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Preface

We would like to present, with great pleasure, the inaugural volume-8, Issue-1, January 2022, 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.

Environmental Research:

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

Agriculture Research:

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

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

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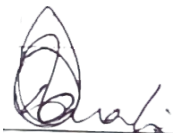
(Managing Editor)



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Once again CGG Repeat Patients, Hypospadia: A Systematic Review

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Abstract— *High prevalence of hypospadias is only the peak of the iceberg, because there are stigma and isolation, which make not all patients seek a doctor to check up and do counseling. However, failure of psychical, anatomical and metabolomic development should be faced from the childhood age, adolescent, through the puberty years. One hundred years of congenital adrenal hyperplasia (CAH) in Sweden, open the failure of this labium minor fusion is associated with hypermethylation which have many names. Familial cases, in female, bring this case a gender reversal and reconstructive urology repair, were successful, while the cause, CYP21 mutation/blockade/deficiency also known as cytochrome P450 deficiency is neglected. In prevention, lose with reconstruction, psychologic and environmental which reported due to pesticide are favorite. Digging the cause of labium minus fusion failure, which given the real cause, is need to be chased for prevention in the population.*

Method: Systematic review (SR) using Science Direct search engine cross by PubMed, preferable SR and meta-analysis (MA) design than the other. Keywords: hypospadia-fragile (10), -CYP21 mutation (2). CYP21 deficiency-CAH is the core of this study.

Result: Twenty-one references flowchart and table with 4 references in SR and MA which supported Hypospadia-CYP21 deficiency-CAH. More than 3126 cases were studied.

Discussion: The association of CAH hormone and behavior (family-genetic), hypospadia-Parkinson (12), -pesticides, gen chromosome 6p, -autism (59), -bipolarism (22) bring to epigenetic hypermethylation cases. The CGG repeat-hypermethylation-gene silencing which produce protein enzyme is the cause of hypospadia. Geographical of Brazil, Latin American, incl. Argentine, SEA such as Thailand, Indonesia, and also Sweden, which all are in wet and warm climate area, has high hypermethylation prevalence due to RNAi in GMO green activity.

Keywords— CAH, gene silencing, Martin-Bell syndrome, Escalante syndrome, FRAXA.

I. INTRODUCTION

It is already known that CGG repeat large amount give FMR1 full mutation, while 55-200: permutation associated with psychological and behavior disorder, and FMR1 intermediate, grey zone permutation, and small CGG repeat has also been studied. This epigenetics hypermethylation cases underlie many diseases such as Fragile-X Syndrome, parkinson,[1] bipolar,[1]

autism,[1,2] etc. due to silence of DNMT enzyme.[3] Hypospadia cause by hormonal disorder, have a Congenital Adrenal Hyperplasia (CAH) disorder (CYP21 deficiency),[4] is reported genetics,[5] but never epigenetic. Hypospadia could be associated with CGG repeat/ CpG island hypermethylation, -FMR1, -CYP21 mutation. The later is cause by failure of neurosteroid production.[6] Hypospadia is a structural marker of FX syndrome also in CAH, and CAH 100 year in Sweden has been reported.[7] The high prevalence of hypospadias is only the tip of an iceberg, because the stigma and exclusion, prevent most sufferers from getting checked by a doctor.[8] They avoid seeking medical advice. However, psychological disorder, and fail in anatomical development, as well as metabolism, must be faced by the subjects since childhood, adolescence through puberty.[9,10,11,12] Familial, in Congenital Virilizing Adrenal Hyperplasia (CVAH) women the case of gender reversal to male in male rearing are successful managed as male,[13] and three reconstructive pediatric urological condition: hypospadia, CAH, and bladder exstrophy have been reported from the perspective of changing expectations and outcomes,[14] while the main cause of Cyp21 mutation/blockade/deficiency or cytochrome P450 deficiency is underestimated and become inferior than reconstruction handlers. The psychological and environmental prevention are mentioned due to pesticide in this epigenetics cases.[15,16] whereas knock out the enzyme production give hypospadia[2] and antenatal androgen excess.[17] Exploring the labium minus cause of failure to adhere (hypospadia), the real cause needs to be pursued for mainly prevention purposes.

The term CAH covers a number of people of disruptions caused by imperfection in hydroxylation of cortisol precursors. The missing enzyme could be 21-hydroxylase, 17- hydroxylase, 18-hydroxylase, etc. The CYP21 is the mainly steroid missing enzyme gene due to mutation, hypermethylation on CpG islands, blocking, or deficiency. Extremely increasing rate 90% of CAH subjects are made by the alteration of the CYP21 gene on a DNA double helix in 6p21.3.[18,19]

When a serious autosomal recessive (AR) disorder, which results in influenced homozygotes, has a large occurrence of wide scope population, the account that makes something clear must lie in either a very high mutation rate or in heterozygote advantage. Yupik Eskimos has been reported resistance or advantage in Influenza B in CAH disorder, an AR genetic disorder.[19] The art icon mask of Yupik Eskimo depicting the bad spirit of the mountain/river, is similar with Escalante syndrome in Argentina

(https://commons.wