapidae)	6	2	D	r	PF	Y
Mesostigmata, Laelapidae (sp.1)	6	2	D	r	PF	Y
Prostigmata, Heterostigmatina, *Scutacaridae* (sp.1)	6	4	D	r	PF	W
Gaeolaelaps sp.2 (Mesostigmata, Laelapidae)	5	2	ND	r	PF	Y
Hypoaspis sp.1 (Mesostigmata, Laelapidae)	5	2	ND	r	PF	Y
Mesostigmata, Gamasina, Uropodina, Trachytidae	5	2	ND	r	PF	Y
Tyrophagus sp. (Astigmatina, Acaridae)	5	4	ND	r	PF	W
Eremobelba zicsii (Oribatida, Eremobelbidae)	5	2	ND	r	PF	Y
Acrotritia (*Rysotritia*) *peruensis* (Oribatida, Euphthiracaridae)	5	2	ND	r	PF	Y
Oribatida (immature) (sp.14) (Suborder)	5	5	ND	r	PF	W
Microppia minus (Oribatida, Oppiidae)	5	1	ND	r	PF	Z
Mesostigmata, Dermanyssina, Digamasellidae	5	4	ND	r	PF	W
Asca sp.1 (Mesostigmata, Ascidae)	4	3	ND	r	PF	Y
Mesostigmata, Gamasina, Ologamasidae (sp.2)	4	3	ND	r	PF	Y
Proprioseiopsis sp.2 (Mesostigmata, Gamasina, Phytoseiidae)	4	2	ND	r	PF	Y
Rhodacarus sp. (Mesostigmata, Rhodacaridae)	4	2	ND	r	PF	Y
Pseudoamerioppia barrancensis paraguayensis (Oribatida, Oppiidae)	4	3	ND	r	PF	Y

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Rhagidia sp.1(Prostigmata, Eupodina, Rhagidiidae)	4	2	ND	r	PF	Y
Mesostigmata, Gamasina, Eviphidae	3	3	ND	r	PF	Y
Mesostigmata, Gamasina, Uropodina (sp.6) (Cohort)	3	3	ND	r	PF	Y
Astigmatina (Cohort)	3	1	ND	r	PF	Z
Eohypochthonius sp. (Oribatida, Hypochthoniidae)	3	1	ND	r	PF	Z
Tegeozetes sp. (Oribatida, Tectocepheidae)	3	1	ND	r	PF	Z
Prostigmata, Erythraeidae	3	2	ND	r	PF	Y
Protigmata, Heterostigmata, Microdispidae	3	2	ND	r	PF	Y
Prostigmata, Eupodina, Rhagidiidae (sp.1)	3	3	ND	r	PF	Y
Prostigmata, Heterostigmata, *Scutacaridae* (sp.2)	3	2	ND	r	PF	Y
Stigmaeus sp. (Prostigmata, Eleutherengona, Stigmaeidae)	3	3	ND	r	PF	Y
Asca sp.2 (Mesostigmata, Ascidae)	2	2	ND	r	PF	Y
Asca sp.3 (Mesostigmata, Ascidae)	2	2	ND	r	PF	Y
Cosmolaelaps sp.1 (Mesostigmata, Laelapidae)	2	2	ND	r	PF	Y
*Gaeolaelaps s*p.3 (Mesostigmata, Laelapidae)	2	1	ND	r	PF	Z
Hypoaspis sp.3 (Mesostigmata, Laelapidae)	2	1	ND	r	PF	Z
Chelaseius sp. (Mesostigmata, Phytoseiidae)	2	1	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.10) (Cohort)	2	1	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.7) (Cohort)	2	2	ND	r	PF	Y
Mesostigmata, Gamasina, Uropodina (sp.9) (Cohort)	2	1	ND	r	PF	W
Fosseremus quadripertitus (Oribatida, Damaeolidae)	2	1	ND	r	PF	Z
Oribatida (immature) (sp.22) (Suborder)	2	1	ND	r	PF	Z
Oribatida (immature) (sp.29) (Suborder)	2	1	ND	r	PF	Z
Oppiella nova (Oribatida, Oppiidae)	2	1	ND	r	PF	Z
Prostigma, Anystina, Anystidae	2	2	ND	r	PF	Y
Ctenacarus sp. (Oribatida, Ctenacaridae)	2	1	ND	r	PF	Z
Rhagidia sp.3 (Prostigmata, Eupodina, Rhagidiidae)	2	1	ND	r	PF	Z
Scutacarus sp. (Prostigmata, Heterostigmata, Scutacaridae)	2	2	ND	r	PF	Y
Prostigmata, Eleutherengona, Stigmaeidae (sp.1)	2	2	ND	r	PF	Y
Alycus sp. (Endeostigmata, Alycidae)	1	1	ND	r	PF	Z
Mesostigmata, Gamasina, Ameroseiidae	1	1	ND	r	PF	Z
Cosmolaelaps sp.2 (Mesostigmata, Laelapidae)	1	1	ND	r	PF	Z
Stratiolaelaps sp. (Mesostigmata, Laelapidae)	1	1	ND	r	PF	Z
Mesostigmata, Gamasina, Macrochelidae (sp 1)	1	1	ND	r	PF	Z
Pseudoparasitus sp. (Mesostigmata, Laelapidae)	1	1	ND	r	PF	Z

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Neoseiulus sp. (Gamasina, Mesostigmata)	1	1	ND	r	PF	Z
Proprioseiopsis sp.3 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Proprioseiopsis sp.4 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Typhlodromus sp.2 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Podocinum sp. (Mesostigmata, Podocinidae)	1	1	ND	r	PF	Z
Protogamasellopsis sp. (Mesostigmata, Gamasina, Rhodacaridae)	1	1	ND	r	PF	Z
Astigmatina, Histiostomatidae	1	1	ND	r	PF	Z
Oribatida (immature) (sp.3) (Suborder)	1	1	ND	r	PF	Z
Oribatida, Lohmanniidae	1	1	ND	r	PF	Z
Papillacarus sp. (Oribatida, Lohmanniidae)	1	1	ND	r	PF	Z
Oribatida, Oppiidae (sp.)	1	1	ND	r	PF	Z
Brasilobates bipilis (Oribatida, Xylobatidae)	1	1	ND	r	PF	Z
Quadroppia circumita (Oribatida, Quadroppiidae)	1	1	ND	r	PF	Z
Zetorchestes schusteri (Oribatida, Zetorchestidae)	1	1	ND	r	PF	Z
Prostigmata, Caligonellidae	1	1	ND	r	PF	Z
Mexecheles sp. (Prostigmata, Eleutherengona, Cheyletidae)	1	1	ND	r	PF	Z
Prostigmata, Bdelloidea, Cunaxidae (sp.5)	1	1	ND	r	PF	Z
Prostigmata, Tydeoidea, Ereynetidae	1	1	ND	r	PF	Z
Eupodes sp.2 (Prostigmata, Eupodidae)	1	1	ND	r	PF	Z
Rhagidia sp.2 (Prostigmata, Eupodina, Rhagidiidae)	1	1	ND	r	PF	Z
Prostigmata, Eupodina, Rhagidiidae (sp.2)	1	1	ND	r	PF	Z
Prostigmata, Eleutherengona, Stigmaeidae (sp.2)	1	1	ND	r	PF	Z
Total	2.247					

[1]Dominance: D - dominant, ND - non - dominant. Laroca and Meilke method [26], Moraes et al. [23].
[2] Abundance: ma - very abundant, a - abundant, c - common, d - dispersed, r - rare.
[3] Frequency: PF - little frequent, MF - very frequent, F - frequent.
[4] Constancy: W - constant, Y - accessory, Z - accidental.

3.3 Soil samples from the end of rainy season plus end of dry season

In the analysis performed on the total of mites found at the end of the rainy season plus those found at the end of the dry period of 2013, the families Nanorchestidae (*Spelenorchestes* sp.), Oplitidae (*Oplitis* sp.), Acaridae (*Rhyzoglyphus* sp.), Alycidae (*Bimichaelia* sp.), cohort Astigmatina (in the hypopus phase), Eupodidae (*Eupodes* sp.1), Rhodacaridae (*Multidentorhodacarus* sp.1), Pygmephoridae, Tarsonemidae (*Tarsonemus* sp.), the cohort Uropodina (sp.2) and the suborder Oribatida, presented the maximum rates of faunistic classification, dominant (D), very abundant (ma), very frequent (MF) and constant (W), that is, in the sum of the two samples made (Table 3).

Regarding the dominance, it was verified that of the total of 139 Suborder, cohort, family, genus, species collected, 71 were considered dominant (D). Dominant species have the capacity to modify an impact received from the environment for their own benefit, which may lead to the appearance or disappearance of other species [24].

Regarding the classification of abundance, 105 species, subspecies and families were categorized as rare (r). However, even though they are rare, they are important because they have a high influence on the diversity of ecosystems. The substitutions of species and arrangements in the abundance are part of the development of the ecosystem in search of the equilibrium [27].

In the classification of constancy, 90 species, subspecies and families were accidental (Z). Many species presented small numbers of specimens, and these specimens did not present constancy in the samples. The large number of accidental species associated to the high diversity index, show a balanced environment where interspecific and intraspecific competitions can determine species behavior [28].

TABLE 3
FAUNISTIC ANALYSIS FOR TAXA OF EDAPHIC MITES COLLECTED BETWEEN THE LINES OF THE COFFEE PLANTATION AND FOREST AT THE END OF THE DRY SEASON PLUS THOSE FOUND AT THE END OF THE RAINY SEASON OF 2013. SÃO SEBASTIÃO DO PARAÍSO, MG.

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Rizoglyphus sp. (Astigmatina, Acaridae) (Hypopus)	626	23	D	ma	MF	W
Scheloribates sp. (Oribatida, Scheloribatidae)	279	11	D	ma	MF	W
Galumna flabellifera (Oribatida, Galumnidae)	271	11	D	ma	MF	W
Suctobelbella sp. (Oribatida, Suctobelbidae)	250	14	D	ma	MF	W
Oribatida, Licnodamaeidae	223	10	D	ma	MF	W
Oplitis sp. (Mesostigmata, Uropodina, Oplitidae)	178	19	D	ma	MF	W
Eupodes sp.1 (Prostigmata, Eupodidae)	168	14	D	ma	MF	W
Arcoppia aff. dechambrierorum (Oribatida, Oppiidae)	141	12	D	ma	MF	W
Rhizoglyphus sp. (Astigmatina, Acaridae)	106	10	D	ma	MF	W
Spelenorchestes sp. (Endeostigmata: Nanorchestidae)	100	11	D	ma	MF	W
Multidentorhodacarus sp.1(Mesostigmata, Rhodacaridae)	82	11	D	ma	MF	W
Eremulus crispus (Oribatida, Eremulidae)	78	9	D	ma	MF	W
Epilohmannia pallida americana (Oribatida, Epilohmanniidae)	77	14	D	ma	MF	W
Berlezetes brasilozetoides (Oribatida, Microzetidae)	70	12	D	ma	MF	W
Tarsonemus sp. (Prostigmata, Heterostigmata, Tarsonemidae)	68	10	D	ma	MF	W
Oribatida (immature) (sp.9) (Suborder)	62	12	D	ma	MF	W
Oribatida, Brachichthoniidae	61	9	D	ma	MF	W
Oribatida (immature) (sp.23) (Suborder)	50	14	D	ma	MF	W
Multidentorhodacarus sp.2 (Mesostigmata, Rhodacaridae)	48	7	D	ma	MF	Y
Prostigmata, Heterostigmata, Pygmephoridae	47	5	D	ma	MF	Y
Bimichaelia sp. (Endeostigmata, Alycidae)	46	9	D	ma	MF	W
Mesostigmata, Gamasina, Uropodina (sp.2) (Cohort)	45	8	D	ma	MF	W

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Cultroribula zicsii (Oribatida, Astegistidae)	43	7	D	a	MF	Y
Oribatida (immature) (sp.47) (Suborder)	43	10	D	a	MF	W
Protogamasellus mica (Mesostigmata, Ascidae)	38	10	D	c	F	W
Oribatida, Phthiracaridae	34	9	D	c	F	W

Protogamasellus sigillophorus (Mesostigmata, Ascidae)	33	7	D	c	F	Y
Eupodes sp.2 (Prostigmata, Eupodidae)	33	6	D	c	F	Y
Ramusella (Inculptoppia) sp. (Oribatida, Oppiidae)	30	5	D	c	F	Y
Prostigmata, Heterostigmata, Microdispidae	30	6	D	c	F	Y
Prostigmata, Bdelloidea, Cunaxidae (sp.1)	28	11	D	c	F	W
Mesostigmata, Gamasina, Uropodina (sp.3) (Cohort)	24	5	D	c	F	Y
Mesostigmata, Gamasina, Uropodina (sp.4) (Cohort)	24	6	D	c	F	Y
Lamellobates molecula (Oribatida, Austrachipteriidae)	23	6	D	c	F	Y
Mesostigmata, Gamasina, Ologamasidae (sp.1)	21	5	D	c	F	Y
Asca sp.1 (Mesostigmata, Ascidae)	20	6	D	c	F	Y
Oribatida (immature) (sp.25) (Suborder)	20	7	D	c	F	Y
Striatoppia sp. (Oribatida, Oppiidae)	20	9	D	c	F	W
Rhodacarellus sp. (Mesostigmata, Gamasina, Rhodacaridae)	19	6	D	c	F	Y
Proctolaelaps paulista (Mesostigmata, Ascidae)	16	6	D	d	PF	Y
Mesostigmata, Gamasina, Ologamasidae (sp.2)	16	6	D	d	PF	Y
Tectocepheus velatus (Oribatida, Tectocepheidae)	16	5	D	d	PF	Y
Scutacarus sp. (Prostigmata, Heterostigmata, Scutacaridae)	16	5	D	d	PF	Y
Nanorchestes sp. (Endeostigmata, Nanorchestidae)	15	8	D	d	PF	W
Gaeolaelaps sp.1 (Mesostigmata, Laelapidae)	13	4	D	r	PF	Y
Mesostigmata, Gamasina, Uropodina (sp.5) (Cohort)	13	4	D	r	PF	Y
Oribatida (immature) (sp.14) (Suborder)	13	8	D	r	PF	W
Rostrozetes faveolatus (Oribatida, Haplozetidae)	12	2	D	r	PF	Z
Torpacarus ommitens paraguayensis (Oribatida, Lohmanniidae)	12	1	D	r	PF	Z
Winterschmidtiidae, Astigmata	12	2	D	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.1) (Cohort)	11	5	D	r	PF	Y
Prostigmata, Heterostigmata, Scutacaridae (sp.1)	11	7	D	r	PF	Y
Eremobelba zicsii (Oribatida, Eremobelbidae)	9	3	D	r	PF	Z
Oribatida (immature) (sp.3) (Suborder)	9	9	D	r	PF	W
Xylobates capucinus (Oribatida, Haplozetidae)	9	2	D	r	PF	Z
Nothrus aff. monticola (Oribatida, Nothridae)	9	1	D	r	PF	Z
Eohypochthonius sp. (Oribatida, Hypochthoniidae)	8	1	D	r	PF	Z

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	**Number of specimens**	**Number of samples in which was recorded (n = 48)**	**D[1]**	**A[2]**	**F[3]**	**C[4]**
Pseudoparasitus sp. (Mesostigmata, Laelapidae)	7	2	D	r	PF	Z
Mesostigmata, Gamasina, Uropodina, Trachytidae	7	4	D	r	PF	Y
Tyrophagus sp. (Astigmatina, Acaridae)	7	6	D	r	PF	Y
Rysotritia peruensis (Oribatida, Euphthiracaridae)	7	3	D	r	PF	Z
Malacoangelia sp. (Oribatida, Hypochthoniidae)	7	1	D	r	PF	Z

Neosuctobelba transitoria (Oribatida, Suctobelbidae)	7	3	D	r	PF	Z
Graptoppia sp. (Oribatida, Oppiidae)	7	1	D	r	PF	Z
Stigmaeus sp. (Prostigmata, Eleutherengona, Stigmaeidae)	7	5	D	r	PF	Y
Cosmolaelaps sp.3 (Mesostigmata, Laelapidae)	6	2	D	r	PF	Z
Mesostigmata, Laelapidae (sp.1)	6	2	D	r	PF	Z
Proprioseiopsis sp.2(Mesostigmata, Gamasina, Phytoseiidae)	6	3	D	r	PF	Z
Rhodacarus sp. (Mesostigmata, Rhodacaridae)	6	4	D	r	PF	Y
Oppiella nova (Oribatida, Oppiidae)	6	2	D	r	PF	Z
Prostigmata, Eupodina, Rhagidiidae (sp.1)	6	5	D	r	PF	Y
Mesostigmata, Gamasina, Eviphidae	5	4	ND	r	PF	Y
Cosmolaelaps sp.2 (Mesostigmata, Laelapidae)	5	3	ND	r	PF	Z
Gaeolaelaps sp.2 (Mesostigmata, Laelapidae)	5	2	ND	r	PF	Z
Hypoaspis sp.1 (Mesostigmata, Laelapidae)	5	2	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.7) (Cohort)	5	4	ND	r	PF	Y
Oribatida (immature) (sp.29) (Suborder)	5	2	ND	r	PF	Z
Microppia minus (Oribatida, Oppiidae)	5	1	ND	r	PF	Z
Prostigmata, Bdelloidea, Cunaxidae (sp.5)	5	4	ND	r	PF	Y
Alycus sp. (Endeostigmata, Alycidae)	4	2	ND	r	PF	Z
Oribatida (immature) (sp. 22) (Suborder)	4	2	ND	r	PF	Z
Oribatida, Lohmanniidae	4	4	ND	r	PF	Y
Pseudoamerioppia barrancensis paraguayensis (Oribatida, Oppiidae)	4	3	ND	r	PF	Z
Prostigmata, Tydeoidea, Ereynetidae	4	3	ND	r	PF	Z
Prostigmata, Erythraeidae	4	3	ND	r	PF	Z
Rhagidia sp.1(Prostigmata, Eupodina, Rhagidiidae)	4	2	ND	r	PF	Z
Prostigmata, Heterostigmata, *Scutacaridae* (sp.2)	4	3	ND	r	PF	Z
Prostigmata, Tydeidae	4	N	ND	r	PF	Z
Asca sp.2 (Mesostigmata, Ascidae)	3	3	ND	r	PF	Z
Cosmolaelaps sp.1 (Mesostigmata, Laelapidae)	3	3	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.6) (Cohort)	3	3	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.9) (Cohort)	3	2	ND	r	PF	Z

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Fosseremus quadripertitus (Oribatida, Damaeolidae)	3	2	ND	r	PF	Z
Eohypochthonius sp. (Oribatida, Hypochthoniidae)	3	1	ND	r	PF	Z
Astigmatina (Cohort)	3	1	ND	r	PF	Z
Quadroppia circumita (Oribatida, Quadroppiidae)	3	2	ND	r	PF	Z
Tegeozetes sp. (Oribatida, Tectocepheidae)	3	1	ND	r	PF	Z
Rhagidia sp.2 (Prostigmata, Eupodina, Rhagidiidae)	3	2	ND	r	PF	Z

Rhagidia sp.3(Prostigmata, Eupodina, Rhagidiidae)	3	3	ND	r	PF	Z
Mesostigmata, Gamasina, Ameroseiidae	2	2	ND	r	PF	Z
Asca sp.3 (Mesostigmata, Ascidae)	2	2	ND	r	PF	Z
Gaeolaelaps sp.3 (Mesostigmata, Laelapidae)	2	1	ND	r	PF	Z
Hypoaspis sp.3 (Mesostigmata, Laelapidae)	2	1	ND	r	PF	Z
Chelaseius sp. (Mesostigmata, Phytoseiidae)	2	2	ND	r	PF	Z
Podocinum sp. (Mesostigmata, Podocinidae)	2	2	ND	r	PF	Z
Mesostigmata, Gamasina, Uropodina (sp.10) (Cohort)	2	1	ND	r	PF	Z
Oribatida (immature) (sp.8) (Suborder)	2	2	ND	r	PF	Z
Bdella (sp.1) (Prostigmata, Eupodina, Bdellidae)	2	1	ND	r	PF	Z
Prostigmata, Bdelloidea, Cunaxidae (sp.3)	2	1	ND	r	PF	Z
Rhaphignatus sp. (Prostigmata, Raphignathidae)	2	1	ND	r	PF	Z
Prostigma, Anystina, Anystidae	2	2	ND	r	PF	Z
Ctenacarus sp. (Oribatida, Ctenacaridae)	2	1	ND	r	PF	Z
Prostigmata, Eleutherengona, Stigmaeidae (sp.1)	2	2	ND	r	PF	Z
Protogamasellus sp.2 (Mesostigmata, Ascidae)	1	1	ND	r	PF	Z
Stratiolaelaps sp. (Mesostigmata, Laelapidae)	1	1	ND	r	PF	Z
Mesostigmata, Gamasina, Macrochelidae (sp1)	1	1	ND	r	PF	Z
Proprioseiopsis sp.3 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Proprioseiopsis sp.4 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Typhlodromus sp.1 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Typhlodromus sp.2 (Mesostigmata, Gamasina, Phytoseiidae)	1	1	ND	r	PF	Z
Neoseiulus sp. (Gamasina, Mesostigmata)	1	1	ND	r	PF	Z
Protogamasellopsis sp. (Mesostigmata, Gamasina, Rhodacaridae)	1	1	ND	r	PF	Z
Adelphacarus sp. (Oribatida, Aphelacaridae, Adelphacaridae syn.)	1	1	ND	r	PF	Z
Astigmatina, Histiostomatidae	1	1	ND	r	PF	Z
Brachioppia sp. (Oribatida, Oppiidae)	1	1	ND	r	PF	Z
Oribatida, Oppiidae (sp.)	1	1	ND	r	PF	Z
Brasilobates bipilis (Oribatida, Xylobatidae)	1	1	ND	r	PF	Z

Continua…

Continuação

Taxa (Suborder, cohort, family, genus and species)	Number of specimens	Number of samples in which was recorded (n = 48)	D[1]	A[2]	F[3]	C[4]
Papillacarus sp. (Oribatida, Lohmanniidae)	1	1	ND	r	PF	Z
Bdella (sp.2) (Prostigmata, Eupodina, Bdellidae)	1	1	ND	r	PF	Z
Mexecheles sp. (Prostigmata, Eleutherengona, Cheyletidae)	1	1	ND	r	PF	Z
Cryptognathus sp. (Prostigmata, Cryptognathidae)	1	1	ND	r	PF	Z

Mesostigmata, Digamaselidae	1	1	ND	r	PF	Z
Prostigmata, Erythraeidae (sp.) (adulto)	1	1	ND	r	PF	Z
Astigmatina, Pyemotidae	1	1	ND	r	PF	Z
Prostigmata, Eupodina, Rhagidiidae (sp.2)	1	1	ND	r	PF	Z
Zetorchestes schusteri (Oribatida, Zetorchestidae)	1	1	ND	r	PF	Z
Prostigmata, Eleutherengona, Caligonellidae	1	1	ND	r	PF	Z
Prostigmata, Eleutherengona, Stigmaeidae (sp.2)	1	1	ND	r	PF	Z
Total	4.035					

[1]Dominance: D - dominant, ND - non - dominant. Laroca and Meilke method [26], Moraes et al. [23].
[2]Abundance: ma - very abundant, a - abundant, c - common, d - dispersed, r - rare.
[3]Frequency: PF - little frequent, MF - very frequent, F - frequent.
[4]Constancy: W - constant, Y - accessory, Z - accidental.

Oribatid mites generally have little capacity in response short term environmental changes, i.e., their populations decline rapidly when habitats are altered, a feature that may allow their use to detect environmental degradation [29] [30]. Changes in the dominance structure of a soil microarthropods community may be a pre-indicator of environmental stress [31].

The decrease of oribatid mites number in the soil can compromise, in medium and long term, the processes of decomposition and mineralization of organic matter, affecting the quality of the soil and, consequently, the entire ecological system. Mite debris provides a large area for decomposition and, in turn, is an integral part of the soil structure, with direct or indirect effects on the formation and maintenance of soil structure [29].

The species *Oplitis* sp. (Mesostigmata, Uropodina, Oplitidae) was found in all samples, however, this species was most representative at the end of the dry season of 2013. The oribatid mites of the family Oppiidae, which in this work were found both at the end of the dry season and at the end of the rainy season, are considered environmentally insensitive, and are commonly found in disturbed habitats [32].

IV. CONCLUSIONS

There is a difference in the abundance of soil mites when compared the end of the dry period with the end of the rainy season, and the end of the dry period is generally more favorable to the edaphic mite community.

The cohort Astigmatina (family Acaridae) followed by the suborder Oribatida of mites, present the largest numbers of edaphic species, both at the end of the dry period and at the end of the rainy season, and can be worked to be an indicative of the soil quality.

ACKNOWLEDGEMENT

To *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - Coordination of Improvement of Higher Level Personnel-CAPES, and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* - National Council for Scientific and Technological Development - CNPq and for granting scholarships.

To Dr. Pavel Klimov, University of Michigan, USA, for the identification of Acaridae family mites; Dr. Antônio Carlos Lofego, from the State University of São Paulo, Campus of São José do Rio Preto, São Paulo, Brazil, for identification of the mites of the Tarsonemidae family.

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Nondestructive testing of sliding bearings

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***Abstract*— *A validation of electro-physical probing method usage is given for a sliding bearings diagnostic at a boundary friction. Electric circuits and a way of sliding bearings diagnostic, where an analysis of a boundary lubricating layer (BLL) thickness control is carried out on contact resistance parameters indirectly. A sliding bearing lubricating state is defined on previously installed threshold values which achievement defines its running regime.*

***Keywords*— boundary lubrication layer, contact resistance, diagnostic, criteria, phenomenological model.**

I. INTRODUCTION

A diagnosing task of a boundary lubricating sliding bearing mode, containing a shaft and an insert, in the internal-combustion engine (ICE), for example, of all-purpose power unit (APU) at its loading under operating conditions is actual. As a result of forced operations ICE increasing in operation modes, increased requirements are laying claim to engine oils operational properties, therefore creation of the way, allowing to control the state and properties of a boundary lubricating layer (BLL), is rather necessary and actual [1].

The service load and high-speed ICE parameters increasing especially in a high-forced mode (GOST 17479.1-2015) are laying increased requirements to nanometer lubricating layers thickness, i.e. to boundary lubricating layers (BLL). BLL state is a complex value defined by its structural changes and causing many measured tribotechnical parameters (strength, antifrictional, antiscuff, thermo-oxidative stability etc.) which are defined operational properties of lubricating material (LM) in aggregate.

In connection with a degradation of producing LM a problem of their quality assessment is actual. Moreover, before machinery producers is a nagging problem of import on domestic oils replacement with an optimum combination price and quality. On-stream, LM quality analysis is carry out on 15 physical and chemical parameters (GOST 8581-78), basic of which are kinematic viscosity, flash and chilling temperature, a mass fraction of water and mechanical impurities, alkali neutralization number, sulfonate ash content etc. Operational LM properties complex estimation is carried out at benchmark test on driving axles, different installations and internal-combustion engines and also field test [2]. Usually a LM user is interesting in its quality, but not a viscosity class and operational properties which define necessary, but not sufficient service oil conditions in specific installation. Under sufficient conditions we understand not that it is declared in the certificate at LM production, but those real LM characteristics, which define a sliding bearing state at specific load and speed service modes, especially during the moment of ICE start and stop or the forced acceleration in real time.

The work purpose is an estimation of operational properties and thermooxidative oil stability for its replacement age definition, and sliding bearing diagnostic on a boundary lubricating layer state to raise control reliability and its operation modes management in real time.

The method based on registration of a point contact conductivity parameters, was successfully applied by Bowden, Tabor [3], W. H. Abbot [4, 5], M. Antler [6, 7], J. F. Archard [8] for frictional behavior of "dry" materials used in electric contacts to research. Anti-wear properties of noble metals: platinum, gold, silver and their alloys were studied generally. It is installed, that in the absence of lubricants and comparatively low contact pressures the electric current passing promotes wear rising, acceleration of adsorbed films formation and chemical reactions passage on a surface. The results gained by them had qualitative character in the main, without a quantitative estimation of thickness and boundary lubrication continuity.

II. EXPERIMENTAL TECHNIQUE

In the general case conductivity through the molecular scale contact gap can be carried out by means of tunnel effect, thermionic and intrinsic conduction substance of an intermediate layer. The contraction resistance theory and thermionic conductivity are observed in this work [9]. The quantum-mechanical tunnel effect theory in metal-dielectric-metal system is observed for the first time in Sommerfeld and Bethe works [10], concerning to idealized square potential barrier, and has gained further development in the work [9].

In the presence a continuous flash lubricating layer *d* of nanometric thickness in a contact zone, its specific conductance is defined by tunnel conductivity generally [9, 10]:

$$\sigma_{\text{specific}} = \frac{e^2}{h^2 \cdot d}\sqrt{2 \cdot m \cdot \varphi}\, \exp\left(-\frac{4\pi d}{h}\sqrt{2 \cdot m\varphi}\right) \tag{1}$$

where e is an electron charge, m is an electron mass, h is a Planck constant, φ is an electron liberation work, d is an electrode spacing.

Mathematical factors calculation in an expression (1), where *d* is measured in nms (nanometers), and the electron liberation work in eV allows to simplify the expression (1) and to write down it in the form (2):

$$R_t = \left(\frac{10^{-14} d}{a^2 \varphi^{1/2}}\right) \exp\left(10{,}24 \varphi^{1/2} d\right) \tag{2}$$

where φ is an effective electron liberation work, *a* is a contact point radius, *d* is lubricating layer thickness.

In the general case conductivity through the molecular scale contact gap can be carried out by means of tunnel effect, thermionic and intrinsic conduction substance of an intermediate layer. The dependence conductivity analysis from temperature has shown that at temperature tests to ≈ 300° C thermionic and intrinsic conduction in a boundary lubrication rate it is possible to neglect [11]. The experimental data analysis can be carried out, using the theory of tunnel conductivity and the contraction theory, for a contour ground with an indissoluble lubricant layer which thickness is up to ≈ 3 nm [11].

R. Holm studied electric current passage processes in case of point and multiple contacts of conjugate objects. In a basis of voltage drop measuring on a searched object, having unknown contact resistance R_c a 4-wiring circuit (figure 1) [9] has been accepted.

E is a current source, R_1 is calibrating resistance; R_2 is a resistance box; R_c is contact resistance; V is a voltmeter; ADT is an analog digital transducer; PC is a personal computer.

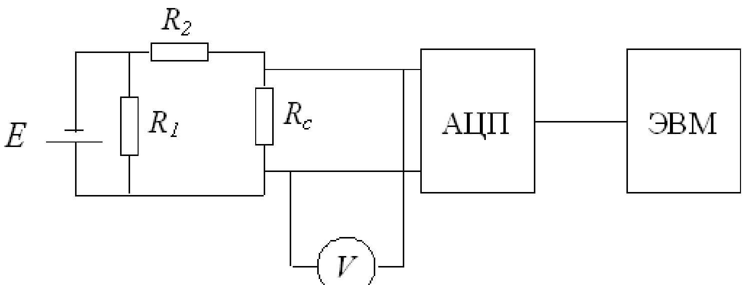


FIGURE 1. 4-WIRING CIRCUIT OF CONTACT RESISTANCE REGISTRATION.

Conjugate objects greasing point contact state analysis with using of all possible alternatives, given in the figure 1, namely: metallic; mixed or cluster and sieve; indissoluble lubricant layer was carried out in the beginning. Each alternative has its type, the electric conduction value accordingly and it is calculated on the basis of matching mathematical expressions [12]. It is necessary to calculate an actual contact point radius for a quantitative estimation of BLL nanometer thickness. The contact point radius is estimated on the basis of Hertz theory relationships for objects elastic (3) deformations; it is defined by loading value, objects' mechanical properties and their geometrical sizes [12]:

$$a = 1.11(NR/E^*)^{1/3} \tag{3}$$

where N is a loading, r is conjugate objects effective radius; E* is an effective conjugate objects elastic modulus. At calculation of BLL thickness it is necessary to consider also, that an effective electron liberation work φ at BLL thickness less than 1.5 nm is 2.025 eV, and at 2.0–3.0 nm is 1.8 eV [11, 12].

An effective elastic modulus E* and a radius R are calculated from correlations (4) and (5) [13].

$$1/E^* = (1-\nu_1^2)/E_1 + (1-\nu_2^2)/E_2 \tag{4}$$

where E_1 and E_2 are elastic modulus, and ν_1 and ν_2 are both conjugate objects Poisson's ratios. At contact of two spheres with radiuses R_1 and R_2, an effective radius R, using for a calculation, is defined from correlation [13]:

$$\frac{1}{R} = \frac{1}{R_1} \pm \frac{1}{R_2} \tag{5}$$

where R_1 and R_2 are conjugate objects radiuses, we take the sign plus (+) at convex objects contact, and the sign minus (-) at the cylinder and the matching cylindrical cavity contact [13]. The roller width made ≈ 0.01 m. As a roller and a segment are executed from one material, steel 45, and a ball from steel ШХ 15, we considered an effective module value equal to the elastic steel modulus value ($E \approx 2.6 \cdot 10^{11}$ Pa). The calculated effective radius *R* for the circuit roller-sphere makes $3.45 \cdot 10^{-3}$ m.

Parameters calculation contact (table 1) data with a theory of Hertz correlation using are given in table 1 [14].

TABLE 1
CONTACT PARAMETERS CALCULATION FOR THE CIRCUIT DESIGN ROLLER-SEGMENT (/), ROLLER-PLANE (/) AND ROLLER-SPHERE

N, H	a $*10^{-6}$, m	p_{cp}, MPa	R_s, mOm	$R_{ок}$, mOm	R_c, mOm
20	155/ 15.7/ 71	6.5/ 64/ 1300	1/ 9.6/ 2.1	0.3/ 3.2/ 63	1.3/ 12.8/ 65
100	346/ 35/ 122	14.5/ 143/ 2100	0.4/ 4.3/ 1.2	0.1/ 1.4/ 21.4	0.5/ 5.7/ 22.6
200	490/ 49.5/ 154	20.4/ 202/ 2700	0.3/ 3/ 0.9	0.1/ 1/ 13.5	0.4/ 4/ 14.4
400	693/ 70/ 194	28.9/ 286/ 3400	0.2/ 2.1/ 0.8	0.07/ 0.7/ 8.5	0.7/ 2.8/ 9.3
600	849/ 85.7/ 222	35.3/ 350/ 3900	0.18/ 1.8/ 0.7	0.06/ 0.6/ 6.5	0.24/ 2.4/ 7.2
800	980/ 99/ 244	40.8/ 404/ 4300	0.15/ 1.5/ 0.6	0.05/ 0.5/ 5.4	0.20/ 2.0/ 6
1000	1100/ 111/ 263	45.7/ 452/ 4600	0.14/ 1.4/ 0.57	0.04/ 0.45/ 4.6	0.18/ 1.9/ 5.2
1200	1200/ 121/ 279	50.0/ 495/ 4900	0.13/ 1.2/ 0.54	0.04/ 0.04/ 4.1	0.17/ 1.2/ 4.6
1400	1300/ 131/ 294	54.0/ 535/ 5200	0.12/ 1.15/ 0.51	0.04/ 0.04/ 3.7	0.16/ 1.2/ 4.2
1600	1390/ 140/ 307	57.7/ 571/ 5400	0.11/ 1.1/ 0.49	0.04/ 0.04/ 3.4	0.15/ 1.1/ 3.9
1800	1470/ 149/ 319	61.2/ 606/ 5600	0.10/ 1/ 0.47	0.03/ 0.03/ 3.1	0.13/ 1.1/ 3.6
2000	1550/ 157/ 331	64.5/ 639/ 5800	0.10/ 1/ 0.45	0.03/ 0.03/ 2.9	0.13/ 1/ 3.4

**** Notes. The real contact point radius (a), the actual average (p_{cp}) value, the contact contraction resistance (R_s), the oxide film ($R_{ок}$) and the recorded contact resistance (R_c) value. In each column row three figures are given through the slash. The first figure matches for the circuit roller-segment, the second figure matches for the circuit roller-plane and the third figure matches for the roller-sphere circuit.***

Lubricated contact interfaces models are given in the figure 2.

А) $R_s = \rho/2a$; Б) $R_s = \rho/2n\alpha$; В) $R_f = \frac{10^{-14}\, d}{\alpha^2 (\varphi)^{1/2}} \exp(10{,}24\, (\varphi)^{1/2} d)$

OXIDIZED METAL

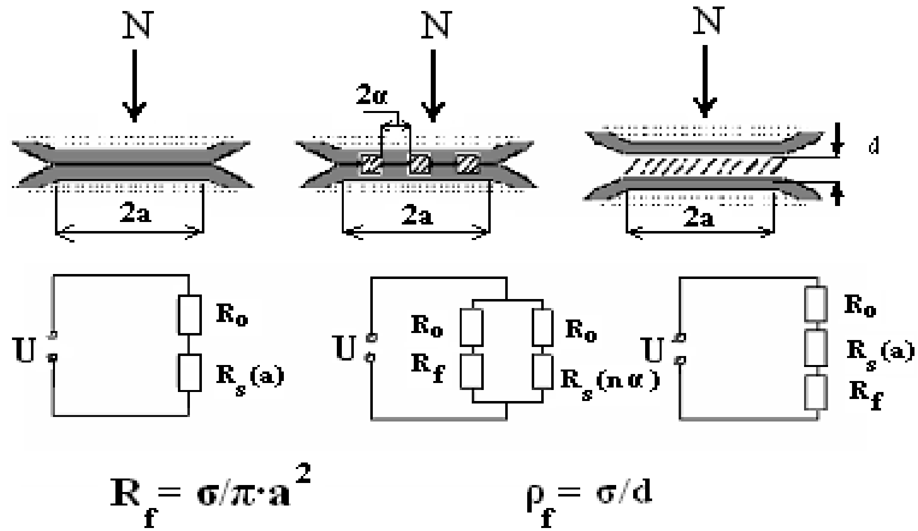


FIGURE 2. LUBRICATED CONTACT INTERFACES MODELS

The passage from point to multiple contact at oils scoring resistance analysis of the various nature and functionality (hydraulic, motor, transmission, geared) was carried out with the flow-chart using, given in the figure 3.

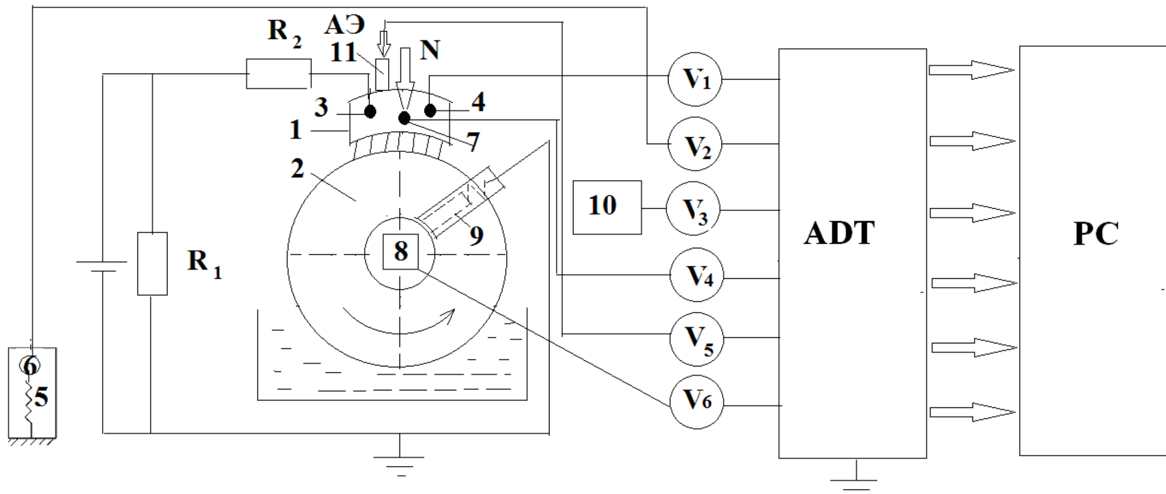


FIGURE 3. Flow chart device, where 1 is a backplate in a segment form; 2 is a mobile electrode in a roller form; 3 are current electrodes; 4 are potential electrodes; 5 is a load node; 6 is a load cell; 7 is a thermocouple; 8 is an inductive sensor for a friction torque measurement; 9 is spring-loaded copper-graphite brush; 10 is a drive with a velocity sensor; 11 is an acoustic emission sensor; ADT is an analog digital transducer; PC is a personal computer.

III. RESULTS AND DISCUSSION

A recorded contact resistance value (R_c) at experiment execution is equal to the contraction resistance sum R_s and oxide film $R_{ок}$. Calculated resistance values are given in the table 1. It is necessary to note, that the average contact pressure codomain changed within: 6.5 – 64.5 MPa – for the circuit roller-segment; 64 – 639 MPa – for the circuit roller-plane; 1.3 – 5.8 GPa – for the circuit roller-sphere.

Recorded contact resistance decreasing in experiment to level values characteristic for contraction resistance (Rs), given in the table 1, means BLL destruction on real contact points and presence of "dry" metal contact with a subsequent mating surfaces of the roller and the segment gripping.

In works [15, 16] operating modes at a step radial loading rolling bearings depending on a lubrication condition and structure of a steel surface are experimentally defined. It is shown, that the one cycle time period of a metal surface reinforcement and destruction is in many aspects defined by high-speed loading conditions [17], physical and chemical lubricant nature [17] and, as consequence, BLL tribological properties and structural changes kinetics accumulation [18-20]. The most typical

sliding bearings and rolling bearings diagnostics difference is that at loading on the shaft increasing or in a turbine start and stop period or the forced ICE power increasing a regime with an aggravation at which hydrodynamic, and then a boundary lubricant layer destruction are accompanied by catastrophic jamming and the sliding bearing destruction with all consequences can occur in result.

The calculation analysis dependence on tunnel resistance (R_t), and BLL nanometric thickness, and a real contact area shows, that at loading increasing by two orders the actual contact area changes by one order, and the contribution from the BLL thickness in calculated value Rt increases by ten orders [21]. The basic contribution in the tunnel resistance calculated value is brought the BLL thickness, but not the real contact area, that allows estimating mechanical and frictional properties of boundary lubricant layers (BLL) at elastic contact interacting of conjugate objects at their relative movement. The last has defined a possibility to use an electrophysical probing method for an antiscuff various functionality oils properties estimation: motor [22], transmission [14], hydraulic [23], geared [21], etc.

Modeling tests on BLL formation and destruction kinetics, with friction machine CMT-1 using with step load increment have been made for criterion development. The circuit roller-segment was used in the experiment where the roller (Ст 45) modeled the shaft, and the segment (Ст 45) modeled the support insert. Linear roller rotational velocity made 0.5 m/s, the segment area was $2{\cdot}10^{-4}$ m^2.

Let's instance antiscuff and operational properties of hydraulic oils features estimation (figure 4) [23]. The roller was located in a tray with analyzed oil before a test operation. One operational class hydraulic oils were test subjects on classification API: ZF-46 (ТУ 0253-014-44918199-2005); МГЕ-46В (ТУ 38.001347-2000); HVLP-46 (ТУ 0253-028-44918199-2006); HLP-46 (ТУ 38.301-41-180-2001).

The contact resistance value R_c between the shaft and the insert, measured on the four-wire circuit (figure 1) [9] has been chosen as measured sliding bearing lubrication parameter state at a boundary friction diagnostic. It is caused by that the BLL thickness estimation is carried out on contact resistance R_c value indirectly.

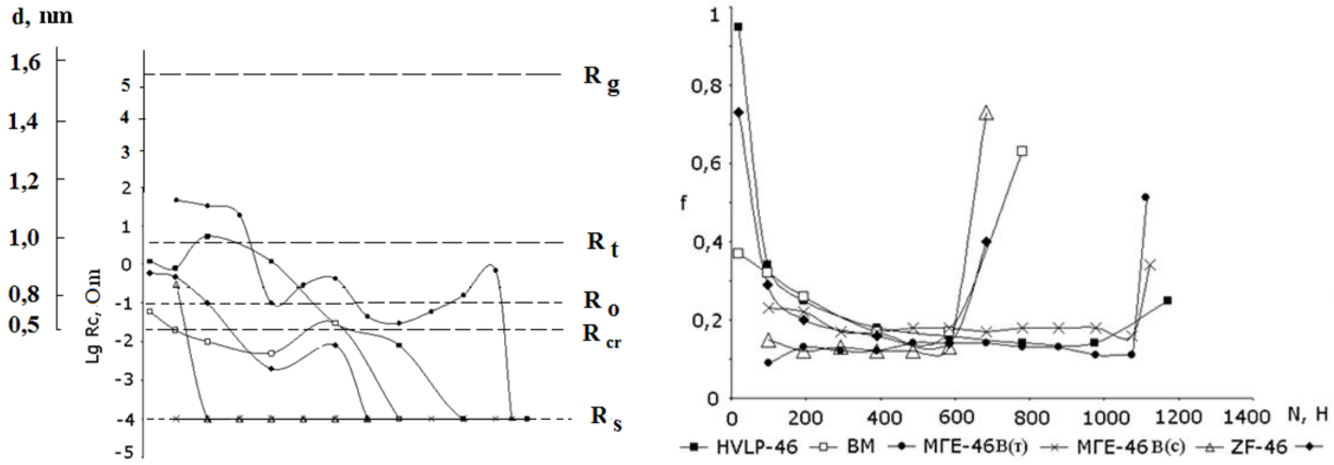


FIGURE 4 – A contact resistance (R_c) and a friction factor (f) on loading (N) dependence.

The estimation results generalization of antiscuff oils durability various functionality (motor, transmission, hydraulic, geared, etc.) by influence on them external load and high-speed factors are presented in the figure 5. It is experimentally installed, that a boundary lubricant layer [24] presented, for example, by engine oil, and metal conjugate objects surfaces of the tribosystem under some load and high-speed (P, V) factors influence will occupy some equilibrium and stable structural states characterized by certain points (O; A; B; C; D; K) on the circuit (figure 5) [14]. Each structural section surface condition causes a certain contact resistance (R_c) dependence, i.e. each point O, A, B,… will match its average contact resistance (R_c, about; $R_{c,\,A}$; $R_{c,\,B}$; $R_{c,\,C}$; $R_{c,\,D}$; $R_{c,\,K}$) value level. Let there is some minimum load and high-speed influence (for example, a friction knot idling) on an interface which is characterized by O point. Then with electrophysical probing parameters using we will develop diagnostic estimation criterion of lubricants antiscuff properties at step loading which is applied at an estimation of engine oils antiscuff properties. The given complex criterion can be used only at registration, external load and high-speed factors as well as internal tribosystem factors, for example, a contact resistance (R_c), characterizing structural changes of conjugate objects interface.

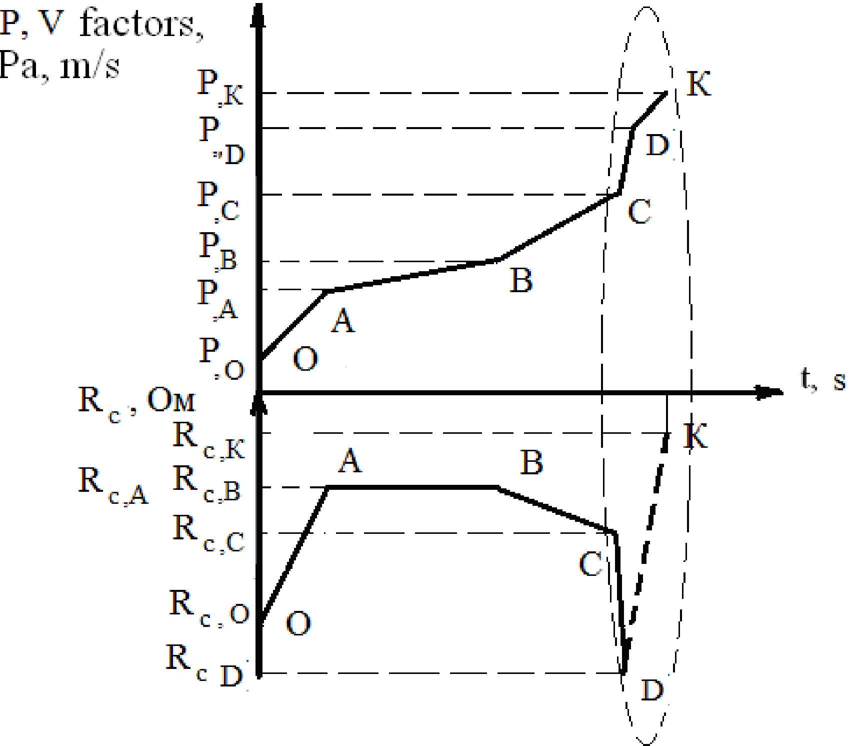


FIGURE 5. An external load and high-speed (P, V) factors on tribosystem depending on a time (t), where structural section surface conditions are characterized by points (O, A, B, C, D, K) with matching to them contact resistance (R_c) levels.

The diagnostic invariant criterion is developed, which does not depend on load and high-speed modes or conjugate objects contact circuit (point, multiple) [14, 25]. The criterion consists in the following:

1. The BLL self-organizing mode occurs in the real pressure to ≈ 35 MPa field and is characterized by connection BLL molecules with a surface transition from a physical adsorption to stronger chemical adsorption. BLL structure change is accompanied by increasing its thickness and, as consequence, rising contact resistance R_c level. The recorded contact resistance (R_c) to an initial metal surface (without lubricant) relation with an oxide film ($R_{ок}$) $R_c/R_{ok}>> 1$ resistance characterizes the BLL state in which the polymolecular component remains;

2. The BLL dynamic equilibrium mode. In the formation and mechanical destruction chemisorbed layer process occurs dynamic equilibrium in time. The polymolecular BLL component remains in this case, and the layer thickness by reason of its wear decreases a little in comparison with the first mode. The chemisorbed layer modulus can attain the value ≈ 1.4 GPa, that is comparable, on the order value with a rubber elasticity modulus (≈ 5 GPa). Resistance values relation is more than one, i.e. $R_c/R_{ok}> 1$ for this mode;

3. Physical and chemical processes complex (mechanodestruction, thermodestruction, etc.) causes decreasing values of contact resistance level in view of BLL wearing. In this case it is possible to assume, that a polymolecular BLL component is destroyed, and the monomolecular layer component ≈ 0.5 nm (hydrocarbon molecules cross-section size) remains. In view of the fact that an oxide film contact resistance value is comparable with a tunneling conductance value for the given layer thickness, the resistance values relation becomes about one, i.e. $R_c/R_{ok} \approx 1$;

4. At the friction critical behavior, score forestalling, the monomolecular BLL component is destroyed, that proves by the further contact resistance value decreasing. At the same time, depending on the metals plastic deformation nature, two variants can occur: the intensive surface oxidation accompanied, in the running-in period of surfaces interaction, by contact resistance level by two-three orders increasing, with a subsequent an elastic energy surface layer accumulation and developed dislocation structure formation with the subsequent surface layer destruction; surface layer destruction and a juvenile surface uncovering without preliminary its intensive oxidation. For the given mode recorded contact resistance R_c decreasing to contraction resistance (R_s) values level is characteristic. The recorded contact resistance (R_c)

to contraction resistance (R_s) relation becomes equal about one, i.e. $R_c/R_s \approx 1$. In a knot operating mode occurs, local in a time, the friction surfaces gripping accompanied debris formation in a contact zone and conjugate surfaces separating by them. At the same time, fluctuations R_c level increases sharply to an upper limit measurement (the boundary line is set by electrical circuit and set current source parameters). Friction torque value and temperature increase sharply at this time.

The given criterion can be used for a forecasting conjugate objects surface section lubrication state. The developed criterion is in a basis of control algorithms conjugate metal objects surface section state in working friction knots conditions (rolling and sliding bearings etc.), that is important for diagnostic and management by their operation modes.

Let's instance the developed actuality criterion and the way presented in the article, for a diagnostic internal-combustion engine by "Mercedes" manufactured, type OM 502, operated in composition with a fodder harvester КВК 8060. At a high-cycle spring compression and releasing which end rested against a valve cover, there was a fatigue failure of its end cover and spring releasing. The last has led to engine oil supply in the motor trouble. A lack of liquid-film lubrication rate or a hydrodynamic regime violation has led to a piston flashing in the cylinder, to inserts crank and crankshaft jamming (Figure 6).

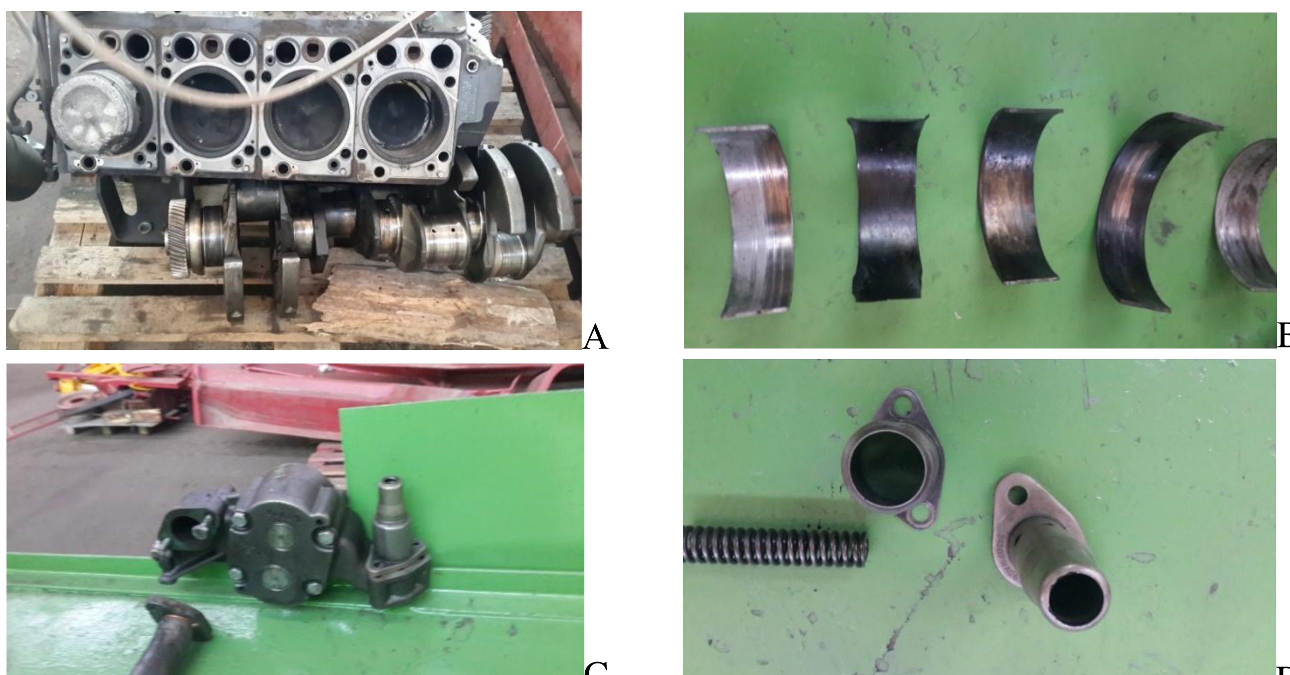
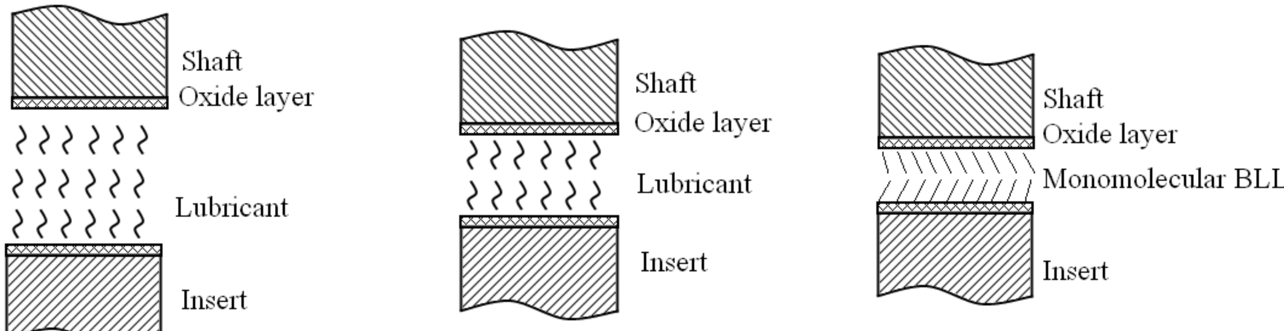


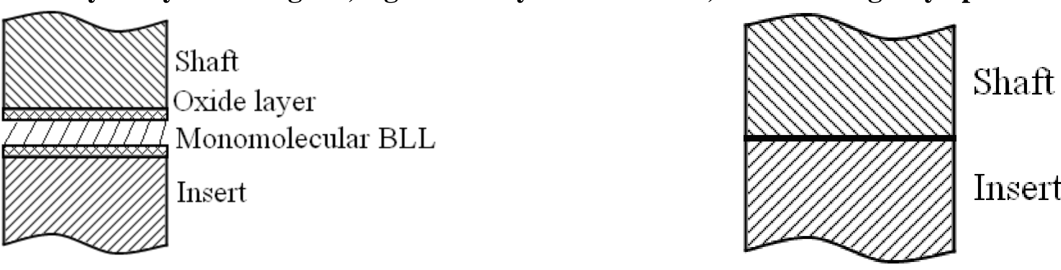
FIGURE 6. An aspect of the jammed engine at its dismantling: A – the system cylinder-piston and the crankshaft with necks (inserts); B – necks or crankshaft inserts; C – the valve controlling oil supply in the engine; D – the valve cover destruction.

An experimental data values analysis of the contact resistance at load step increasing has allowed us to mark out four boundary operating modes of sliding bearings connected with its lubrication state (figure 7) [26]:

- the hydrodynamic regime: a lubricant multilayer [27] between the shaft and the sliding bearing is "enough thick";
- the boundary friction mode: a lubricant multilayer is between the shaft and the sliding bearing and the tunneling electric conductance is realized between the shaft and the bearing;
- the preemergency operation: the bearing is in a boundary friction mode with conservation of several monomolecular BLL. The tunneling conductance occurs;
- the critical behavior: conservation is minimum possible on the thickness BLL monomolecular ≈ 0.3 nm with the maximum possible real contact lapped area;
- the bearing destruction: BLL absence, testifying to "dry" metal contact, with oxide films destruction between the shaft and the sliding bearing which is accompanied by the scoring and conjugate surfaces gripping.



Hydrodynamic regime, Rg Boundary friction mode, Rt Preemergency operation, Ro



Critical behavior, R_{cr} **Destruction, Rs**

FIGURE 7. Sliding bearings operating modes

Comparison of recorded contact R_c value with theoretically counted threshold values R_g, R_t, R_o, R_{cr}, R_s (fig. 4) characterizes conjugate objects lubrication state. The developed invariant test to rolling and sliding bearings using allows elaborating a way of the lubrication state control [14]. The criterion is based on comparison of measured contact resistance R_c value with theoretically counted contact resistance R_g, R_t, R_o, R_{cr}, R_s threshold values characterizing the sliding bearings operating modes observed above.

We measured a contact resistance R_c quantity in the course of experiment at the step loading and we recorded kinetics of its changing during 300 s. for each loading value, that was necessary for stabilization of physical and chemical processes passing in BLL [28]. The measured value R_c was compared with counted threshold values R_s, R_{cr}, R_o, R_t, R_g, and we defined the bearing lubrication mode.

Contact resistance R_c and friction coefficient *f* from loading *N* dependences are presented in the figure 3, received contact resistance threshold values are shown by dashed lines. A correlation between friction coefficient from loading and measured value contact resistance R_c dependence is visually presented in the figure 3. Hydraulic oil of marketable delivery МГЕ-46В (ТС 38.001347-00) was the object of research.

We accept a boundary lubricant layer thickness matching to transition from a hydrodynamic friction to a boundary friction mode – 2.0 nm for testing oil, and the boundary lubricant layer thickness is matching to the monomolecular lubricant minimum thickness layer – 1 nm.

Contact resistances R_g and R_t values, matching to hydrodynamic and boundary sliding bearings operating modes and differing by lubricant layer various values d thickness are calculated with a formula (1).

We counted by formula (6) the contact resistance R_o value, installing the contact resistance boundary line of metal contact with an accounting oxide films presence and corresponding to emergency bearing operation mode:

$$R_o = \sigma/\pi a^2 \qquad (6)$$

where σ is a sheet specific resistance, a is a real contact point radius.

Considering that the sheet resistivity level defined from reference data, can differ considerably for really used contact materials surfaces with various process technology (carburizing, nitriding etc.), the contact resistance R_o value corresponding

to metal contact with a glance oxide films presence, it is necessary to measure in a statics at a bearing loading under a dead load in lubricant absence.

We defined R_{cr} experimentally at BLL monomolecular component destruction as it was possibly it to count, but its calculated value will be equal ≈ R_o in signification. We defined the R_{cr} value experimentally at monomolecular BLL component destruction. It is experimentally installed, that a voltage drop decreasing to ≈ 2 – 3 mV means the BLL monomolecular component destruction.

The resistance contraction R_s value installing a contact resistance boundary line of metal contact with a glance mechanical materials properties of which the bearing is made, it is necessary to calculate the radius of a real contact point (*a*) and the actual contact area (*S*) for the specific bearing circuit, proceeding from the classical Hertz theory relationships (7).

$$R_s=\rho/2a \quad (7)$$

where ρ is an electric specific resistance, a is a real contact point radius.

We have theoretically counted threshold values R_g, R_t, R_o, R_{cr}, R_s and have obtained R_g = 166722 Om, R_t = 5 Om, R_o = 0.1 Om, R_{cr} = 0.04 Om, R_s = 0.001 Om (fig. 4).

We measured contact resistance R_c at a step segment loading on a roller for tested oil and recorded kinetics of its change in the course of 300 s. on each loading step. We compared recorded $\mathbf{R_c}$ value and counted threshold values R_g, R_t, R_o, R_{cr}, R_s (fugure 4). It is necessary to note, that R_g value at experiment executing has not been attained, as in the loadings field realized on the friction test machine CMT-1, a boundary friction mode become at once.

We are not following results (figure 4) for hydraulic oil marketable delivery of the brand МГЕ-46В. The measured value R_c = 33 Om was for loading 200 N.

We have compared the gained value and counted threshold values and as $\mathbf{R_t}$ <$\mathbf{R_c}$ <$\mathbf{R_g}$ (5 Om <33 Om <166722 Om), we conclude, that the bearing is in a boundary friction mode, the BLL thickness makes more than 1 nm, i.e. is in a normal running regime.

The measured value $\mathbf{R_c}$ = 5 Om is for loading 350 N. We have compared the got value and counted threshold values and as $\mathbf{R_c}$ = $\mathbf{R_t}$ (5.2 Om = 5 Om), we conclude, that the bearing is in a boundary friction mode, the BLL thickness makes 1 nm, i.e. is in a normal running bearing regime.

The measured value $\mathbf{R_c}$ = 0.43 Om is for loading 600 N. We have compared the got value and counted threshold values and as R_o <R_c <R_t (0.2 Om <0.43 Om <5 Om), we conclude, that the bearing is in a boundary friction mode, the BLL thickness is less than 1 nm, but BLL keeps its integrity.

The measured value $\mathbf{R_c}$ = 0.2 Ohm is for loading 650 N. We have compared the got value and counted threshold values and as R_c = R_o (0.2 Om ≈ 0.1 Om), we conclude, that the bearing passes in the preemergency operation mode at which there is a boundary lubricant layer destruction and an electric conduction appearance through real points of metal contact with a glance of oxide films.

The measured value $\mathbf{R_c}$ = 0.03 Om is for loading 800 N. We have compared the got value and counted threshold values and as $\mathbf{R_s}$ <$\mathbf{R_c}$ <R_{cr} (0.001 Om < 0.04 Om <0.1 Om), we conclude, that the bearing is in an emergency operation mode at which a boundary lubricant layer monomolecular component destruction and points with metal contact predominance occurs.

The measured value $\mathbf{R_c}$ = 0.0001 Om is for loading 1120 N. We have compared the got value and counted threshold values and as R_c = R_s (0.0001 Om), we conclude that conjugate surfaces grabbing regime of sliding bearing occurs.

To control developed criteria we created the stand simulating plain bearings work, with recorded parameters output to the computer (figure 8).

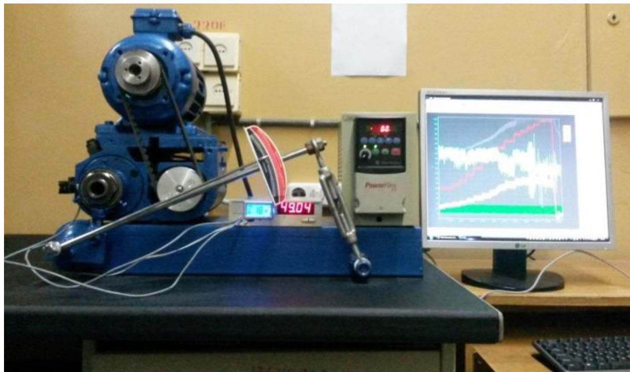


FIGURE 8. Stand to diagnose plain bearings lubrication state

The basic technical features: a test category – friction; loading and force measurement – mechanical; force measurement range – 20... 4000 N; the force dynamometer makes 2 %; the frictional torque type gauge is electronic; a frictional torque error measurement makes 3 %; rpm measurement range is – 15... 3600 min^{-1}; a power consumption is no more than 1 kw; overall dimensions are: 700x450x500 mm; weight is 43 kg.

The stand contains an adjustable electric drive, power expenses measurement device, real frictional unit equipped with a boundary lubricant layer state electrophysical control circuit (hydrodynamic or hydrostatic journal bearing), hydrostation for lubricant supply in frictional units and adjustable loading. The stand circuit is presented in the figure 9.

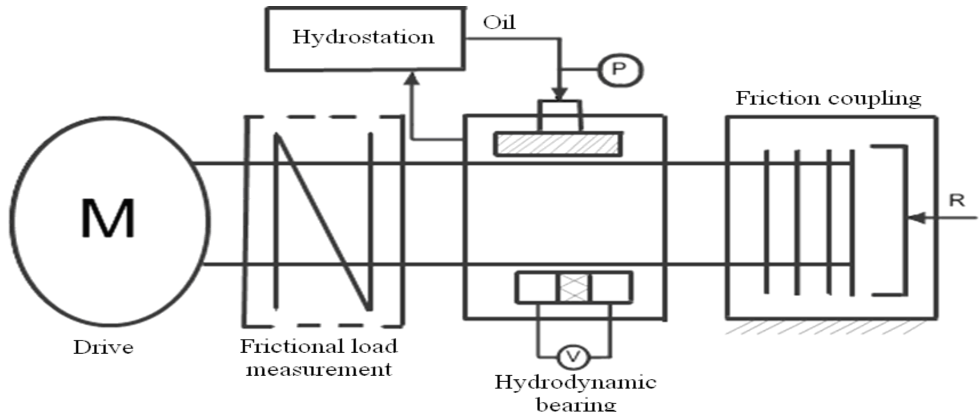


FIGURE 9. Stand circuit for plain bearings diagnostic

One of electrical schematics for voltage drop between the shaft and one of isolated sliding measurement is presented in the figure 10, where N is a loading on the shaft, and I are electric current lines between the shaft and the sliding bearing.

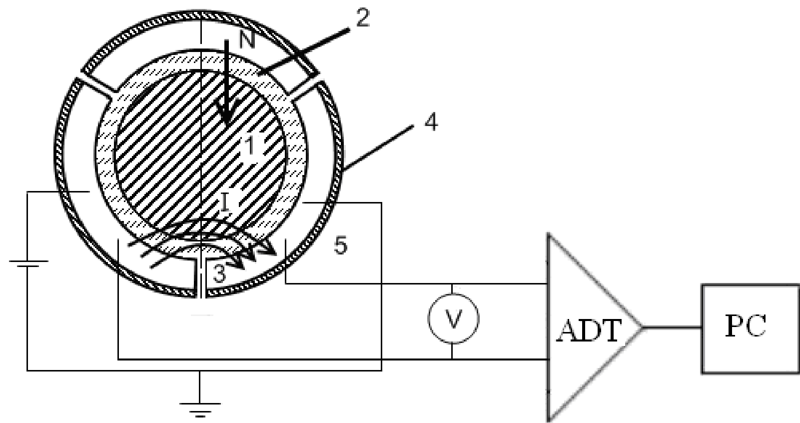


FIGURE 10. Sliding bearing electrophysical probing circuit where 1 is a shaft, 2 is lubricant, 3 is an insert, 4 is an insert isolation, 5 is a bearings case

Another electric circuit for voltage drop between the earthed shaft and one of dielectric isolated sliding bearing measurement is presented in the figure 11.

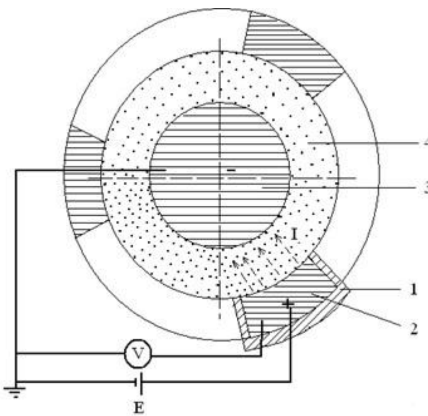


FIGURE 11. Circuit for plain bearing diagnostic on a boundary lubricant layer state, where 1 is dielectric, 2 is a hydrodynamic bearing, 3 is a turbine rotor, 4 is a physical wedge, E is EDS = 50 mV, V is a voltmeter, I are streamlines.

Other circuits for voltage drop between the shaft and sliding bearings measurement are possible also.

We used as tested oils turbine oil ТП-22С and transmission oil ТАД-17и also. These oils have a wide practical application at turbines operation in RUP «Gomelenergo» and in drive train components of fodder and grain harvesters, produced by JSC "Gomselmash". Estimation results of antiscuff and operational oils properties are presented in the figure 12.

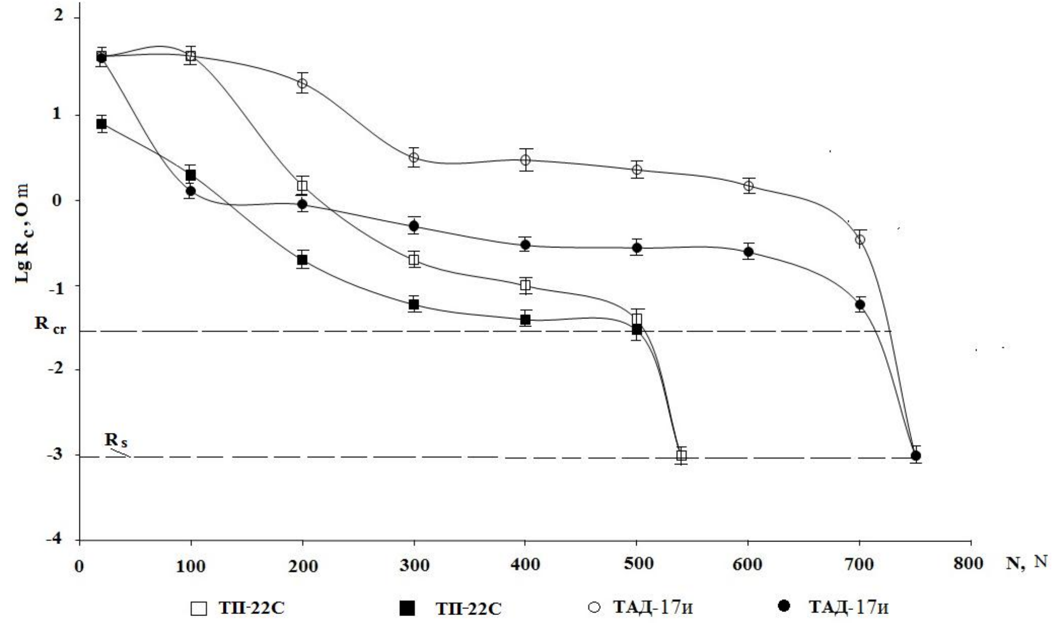


FIGURE 12. Contact resistance on loading for turbine oil ТП-22С and transmission oil ТАД-17и dependence

It is installed as a result of tests, that theoretically prognosticated contact resistance criterion levels defining sliding bearings lubrication state, completely have found practical evidences. In case of the shaft dry metal contact (Ст 45) and the bronze insert or a sliding bearing (БрОЦС-5-5-5) a voltage drop between them made ≈ 0.1 mV. It is experimentally installed at step load increasing, that voltage drop decreasing to ≈ 2 – 3 mV means monomolecular BLL component destruction, presented both turbine, and transmission oils. After achievement the given critical value R_{cr} conjugate surfaces grabbing and an electric motor rotating the shaft jamming were noted. The last was accompanied by spasmodic decreasing of recorded contact resistance to contraction resistance.

IV. CONCLUSION

Electro-physical probing methods using foundation is given for sliding bearings operation modes estimation on BLL state. A calculation dependence analysis of tunnel resistance (R_t) from BLL nanometric thickness and the real contact area shows, that at load increasing by two orders the real contact area changes by one order, and the contribution from a BLL thickness in

design value R_t increases by ten orders. The BLL thickness brings a basic contribution in the calculated value tunneling resistance, instead of real contact area that allows to estimate mechanical and frictional properties of boundary lubricant layers (BLL) at elastic contact interacting of conjugate objects at their relative moving.

Electric circuits and methods, allowing estimating BLL operational properties, are developed. This method allows to test BLL formation and destruction kinetics in real sliding bearings direct at their operation.

On the experimental data analysis basis an antifrictional and antiscuff oils and plastic lubricants of a various functional purpose properties estimation (transmission, motor, hydraulic, geared), the recorded contact resistance value relatively to an initial metal surface resistance value or contraction resistance is developed the diagnostic estimation state criterion of metal interface, namely at BLL formation and its self-organizing $R_c/R_{ok}>> 1$ at a dynamic equilibrium between BLL formation and destruction $R_c/R_{ok}> 1$, BLL destruction $R_c/R_{ok} \approx 1$, metal interface destruction $R_c/R_s \approx 1$. The developed criteria are the basis for creating phenomenological models for predicting the state of lubrication of the interface surface of conjugated bodies during rolling and sliding. These criteria are laid in the basis of algorithms for monitoring the state of the interface of conjugated metal bodies under the conditions of rolling and sliding bearings, which is important for the diagnosis and management of their operation modes.

Contact resistance threshold, on which values it is possible to estimate sliding bearings operation modes, connected with its lubrication state, namely, hydrodynamic regime R_g, boundary friction mode R_t with polymolecular BLL conservation, preemergency operation with monomolecular BLL (R_o) conservation, emergency operation mode (R_{cr}) with very thin (≈ 0.3 nm) monomolecular BLL conservation, destruction mode (R_s) are counted. Recorded contact R_c value with theoretically counted threshold values (R_g, R_t, R_o, R_{cr}, R_s) comparison characterizes conjugate objects lubrication state. The proposed way allows to estimate by means of nondestructive testing sliding bearing lubrication state at boundary friction that gives a chance, depending on conjugate surfaces lubrication state to introduce amendments in its operation modes, to define oil replacement age and thus to provide reliability and durability of its work.

Thus, the electro-physical probing method using foundation for sliding bearings diagnostic on the boundary lubricant layer state is given. The way for the boundary sliding bearing lubricant control mode, for example, internal-combustion engine (ICE) crankshaft journal at its loading in operating conditions, and also for an incoming control or engine oils quality estimation is developed. The given diagnostic way allows to make an incoming quality oils inspection (hydraulic, turbine, motor), their antifrictional, antiscuff, and operational properties estimation at an early stage before critical, emergency sliding bearing running, to operate its work mode. The developed way has a big practical importance, as on its basis the device which in real time carries out a quality control of used engine oil in the internal-combustion engine. The given device using, for example, in fodder harvesters will allow to the machine operator to carry out not only a quality control of bought engine oil in the market, but also to do its replacement in due time, on the basis of its thermal-oxidative ability.

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Efficiency of Cooperative Societies in Credit Delivery to Agricultural Enterprises in Yakurr Local Government Area, Cross River State, Nigeria

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Abstract— *The study evaluates the efficiency of cooperative societies in credit delivery to agricultural enterprises in Yakurr Local Government Area, Cross River State. The specific objectives were to; describe the socio-economic profile of cooperatives societies, identify the sources of finance that are available and utilize for credit by cooperative societies, analyze the efficiency of cooperatives using the arrival rate of loan request and the service rate and identify the challenges militating against cooperatives as a means of providing credit facilities to farmers in the study area. random sampling method was used to select 30 Cooperative Societies in the Local Government Area. Data were obtained using well structured questionnaire and were analyzed using descriptive statistics and queue theory. Results from the study showed that most of the cooperatives were formed in 2011 with 16-20 members at inception, which stood currently at 21-40 members. The benefits derived from the society ranges from, provision of input for production, accessibility of loan and marketing of products. The large proportion of the amount disbursed to member's ranges from 11000 - 31000naira. The result revealed that the sources of finance available to members was mainly from members contributions .The result further showed that cooperatives were not effective and efficient in queue management because the average idle time (-0.26) and the average traffic intensity was more than one (1.26).*

Also, findings showed that insufficient funds for disbursement(3.33), lack of qualified personnel (3.23), insincerity of members in credit management (3.16) and changes in government credit policies (3.16) were serious challenges that affected efficient delivery of credit by cooperative societies to agricultural enterprises in the study area, The study therefore recommended capacity building for cooperative members to enable them adequately source for funds and efficiently manage loan disbursement and repayment by members. Also, relevant government and nongovernmental financial institutions should be encouraged to channel credit facilities through cooperatives in other to build their financial base and make credit more accessible to agricultural enterprises.

Keywords— ***Efficiency, credit delivery, cooperative societies.***

I. INTRODUCTION

In developing countries like Nigeria, agriculture dominates the economy. It has been established that about 70% of Nigeria population is engaged in agriculture while 90% of Nigeria total food production comes from small farms and 60% of the country population earn their living from these small farms. (Alufohai, 2009; Awotide, Aihonsu and Adekoya, 2011 and Ajah, Itam and Asuquo 2014). The fall in agricultural production could be attributed to inadequate infrastructure, under mechanization and inadequate finance (Oluwatayo, Sekumede and Adesoji, 2008). One of the major problems of agricultural development in Nigeria is that of developing appropriate organizational and institutional framework to mobilize and induce members of the rural sector to a greater productivity effort (International Cooperative Alliance, 2010). As such rural farmers who are characterized by low income, low resources utilization, small farm holdings and scattered nature of farmland, finds it difficult to pool their resources together in order to raise their farm income and substantially improve their living conditions (Ibitoye, 2012).

Inadequate finance has remained the most limiting problem of agricultural production. This is because capital is the most important input in agricultural production and its availability has remain a major problem to small scale farmers who account for the bulk of agricultural produce of the nation and credit has been identified as a major factor in the development of agricultural sector (Ndifon.,Agube and Odok, 2012). Credit is considered as a catalyst that activates other factors of production and makes under-used capacities functional for increased production (Ijere 2008).

Ijaiya and Bello (2009), define credit as financial resources obtained at certain period of time with an obligation to repay at a subsequent period in accordance with the terms and conditions of the credit obtained. Credit could come from banks, government cooperatives or individuals. Agricultural credits on the other hand are loans extended to farmers for production,

storage, processing and marketing of farm products. When farmers face credit constraint, additional credit supply can raise input use, investment and hence output, these they refer to as liquidity. Better agricultural credit facilities can help farmers smooth out consumption and therefore, increase the willingness of risk adverse farmers to take risks as consumption smoothing effect. Hence a better agricultural credit may lead to a higher volume of food output if the increased credit is used to increase fertilizer, private investment in machines and food crops (Edordu, 2010).

Agricultural credit could be obtained from either the formal sources which are the commercial banks and government owned institutions, or the informal sources which are the self-help- group (SHG), money lenders, cooperatives and Non – Governmental Organizations (NGO'S)(International Cooperative Alliance 2005). However, informal source of credit is more popular among small scale farmers which may be due to the relative ease in obtaining credit devoid of administrative delay, non- existence of security or collateral, flexibility built into repayment which is against what is obtained in the formal source. Also, Izekor and Alufohai (2010), noted that informal rural financial sources in Africa perform better than the formal system because the institutional lending system has failed to meet the objectives for which they were set up. The situation has attracted the attention of Nigeria Government and this had led to the creation of specialized institutions such as Nigeria Agricultural and Cooperative Bank (NACB) which later transformed into the Nigeria Agricultural Cooperative and Rural Development Bank (N.A.C.R.D.B) to cater for the credit need in the agricultural sector. However, Alufohai and Ahmadu (2005) studied its queue management and reported its ineffectiveness in credit delivery.

Following from the above, the small scale farmers are forced to source for capital from relations, money lenders and group contribution. All of these are known to be ineffective in providing capital for substantial increase in agricultural production. The last hope for the small scale farmers then lies with the cooperative societies. The cooperative societies have been identified as better channel of credit delivery to farmers in terms of its ability to sustain the loan delivery function (Nweze, 2003). International Cooperative Alliance (1995) defines cooperative society as an autonomous association of persons who unite voluntarily to meet their common economic, social, and cultural aspirations through jointly-owned and democratically controlled enterprises. Cooperatives are established by likeminded persons to pursue mutually beneficial economic interest. They provide services like provision of farm inputs, farm implements, farm mechanization, agricultural loan, and agricultural extension, member's education, marketing of members' farm produce and other economic activities and services. However, regular and optimal performance of these roles is crucial in order to accelerate the transformation of agricultural and rural economic development.

Cooperative societies are formed with the idea of mutual cooperation. Every cooperative society is formed to render services to its members rather than earn profit. In Nigeria, savings of members are usually very small due to low income status of the population (Yusuf and Iyaiya 2009) and as such majority of the cooperatives do not have enough fund to give out as loan to their members. Some give less than what members request for which may not be sufficient for the project members intend to utilize the loans on. Badiru(2010) identified lack of adequate funding of cooperatives as one of the inhabiting factor or the inability of most poverty alleviation strategies to yield result. Agbo (2009) discovered that poor cooperative education and illiteracy has been one of the greatest hindrances to growth of cooperatives. Adeyemo and Bemire (2005), also found out that education, training and re-training of cooperative members in general and officers in particular have been problems of cooperatives in Nigeria (Dogarawa 2005). Agbetunde (2007) stated that cooperative awareness is high in Nigeria but knowledge of the cooperative principle values, ideas and practices is very low. As such issues are handled as they come without proper knowledge and skills necessary to handle them. Infact some of them lack appropriate documentation, which continues to breed corruption within the organization.

The perceived benefits and problems of cooperative societies in the financial sector is worthy of exploration. Studies carried out by Izekor and Alufohai, (2010) and Ajah *et al.* (2014) on the effectiveness of cooperatives societies in agricultural credit delivery in IkpobaOkha Local Government Area, Edo and Cross River State respectively have shown that cooperative carry out the function of credit delivery to farmers but there is ample evidence that farmers still face difficulties in obtaining credit. More so the problem of sourcing for capital by agricultural enterprises still lingers. This may be unconnected to cooperative societies efficiency in credit delivery. It is against this backdrop that this study is designed.

II. OBJECTIVES OF THE STUDY

The objectives are to;

1. Examine the socio-economic profile of cooperatives societies.

2. Identify the sources of finance that are available and utilize for credit by cooperative societies in Yakurr Local Government Area.
3. Analyze the efficiency of cooperative using the arrival rate of loan request and the service rate
4. Identify the challenges militating against cooperatives as a means of providing credit facilities to farmers.

III. LITERATURE REVIEW

The theory that forms the framework for studies on credit delivery is the credit market clearing theory. It postulates that lending rate is the major and significant determinant of the amount of credit dispensed by the banking sector to the credit market. If collateral and other restrictions remain constant the interest rates is the only price mechanism.

An increase in demand for credit and customers supply leads to an increase in interest rate while a reduction in credit demand will reduce interest rates. There exist a positive relationship between the default probability of a borrower and the interest rate charged on the advance (Awoke 2004). It creates the impression that collateral has no effect on lending rate, and if a risky borrower would wish to face the same lending rate as a borrower with a lower risk. This brings about the moral hazard and adverse selection phenomenon. Firstly because of information asymmetry existing between the lender and borrower (Zella and Sharma, 1998).

Several studies have been carried out on effectiveness and efficiency of credit delivery

Awotide, Alhonsu and Adekoya (2011), in their study on Cooperative Society Effectiveness in Credit Delivery for Agricultural Enterprises in North Local Government of Ogun State discovered that the cooperative societies had approval rate of 88.4% with an average traffic intensity of 1.05 and an idle time of 0.05. This shows that the cooperatives were not very efficient in the queue management because the idle time was not zero and were not very effective in credit delivery because the approval rate is less than 100%

Izekor and Alufohai (2010), in their study on Assessment of Cooperatives Societies Effectiveness in Agricultural Credit Delivery revealed overall approval rate of 99.16%, arrival rate of 45%, service rate of 43 per month which resulted in a traffic intensity of 1.05 and idle time of -0.01. Empirical study showed that the cooperatives were effective in credit delivery.

The study by Alufohai (2006) on Sustainability of Farm Credit Delivery by Cooperatives and NGO's in Edo and Delta showed low capital formation rate of 0.1815 and 0.123 for cooperatives and NGOs respectively, cooperatives had zero subsidy Dependence Index (SDI) having no subsidies though with low loan volume. He also showed that cooperatives are more likely to sustain the credit delivery function than the NGOs but they may need to improve their capita. Also, Ajah, Itam and Asuquo (2014), in their studies on Analysis of Cooperative Societies Effectiveness in Credit Delivery to Agricultural Enterprises that are not less than 5 years in operation, revealed that cooperative societies had an average approval rate of 94.5% with an average traffic intensity of 1.06 and an idle time of -0.14. This showed that cooperative societies were not very efficient in the queue management.

Grace and Tosan (2013), in their studies on Assessment of Beneficiaries Satisfaction of the Management of Loan Contract Components by Farmers Cooperative Societies of Edo State, Nigeria, revealed that the main loan contract components to the loan volume, repayment regime, interest rate charged, default management, collateral required, timeliness and loan monitory. Average beneficiaries index was 4.28 out of 5 indicating high satisfaction originating from good queue management with traffic density of 1.12, moderate interest rate of 9%, actual loan monitoring, physical collateral, timely disbursement of loan and accommodation repayment regime. Only individual loan volume was low as a result of inadequate loan able fund.

Yusuf and Ijaiya (2009), in their study on the Informal Financial Institution and Poverty Reduction in the Informal Sector of a Town Kwara State, observed that Cooperatives have three main sources of finance. The most important sources are members as users and investors. Without this base, it is difficult to attract funds from others. The second source is redeemed especially in unallocated funds that are not assigned for distribution to members. These are known as institutional capital, which belongs to the cooperatives and can be liquidated only if the cooperatives incure losses or dissolves. Finally, external funding can also be readily obtained from commercial sources (though usually at a high cost) in a number of forms that include: Loans, equipment financing and even equity capital. In contract, external funding from donor or government sources is shrinking.

According to Fame and French (2000), in their study on Testing Trade-off and Pecking Order Prediction about Dividends and Debt revealed that Cooperatives can get tenancy to organize, operate and expand from two sources: equity capital and

borrowed capital. In a cooperative, equity capital is the portion of assets owned by members. It is also described as the risk capital because all other obligations must be met in case of liquidation before any equity capital is returned to its members. Borrowed capital is capital borrowed through the member's equity in the cooperative.

Inya, Solomon and Otu (2014), in their study on sources of capitalization of cooperative societies in Ebonyi State, Nigeria revealed that the sources of cooperative societies were mostly equity-based, with membership fees and membership certificate recorded 27.15% and 21.9% followed by deferred petrionese 14.43%, retention of unallocated reserve 14.16% revolving funds 14.6% common stock 1.4% and preferred stock 1.0% respectively. Asogwa, Umeh and Penda (2011), study on Analysis of Economic Efficiency of Nigeria Small Farmers observed that high level of cost inefficiency is highly attributed to the low profitability that result from inadequate organization of farmers into collective farmers institutions that can provide opportunities for risk sharing and improved bargaining power. According to Kareem, Arigbabu, Akuturo and Badmub (2012) in their study on Impact of Cooperative Society on Capital Formation: A Case Study of Yemidere Cooperative and Thrift Society Iyebu-Ode, Ogun State discovered that the challenges mostly faced by cooperative society was inadequate amount of capital that can be raised from the members of the cooperatives when compared to the need of small scale industrialists.

Odetola, Awoyemi and Ajijola(2015), studied Impact of Cooperative Society on Fish Farming Commercialization in Lagos State, Nigeria and discovered that cooperative society do not function efficiently due to lack of managerial talent. The members or their elected representative are not experienced enough to manage the society because of limited capital they are not able to get the benefits of professional management. According to Ayegba and Ikani (2013), the major limitations or challenges faced by agricultural cooperatives are high interest rates, bureaucratic bottlenecks, late approval of loan, and unnecessary request for guarantors and collateral. Philip, Nkonya, Pander and On (2009), stated that high interest rate and the short term nature of loan with fixed repayment periods do not suit annual cropping and thus constitute a hindrance to credit delivery. Although cooperatives have proved relatively successful in meeting the credit needs of agricultural enterprises, their limited resources restrict the extent to which they can effectively and sustainably satisfy the credit needs of theses farmers. This is because as agricultural enterprises expand in size the characteristics of loans they require become increasingly difficult for cooperatives to satisfy. (Aryeetey 1996).

IV. MATERIALS AND METHODS

4.1 Study area

The study area is Yakurr Local Government Area which comprise of 13 wards. Yakurr is one of the LGA in Central Cross River State. The Local Government Area was carved out of Obubra in 1987. Yakurr lies between Latitude 5^0 37 and 5^0 58 North of the equator and Longitudes 8^0 00 and 8^0 19 East of the Greenish meridian. It is bounded to the North by Obubura Local Government Area, South by Biase LGA, East by Abi LGA and West by Akamkpa LGA. It has an area of 670km^2, density of 338.66inh/km^2. They speak loka with a population of 196,271 as at 2006 (National Population Commission 2006). The people of Yakurr Local Government Area are largely farmers .The arable crops grown in the area include: yam, cassava, plantain, okra, beans, maize, pumpkin, water yam, and cocoyam. The cash crops includes: oil palm, cocoa, cashew, ground-nut, raffia palm and rubber. The location of the Local Government within the tropical rainforest gives it the ecological basis for population of a wide range of tropical agricultural crop with wide range of potential for industrial convention.

4.2 Sample procedure and sample size

The population of the study consists of all registered Agricultural Cooperatives Societies in Yakurr Local Government Area (LGA), Cross River State. Simple random sampling method was used to select thirty (30) cooperative societies (representing 66.6%) out of forty five (45) cooperative societies in the local government area, from the list of all agricultural cooperative as obtained from the Cross Rivers State Ministry of Agriculture in Yakurr and Agricultural Development Programme (ADP) in Calabar.

4.3 Instrument of data collection

Data were obtained using primary and secondary sources. For the primary source, questionnaires were designed in line with the objectives of the study and used to obtain information from executives of the cooperative societies while the secondary source included information obtained from the records of the cooperative societies on loan request and loan approval within a period of two to four years. Descriptive statistics such as frequency and percentages were used to analyze the socio-economic profile of the cooperatives in the area, Sources of finance, farmer's access to cooperative loans and constraints they face in

the provision of credit facilities. The Queue model was used to analyze the efficiency in credit delivery to agricultural enterprises.

4.4 The queue theory

The queue is a waiting line; it is an array of items waiting to be served. The queue model is usually employed to determine the effectiveness of the performance of an organization (Olayemi and Onyewaku, 1999). The Queue model was used to determine the arrived rate of loan request, the service rate, the idle time and the traffic intensity of Cooperative Societies. This was computed using the formula in the equation below. Omotosho (2002), Alufohai and Ahmadu (2005), Izekor and Alufohai (2010), Olayemi and Onyenweku (1999), Awotide*et al* (2011) Ajah*et al*., (2014) and Webster (1992).

$$\text{Traffic Intensity} = \frac{Arrivalrate}{Servicerate}$$

$$\text{Arrival rate} = \frac{Number\ of arrival}{Time}$$

$$\text{Service rate} = \frac{Numberserved}{Time}$$

Idle time = 1 – Traffic Intensity

The arrival rate depicts the number of loan request per year, the service rate represent the number of application accepted, considering the loan actually provided. Idle time refers to the period when no application was attended to, even when they had been submitted. Efficiency in Queue-management is achieved when the traffic intensity is unity that is arrival rate is equal to service rate and idle time is zero.

V. RESULTS AND DISCUSSION

Results from this study show ocio-economic profile of cooperative societies in the study area. The result revealed that most (56.7%) of the cooperative societies were formed in the year 2011, (23.3%) in 2016, (10%) in 2012, while 2013, 2014, 2015 accounted for 1% each.(Table 1)

The result further revealed that most (50%) of the cooperatives had from 16-20 members at inception. This was followed by 23.3% who had from 5-10 members and 16.7% with 17-25 members at inception. The increase in membership at inception is due to the motivational packages or benefits that could entice more members. Awotide *et al.* (2011) obtained similar result. Their study revealed that the average membership at inception was 20.3% and 80% of the cooperative societies had more than 18 members at inception.

Result revealed that most of the cooperatives currently have large membership. Specifically, 40% had members ranging from 21-40, 23.3% have between 20-30 members, 20% have 51-60 members, while 16.7% currently have 41-50 members. The result showed that there was an increase in membership strength as compared to the period of inception. This could be due to enticing packages. The result is in line with that of Ajah *et al.* (2014). Their study revealed that 30% of the cooperative societies had 40.05 more members presently.

The study showed that the reason most members leave the cooperative was due to lack of awareness (43.3%), 30% was due to late approved rate, while 26.7% left owing to high interest rate. Most of the people join cooperatives without a good knowledge of their organizational objectives and had to leave because they do not believe the cooperatives can solve their problems. The result is in line with that of Agbo (2009) who concluded that people leave cooperative because they don't know about the cooperatives and as such, the trust is not there.

The result also showed that majority of the respondents operate in multipurpose (53.3%) cooperative, 26.7% operate in farmers cooperative, while 20% operate in thrift and credit cooperative. They high proportion of multipurpose is due to the fact that they have enough capital to help her members improve on their businesses.

The benefits derived from the society ranges from, provision of input for production (46.7%), accessibility of loan (26.7%) and marketing of products (20%). This is line with the objectives of multipurpose cooperative society.

The result of the study further revealed that most of the cooperatives (50%) perform the function of crop production, 26.7% are involved in agricultural marketing, and 13.3% are involved in livestock production, while 6.7% are into fisheries. The result shows that procurement of inputs and marketing of crop output is more favorable to that of livestock and fisheries. This

is in line with the findings of Sifa (2012), who concluded that the main categories of agricultural co-operatives fall into mainstream activities of agriculture including supply of agricultural inputs, joint production and agricultural marketing.

The result revealed that a total of ₦11,000- ₦30,000 (43.3%) was disbursed last year, followed by ₦31,000- ₦50,000 (26.7%) and ₦51,000- ₦70,000 (16.7%). This shows that relatively small amount was disbursed and this was used to finance small scale business.

The result also revealed that the membership strength of cooperatives increased from 2012 to 2016. The highest proportion of members was in 2016 (1521). Also, male accounted for the highest proportion of members. This was in line with that of Agbo (2009).

TABLE 1
SOCIO-ECONOMIC PROFILE OF COOPERATIVES SOCIETIES

Variable		Frequency	Percentage
Year of formation	2011	17	56.7
	2012	3	10.0
	2013	1	3.3
	2014	1	3.3
	2015	1	3.3
	2016	7	23.3
	Total	**30**	**100**
Number of members at inception	5-10	7	23.3
	11-15	15	50
	16-20	5	16.7
	21-25	1	3.3
	26-35	1	3.3
	Above 35	1	3.3
	Total	**30**	**100**
Number of members currently	20-30	7	23.3
	21-40	12	40
	41-50	5	16.7
	51-60	6	20
	Total	**30**	**100**
Reason for leaving	High interest rate	8	26.7
	Late approved rate	9	30
	Lack of awareness	13	43.3
	Total	**30**	**100**
Type of cooperative society	Farmers	8	26.7
	Thrift and credit	6	20
	Multipurpose	16	53.3
	Total	**30**	**100**
Benefits derived	Accessibility of loan	10	33.3
	Provision of input for	14	46.7
	Marketing of products	6	20
	Total	**30**	**100**
Types of function performed	Crop production	15	50
	Livestock production	4	13.3
	Fisheries	2	6.7
	Agricultural marketing	8	26.7
	Farm input supply	1	3.3
	Total	**30**	**100**
Amount disbursed last year	<10,000	1	3.3
	11,000-30,000	13	43.3
	31,000-50,000	8	26.7
	51,000-70,000	5	16.7
	71,000-100,000	1	3.3
	>100,000	2	6.7
	Total	**30**	**100**

Source: Field Survey, 2017.

5.1 Sources of Finance Available and Utilized for Credit to Cooperative Societies

Table 2 present the results of the sources of finance available and utilized for credit in the area. The result indicates that most of the respondent's source of finance was through members contributions (43.3%), individual savings (33.3%), members levy and dues (10%), loans from financial institutions (10%) and private money lenders (3.3%). The result revealed that most of the respondent sourced finance from informal sources. This may be due to relative ease in obtaining credit devoid of administrative delay, non-existence of security or collateral, flexibility built into repayment which is against what is obtained in formal sources (Awotide, Alhonsu and Adekoya, 2011). This was in line with that of Yusuf and Ijaiya (2009) who observed that Cooperatives have three main categories or source of finance and concluded that the most important sources are members as users and investors. Inya, Solomon and Otu (2014) also revealed that the sources of cooperative societies were mostly equity-based, with membership fees and membership certificate recording the highest percentages. The low percentage of private money lenders and loans from financial institutions could be due to the high interest rate charged and the rigidity in loan payment.

TABLE 2
SOURCES OF FINANCE AVAILABLE AND UTILIZED FOR CREDIT BY COOPERATIVE SOCIETIES

Source of finance	Frequency	Percentage
Individual savings	10	33.3
Private money lenders	1	3.3
Contributions	13	43.3
Members levy and dues	3	10.0
Loans from financial institutions	3	10.0
Total	30	100

Source: Field Survey, 2017

5.2 Efficiency of credit delivery by cooperatives in the study area

The efficiency of cooperatives in credit delivery was analysed using the arrival rate of loan request and the service rate (Table 3). The result revealed that the cooperatives had an arrival rate of 131 and service rate of 105 for the year 2012 depicting that not all loan requests received were considered and approved. Similarly result was obtained for the year 2013, 2014, 2015 and 2016, the arrival rates were 148, 186, 200 and 234 with their corresponding service rates of 117, 147, 157 and 185 respectively. Indicating that the service rate was not in accord with the loan request, and their traffic intensities were 1.26, 1.27, 1.27 and 1.26, with their corresponding idle time of -0.26, -0.27, -0.27 and -0.26 respectively. This shows that there is need for improvement in credit delivery. The overall results showed that the cooperative were not effective and efficient in the queue management because the average idle time (-0.26) and the average traffic intensity was more than one (1.26). The finding is in line with that of Awotide, Alhonsu and Adekoya (2011), who obtained an approval rate of 88.4% with an average traffic intensity of 1.05 and an idle time of 0.05. Izekor and Alufohai (2010), obtained an overall approval rate of 99.16%, arrival rate of 45%, service rate of 43 per month which resulted in a traffic intensity of 1.05 and idle time of -0.01. Ajah, Itam and Asuquo (2014) also obtained an average approval rate of 94.5% with an average traffic intensity of 1.06 and an idle time of -0.14. All the study reviewed showed that cooperative societies were not very efficient in the queue management.

TABLE 3
EFFICIENCY OF COOPERATIVE USING THE ARRIVAL RATE OF LOAN REQUEST AND THE SERVICE RATE

Year	Arrival rate	Service rate	Traffic intensity	Idle time
2012	131	105	1.25	-0.25
2013	148	117	1.26	-0.26
2014	186	147	1.27	-0.27
2015	200	157	1.27	-0.27
2016	234	185	1.26	-0.26
Total	**899**	**711**	**6.30**	**-1.31**
Average	**180**	**142**	**1.26**	**-0.26**

Source: Field Survey, 2017.

5.3 Challenges militating against Efficient Credit Delivery by cooperatives providing credit facilities to farmers.

The challenges militating against cooperatives as a means of providing credit facilities to agricultural enterprises in the study area were analysed by comparing the responses obtained through likert scale questions to a weighted mean value. A weighted mean value of 3.08 was used as a benchmark to rank the problems of cooperatives. A mean score of 3.08 and above indicate a 'serious' challenge while a mean score of less than 3.08 indicates a ' not serious' challenge. The result show that insufficient funds for disbursement (3.33), lack of qualified personnel (3.23), insincerity of members in credit management (3.16) and changes in government credit policies (3.16) were considered as serious challenges affecting efficiency in credit delivery by cooperative societies to agricultural enterprises in the study area.

TABLE 4
CHALLENGES MILITATING AGAINST EFFICIENT CREDIT DELIVERY BY COOPERATIVES TO AGRICULTURAL ENTERPRISES.

S/N	Challenge	A	SA	D	SD	Cum	Mean
1.	Insufficient fund for disbursement	12(48)	16(48)	2(4)	-	100	3.33*
2.	Lack of qualified personnel	12(48)	14(42)	3(6)	-	97	3.23*
3.	Insincerity of members in credit management	7(28)	21(63)	2(4)	-	95	3.16*
4.	Changes in Government credit policies	7(28)	21(63)	2(4)	-	95	3.16*

Source: Field Survey, 2017..N= 30, n = 8, weighted mean score = 24.61/8 = 3.08, (X ≥ 3.08 = a serious challenge, X<3.08 = not a serious challenge), * = serious challenge, -= no response. A= Agreed, SA = Strongly agreed, D = Disagreed, SD= Strongly, Cum = cumulative frequency.
Figures in parenthesis = the number of those that agreed, strongly agreed, disagreed and strongly disagreed.

VI. CONCLUSION AND RECOMMENDATIONS

The study revealed inefficiency in credit delivery by cooperatives in the study area. This could be as a result of the constraints faced by these cooperatives in sourcing for fund (insufficient fund for disbursement) and lack of capacity of staff in fund management. Cooperative societies could be effective organs for credit delivery to agricultural enterprise, however, there is need for capacity development of cooperative members to enable them adequately source for funds and efficiently manage loan disbursement and repayment by members. Also, relevant government and nongovernmental financial institutions should be encouraged to channel credit facilities through cooperatives in other to build their financial base and make credit more accessible to agricultural enterprises.

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Comparative Effect of Potting Media on Sprouting and Seedling Growth of Grape Cuttings

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Abstract— *A pot experiment was conducted to study the effects of potting media on sprouting and seedling growth of grape cuttings. Three grape varieties viz. Red globe, Thomson seedless and Crimson seedless were planted in four different growth media: CS-Canal silt, CSFYM-Canal silt (75%) + FYM (25%), CSB-Canal silt (25%) + Bagasse (75%) and CSBCP-Canal silt (25%) + Bagasse (50%) + Coco peat (25%). The experiment was conducted in Completely Randomized Design (CRD) along with three replications. The results revealed that almost all observed parameters were significantly influenced by the potting media. However, grape varieties and their interaction with the potting media exhibited non-significant effect for sprouting percentage and most of the seedling related attributes of growth. Minimum days to sprouting (6.78),highest sprouting percentage (84.44), maximum rooting percentage (84.44) and maximum chlorophyll content of leaves (56.23) were observed from the cuttings planted in CSBCP. However, maximum number of sprouts (5.55), number of leaves (13.77), fresh weight of leaves (2.27g), fresh weight of the roots (2.16 g), were observed from CSB. No grape seedling mortality was also observed CSB and CSBCP growth media. On the basis of varietal comparison, Thompson seedless exhibited the best results for number of leaves per cutting (11.50), fresh weight of the roots (1.64 g) and number of roots per cutting (29.17 g) as compared to rest of the grape varieties. The research establishes the potential for locals to use available materials in potting media preparation for healthier and stronger grape seedlings for subsequent improved grape plantation.*

Keywords— Chlorophyll content, growth of grapes cuttings. Potting, sprouting media.

I. INTRODUCTION

Grapes (*Vitisvinifera*) are one of the main fruits cultivated in Pakistan belonging to the family *Vitaceae*. It is commercially grown in subtropical and temperate climates. It is a vine crop and trained on wires on both sides of plant. It is a short duration crop and consumed as fresh and in dried form [1]. This fruit is consumed in a number of varied forms such as wines, juices, jelly, jam and raisins [2]. In Pakistan grapes cultivation is estimated to cover an area of 13,000 hectares and production is about 49,000 tons per year[3]. Greater percentage (70) of grape production is in Baluchistan and the remaining in northern hilly areas of NWFP and Punjab[4].

A potting or growing medium is a substrate where roots of the plants grow and extract nutrients and water from medium, helps in the production of healthy seedlings in containers and bare root production and serve as the sole source of nutrition for the plants [5-7]. So, it is utmost important to select proper potting medium that is a basic step towards successful nursery of any fruit crop. Both the biological and physico-chemical characteristics of a potting medium affect plant and root growth[8]. The proper potting medium that is free from pathogens, have good drainage, water holding capacity and proper

porosity and aeration is good to raise healthy nursery seedlings[9]. Three functions of growing media are; to support plant in soil, to hold and provide water as well as nutrient elements and to enable plant roots to get sufficient amount of oxygen [10].

It is general practice among growers to raise grape seedlings in soil which is a main cause of the pathogen infection as such seedlings are mostly affected by soil pathogens. A modern solution to such a problem is the use of potting medium. Suitable potting media are available in the market but it is difficult for the common grower especially those from developing countries to bare the high cost of the potting media [6]. The best alternative to cope with this problem is to utilize cheap and locally available sources to get good materials. A typical common example is bagasse available in large quantities from sugarcane mills at lowest rates. Other materials like press mud, rice husk, wheat straw, farm yard manure, coconut husk and so many other materials are available in local premises in the country [11]. Meanwhile, growers must know the pros and cons of the material materials being used. The raising of grape seedlings in any potting medium is preferred in containers rather than field production because of easy marketing, long planting, marketing period, easy transportation and rapid product rotation [12].

Choosing the most suitable growing media for the achievement of a successful plant production is very important in potted growth. The growth and survival of the grape seedlings in a nursery is greatly affected by the potting medium. As it is a key source of nutrition and provides root system to the budded plants. Besides, water holding capacity, better aeration, root penetration, presence of organic matter in the growing medium and so many other related factors are greatly influenced by the growing medium [13]. A good potting medium must be easy to supply, process and a cheap source [14, 15]. Many suitable commercial growing media are available for raising healthy and quality seedlings of different crops but unavailability of the potting medium in the local premises of the city makes them more expensive [16]. Import of these potting media is not affordable for a local grower from developing countries like Pakistan. So, there is a need to optimize protocol for potting mix by using cheap source of materials of local premises that are easily available in large scale for rising of healthy and quality grape seedlings. The present study was therefore focusing on comparative effects of potting media on sprouting and seedling growth of grape cuttings.

II. MATERIALS AND METHODS

2.1 Materials

The experiment was carried out at the Agricultural Research Station (North) Mingora Swat, Pakistan. Cuttings of three (3) different grapes varieties; viz. Thompson seedless, crimson seedless and Red globe were used to observe the effect of various potting media on sprouting and seedling growth. The stem cuttings of the three grape varieties were obtained from 8-year-old plants of District Killa Abdullah Balochistan. Local materials (canal silt, farm yard manure, bagasse and coco peat) were obtained from local suppliers.

2.2 Methods

2.2.1 Media preparation

Each potting medium was prepared by mixing canal silt, farm yard manure (FYM), bagasse and coco peat at different percentages (proportions).Four potting mixtures (media)a. CS - canal silt (100%), b. CSFYM - canal silt (75%) + FYM (25%), c. CSB - canal silt (25%) + bagasse (75%) and d. CSBCP - canal silt (25%) + bagasse (50%) + coco peat (25%)(w/w) were prepared for the experiment[17].

2.2.2 Stem cuttings and planting

The stem cuttings were obtained from eight years old plants of the grapes varieties of 8-10 inches in length planted in polythene bags of 4 x 8 inches. While preparing the cuttings, a smooth cut in each cutting was given on distal end and

slanting cut was given at lower end just below the node. Before plantation all the cuttings were showered thoroughly with water to retain the moisture in the cutting and prevent it from drying. The cuttings were planted in potting media and in total 30 cuttings were planted per potting medium. Each replication had ten filled polythene bags and three replications of each variety were kept in the experiment. The mixture of the potting medium was filled in perforated plastic bags of half kg, leaving one-inch space at the top. One cutting was planted in each polythene bag. The cuttings were planted during spring and in a layout of Completely Randomized Design (CRD) with three replications. Data recordings were taken on the following parameters; days to sprouting, sprouting percentage treatment^{-1}, number of sprouts per cutting, mortality percentage per treatment, number of leaves per cutting, fresh weight of the leaves per cutting, rooting percentage per treatment, number of roots per cutting, fresh weight of the roots per cutting, chlorophyll content of leaf and electrolyte leakage of leaf (%). The data was taken each replication and treatment wise.

2.2.3 Growth indicator measurements

Days to sprouting were counted from the day of plantation up to the sprouting of the cuttings. Sprouting percentage of each treatment was checked on every alternative day up to 7th day of plantation and the sprouting percentage was computed per equation (1) as described by Wilson, Stoffella [18];

$$GP(\%) = \left(\sum n/_{N}\right) \times 100 \tag{1}$$

where GP is sprouting percentage, n is number of sprouted cuttings at each counting and N is total number of cuttings in each treatment.

Number of sprouts per cutting were observed and counted daily after plantation for up to the completion of the experiment whereas mortality percentage per treatment. Mortality percentage was observed throughout the whole experimental process and computed after all observation. Number of leaves per cutting was computed such that the number of leaves on each cutting was counted daily up to the completion of the experiment. The fresh weight of the leaves per cutting were determined by separating the leaves from each cutting and weighted in a weighing balance. Rooting percentage per treatment was observed after one week of plantation for up to one month of plantation. The rooting percentage was calculated by using following formula:

$$RP(\%) = \left(A/_{B}\right) \times 100 \tag{2}$$

where RP is rooting percentage, A means number of rooted cuttings while B is total number of planted cuttings.

Determining the number of roots per cutting, the roots on each plant were counted at the end of formation per treatment. For the purpose of this study, the cuttings were taken out from the polythene bags and the adhered soil was discarded then roots were counted per cutting. Furthermore, the fresh weight of roots per cutting was evaluated subsequently when the number of roots per cutting was recorded. The fresh weight of the counted number of roots of each sample was taken in a weighing balance and the data was recorded. Chlorophyll content of leaf was measured by meter (SPAD- 500 plus) in arbitrary units as relative greenness (RG).

2.2.4 Electrolyte leakage of leaf (%)

Electrolyte leakage percentage was measured by taking leaf discs of size 1cm^2 and weight of 0.5 g from randomly selected leaf samples. The leaf discs were washed well with deionised water prior to incubation in 25 ml of deionised water for 3 hours at room temperature. After incubation, the conductivity (value A) of the bathing solution was measured with the conductivity meter. The petal discs were boiled (100^{0}C) with the bathing solution for 15 min to lyse all cells. After cooling at room temperature (31.3^{0}C), the conductivity (value B) of the bathing solution was again measured. The electrolyte leakage was expressed as percent value according to the formula (**Eq. 3**)

$$Electrolyte\ leahage\ of\ leaf\ (\%) = \left({}^{value\ A}/_{value\ B} \right) \times 100 \quad (3)$$

where value A is conductivity of bathing solution at room temperature value B conductivity after boiling.

III. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Days to sprouting

The number of days to sprouting of the cuttings varied significantly in response to the grape variety and potting media (**Table 1**). The interaction of the varieties and potting media was also highly significant (*p<0.05*). It took a minimum of 6 days for Thomson seedless variety to sprout in response to the potting medium CSBCP (**Table 1**). On the basis of potting media, stem cuttings took a minimum mean of 6.78 days for sprouting in similar potting medium. Meanwhile, stem cuttings planted in the CSFYM potting medium took a minimum mean of 14.89 days to sprout as compared to those planted in CS potting medium (10.89). In varietal comparison, cuttings of Thomson seedless sprouted earlier (8.75 days) as compared to Crimson seedless (9.75 days) and Red globe (11.25 days).

3.1.2 Sprouting percentage per cutting

Sprouting percentage was significantly affected by the various potting media (*p<0.05*). However, non-significant results were observed for the varieties and their interaction with the potting media. In **Table 1**, it is portrayed that the highest sprouting percentage (84.44) was observed from the stem cuttings planted in the CSBCP potting. These results are statistically similar with the results obtained from the cuttings planted in the CSB potting medium.

3.1.3 Number of sprouts per cutting

Table 1 again shows that number of sprouts per grape stem cutting ranged between 1.55 to 5.55. The maximum mean number of sprouts (5.55) per cutting were observed from the CSB potting medium where canal silt and bagasse were used at 25 and 75% respectively. These results are statistically similar with the results obtained from the cuttings where potting medium had mixture of canal silt, bagasse and coco peat at 25%, 50% and 25% respectively. On the basis of varietal means, number of sprouts ranged from 3.75 to 3.91. The number of sprouts per cutting was significantly affected by the various potting media (*P<0.05*). No significant differences were observed for number of sprouts from the interaction of varieties and potting media **Table 1**.

3.1.4 Mortality percentage per treatment

According to the results as presented in **Table 1**, the highest mortality percentage of 10% was observed from the cuttings of Red globe variety of grape planted in the potting medium containing canal silt (75%) and FYM (25%). On the basis of potting media, the highest mortality (9.24%) was observed from the same medium (CSFYM) followed by CS with 4.05% with no death of stem cuttings observed on CSB and CSBCP media. Red globe and Thompson seedless had the highest mortality percentage 8.53 and 8.26 on CSFYM potting medium respectively yet non-significant statistically with each other. Mortality percentage was significantly affected by potting media (*P<0.05*). Similarly, the interaction of both the varieties and potting media was also significant.

3.1.5 Number of leaves per cutting

The maximum number of leaves per stem cutting (17.00) was observed from the stem cuttings planted in the potting medium had mixture of canal silt (25%) and bagasse (75%). Statistically similar results were also observed from the cuttings grown in the medium contained canal silt, bagasse and coco peat at 25, 50 and 25% respectively. In comparison to the potting media, more number of leaves per cutting (13.77) was observed from the CSB potting medium as compared CS potting medium with 7.67 leaves per cutting. Thompson seedless had more number of leaves (11.50) in comparison to Red globe

(8.50) and Crimson seedless (8.33). Number of leaves per cutting was significantly affected by the grape varieties and potting media (*P<0.05*) same as the interaction between the grape varieties and potting media (**Table 1**).

3.1.6 Fresh weight of leaves (g)

From **Table 1**, a statistical significant variation was observed in the fresh weight of leaves on the basis of potting media whereas a non-significant result was obtained for the grape varieties and their interaction with the potting media. The results for fresh weight of the leaves as presented in **Table 1**depicts that fresh weight of leaves range from 0.58 to 2.27 g. The highest fresh weight of leaves (2.27 g) was observed from the stem cutting planted in the potting medium had mixture of canal silt (25%) and bagasse (75%) followed 1.79 g obtained from the cuttings planted in the potting medium contained canal silt, bagasse and coco peat at 25, 50 and 25%.

3.1.7 Rooting percentage per treatment

The results in **Table 1** reveals that. CSb and CSBCP potting media had more than 70% rooting in comparison to CS and CSFYM potting media with less than 50%. The highest mean rooting percentage (84.44) was observed from grape cuttings grown in potting media had mixture of canal silt (25%), bagasse (50%) and coco peat (25%). Grape varieties also had significant effect as well as potting media on rooting percentage which ranged from 52.50 to 55.83%. The statistical analysis depicts that the potting media had significant effect on the rooting percentage (*P<0.05*).

3.1.8 Number of roots per cutting

Table 1 depicts presents a result that shows that higher mean number of roots (50.00) were obtained in Thompson seedless grape variety which were planted in CSBCP potting medium. On the basis of mean results of the potting media, lesser number of roots (5.00) was obtained from the cuttings planted in the potting medium contained canal silt (75%) and FYM (25%) followed by 14.55 from canal silt (CS) only. On the basis of varietal comparison, Thompson seedless (29.16) and Crimson seedless (27.41) statistically had similar results as compared to Red globe (20.25). The statistical analysis reveals that number of roots per cutting was significantly affected by the varieties and potting media. Correspondingly, the interaction of both the factors viz. varieties and potting media was also highly significant.

3.1.9 Fresh weight of roots cutting -1

Fresh weight of roots per cutting was significantly affected by the grape varieties and the potting media. However, the interaction of both factors was non-significant. Results in **Table 1** depicts that the highest fresh weight of roots per cuttings (2.16) was observed from the cuttings planted in CSB potting medium having mixture of canal silt (25%) and bagasse (75%). Thompson seedless produced more fresh weight of roots (2.57 g) in response to CSB potting medium. On the basis of varietal comparison, Thompson seedless produced relatively higher fresh weight of roots per cuttings (1.64 g) as compared to Crimson seedless (1.61 g) and Red globe (1.17 g).

3.1.10 Chlorophyll content of leaves

More chlorophyll content of leaves (56.23 % greenness) was observed from the cuttings planted in the potting medium that had mixture of canal silt (25%), bagasse (50%) and coco peat (25%) followed by CSB with (52.50% greenness. On the hand, based on the interaction between potting media and varieties, the leaves of Crimson seedless grape variety had more (57.60%greenness) in response to the CSBCP (**Table 1**). The same variety had also lesser chlorophyll content of leaves from the cuttings planted in the potting medium that had 25% canal silt and 25% FYM. The chlorophyll content of leaves was significantly affected by the potting media as well as its interaction with the varieties (*P<0.05*).

3.1.11 Electrolyte leakage of leaves (%)

On the basis of potting media, mean electrolyte leakage of leaf was in the range of 28.44 and 30.22%. In varietal comparison, it ranged between 28.50 and 30.41%; Crimson seedless with the highest followed by Thomson seedless (29.67%) and Red globe the least 28.50. There was no significant difference in the electrolyte leakage of leaf on the basis of varieties unlike potting media (**Table 1**).

TABLE 1
EFFECTS OF POTTING MEDIA AND GRAPE VARIETY ON GROWTH INDICES OF GRAPE CUTTINGS.

Growth index	Grape varieties	Potting media (M)				Mean
		CS	CSFYM	CSB	CSBCP	
Days to sprouting (days)	Red globe	12.67 ^{a}	16.00 ^{a}	8.33 ^{de}	8.00 ^{ef}	11.25^{A}
	Thompson seedless	10.33 ^{c}	12.00 ^{b}	6.67 ^{fg}	6.00 ^{g}	8.75^{C}
	Crimson seedless	9.67 ^{cd}	16.67 a	6.33 ^{g}	6.33 ^{g}	9.75^{B}
	Mean	10.89 ^{B}	14.89 ^{A}	7.11 ^{C}	6.78 ^{C}	
Sprouting percentage treatment $^{-1}$ (%)	Red globe	46.66^{a}	16.66^{a}	70.00^{bc}	76.66^{bc}	52.50 ^{C}
	Thompson seedless	40.00^{ab}	23.33^{ab}	90.00 ^{a}	83.33 ^{b}	59.17^{A}
	Crimson seedless	40.00^{ab}	16.67^{a}	73.33^{b}	93.33^{a}	55.83^{B}
	Mean	42.22 ^{B}	18.89 ^{C}	77.78 ^{A}	84.44 ^{A}	
№ of sprouts cutting $^{-1}$	Red globe	3.33^{a}	1.66^{b}	6.00 ^{a}	4.66^{bc}	3.91^{A}
	Thompson seedless	3.00 ^{b}c	1.66 ^{b}	5.00^{bc}	5.33^{a}	3.75^{A}
	Crimson seedless	3.33 ^{a}	1.33 ^{a}	5.66^{ab}	5.00 ^{ab}	3.83^{A}
	Mean	3.22 ^{B}	1.55 ^{C}	5.55 ^{A}	5.00 ^{A}	
Mortality percentage (%)	Red globe	3.933 ^{C}	10.94 ^{A}	0.00 ^{D}	0.00 ^{D}	3.72 ^{A}
	Thompson seedless	5.13 ^{C}	8.26 ^{B}	0.00 ^{D}	0.00 ^{D}	3.35^{AB}
	Crimson seedless	3.10 ^{C}	8.53 ^{B}	0.00 ^{D}	0.00 ^{D}	2.91 ^{B}
	Mean	4.05 ^{B}	9.24 ^{A}	0.00 ^{C}	0.00 ^{C}	
№ of leaves cutting -1	Red globe	8.33 ^{ef}	4.33 ^{fg}	10.66 ^{bcd}	10.66 ^{bcd}	8.50 ^{B}
	Thompson seedless	8.67 ^{cde}	3.33 ^{fg}	17.00 ^{a}	17.00 ^{a}	11.50^{A}
	Crimson seedless	6.00 ^{ef}	1.66 ^{g}	13.66 ^{ab}	12.00 ^{bc}	8.33 ^{B}
	Mean	7.67 ^{B}	3.11 ^{C}	13.77 ^{A}	13.22 ^{A}	
Fresh weight of leaves (g)	Red globe	0.76 ^{bc}	0.51 ^{bc}	2.48 ^{a}	2.08 ^{a}	1.46^{A}
	Thompson seedless	0.81^{ab}	0.55 ^{ab}	2.20 ^{bc}	1.50^{bc}	1.26^{A}
	Crimson seedless	0.93 ^{a}	0.69^{a}	2.14 ^{ab}	1.79^{ab}	1.39^{A}
	Mean	0.83 ^{C}	0.58 ^{C}	2.27 ^{A}	1.79 ^{B}	
Rooting percentage (%)	Red globe	46.66^{a}	16.66^{b}	70.00^{c}	76.66^{c}	52.50 ^{C}
	Thompson seedless	40.00^{b}	23.33^{a}	90.00^{a}	83.33^{b}	59.16^{A}
	Crimson seedless	40.00^{b}	16.66^{b}	73.33^{b}	93.33^{a}	55.83^{B}
	Mean	42.22 ^{B}	18.88 ^{C}	77.77 ^{A}	84.44 ^{A}	
№ of roots $^{-1}$	Red globe	14.66 ^{d}	4.00 ^{e}	26.33 ^{c}	36.00 ^{b}	20.25^{B}
	Thompson seedless	14.00 ^{d}	3.66 ^{e}	49.00 ^{a}	50.00 ^{a}	29.16^{A}
	Crimson seedless	15.00 ^{d}	7.33 ^{de}	49.00 ^{a}	38.33 ^{b}	27.41^{A}
	Mean	14.55 ^{B}	5.00 ^{C}	41.44 ^{A}	41.44 ^{A}	
Fresh weight of roots cutting -1	Red globe	0.81 ^{ef}	0.45 ^{f}	1.62 ^{cd}	1.82 ^{bcd}	1.17^{B}
	Thompson seedless	1.56 ^{cd}	0.33 ^{f}	2.57 ^{a}	2.14 ^{abc}	1.64^{A}
	Crimson seedless	1.30 ^{de}	0.43 ^{f}	2.29 ^{ab}	2.44 ^{ab}	1.61^{A}
	Mean	1.21^{b}	0.40 ^{f}	2.16^{a}	2.13^{a}	
Chlorophyll content of leaves (%)	Red globe	35.96^{c}	34.00^{a}	49.72^{c}	54.84^{c}	43.63^{A}
	Thompson seedless	38.30^{a}	34.00^{a}	54.88^{a}	56.25^{b}	45.85^{A}
	Crimson seedless	36.73^{b}	33.11^{b}	52.90^{b}	57.60^{a}	45.08^{A}
	Mean	37.00^{C}	33.70^{C}	52.50^{B}	56.23^{A}	
Electrolyte leakage (%)	Red globe	29.33^{b}	28.67^{c}	28.33^{c}	27.67^{c}	28.50 ^{A}
	Thompson seedless	30.67^{a}	31.00^{a}	30.00^{b}	27.00^{b}	29.67^{A}
	Crimson seedless	28.67^{c}	30.00^{b}	32.33^{a}	30.67^{a}	30.41^{A}
	Mean	29.56^{b}c	29.89^{b}	30.22^{a}	28.44^{c}	

CS: Canal silt, CSFYM: Canal silt (75%) + Farm Yard Manure (25%), CSB: Canal silt (25%) + Bagasse (75%), CSBCP: Canal silt (25%) + Bagasse (50%) + Coco peat (25%). Values are means of three determinations. Means with the same letters (superscript) are not significantly (p>0.05) different.

3.2 Discussion

Four different potting mixtures were used by adding canal silt, FYM, bagasse and coco peat at different percentages [17]. A number of studies have been conducted on the use of growing media for raising better seedlings of different fruit crops. A wide range/variety of materials are used and mixed in different ratios for obtaining an appropriate medium including peat, perlite, sawdust, sand, silt, rice hulls, coconut husk, leaf manure, tree barks, sugarcane waste, spent, sewage sludge which could yield good results as observed in the current study. The results of the current study agrees partially with other studies because of variation in materials[11-13, 19-21].Meanwhile, Mhango, Akinnifesi [12]used soil, sand, peat and spent in different combinations and found sand and peat as appropriate medium in the ratio of 1:1 for better growth of citrus seedlings. Again, Bhagat, Thakur [15] reported that the suitable medium for Uapacakirkiana contained 75% forest soil and 25% sawdust for taller seedlings having larger root collar diameter. The best quality seedlings of Crimean Juniper were obtained by [22]on the media containing forest soil (70%) + humus (15%) and pumice or creek (15%). In the case of grape variety, very rare works have been reported on the effect of potting media on growth [23]. The present study determined that sprouting and seedling growth of grape varieties are greatly affected by the potting media. The potting media composed of canal silt (25%) + bagasse (75%)dented as CSB for the purposes of the current study and canal silt (25%) + bagasse (50%) and coco peat (25%) denoted as CSBCP produced better results for sprouting and proper growth of the grape seedlings. This observation may be due to the presence of coco peat and bagasse in the potting media as this finding corresponds literature [11, 16]. They reported that due to the presence of peat, initiation of roots and rooting percentage was increased. Also, Tariq, Qureshi [19]reported minimum mortality of 8% in plants planted in peat and sand medium in the ratio of 1:1 as compared to maximum (58%) in soil + sand + FYM (1:1:1). They also reported that sand and peat in the ratio of 1:1 the potting media produced for better growth of rough lemon. Our current results also showed that media containing coco peat and bagasse produce better growth in grape cuttings. Misra [24] reported that coco peat and vermi compost improved seed germination of rough lemon. Likewise, Aklibasinda, Tunc [25] reported that sand+ soil+ FYM medium fashioned the best results for maximum length of sprouts and number of leaves per cutting scotch pine. Furthermore, Rani, Akash Sharma [26] also reported more number of leaves per plants in the peat based potting medium for guava propagation.

IV. CONCLUSION

Choice of proper potting media play a critical role in growth and development of plant. Bagasse and canal silt are important sprouting media for grape cultivation as it has positive effect on physiology of grape vines moreover they are cheap and easily available to local growers. The main perspective of this research was to explore the effect of different potting media on sprouting and seedling growth of grape cuttings and to compare and establish the most appropriate potting medium on the basis of the best growth responses. The research therefore concludes based on the results that combination of canal silt (25%) and bagasse (75%) (CSB potting medium) as well as CSBCP [canal silt (25%) + bagasse (50%) and coco peat (25%) potting medium] had produced best results for sprouting and growth of grape seedlings. Media with varied components mixed together improved both germination and then growth compared to sole canal silt medium used in grape nursery. The research establishes the potential for locals to use available materials in potting media preparation for healthier and stronger grape seedlings for subsequent improved grape plantation. This will also help in reduction in production cost as less expenditure will be incurred in terms foreign potting media.

ACKNOWLEDGEMENTS

Authors are appreciative to Horticulture Department of Agricultural Research Station, Mingora Swat, Pakistan for providing funds for this research project.

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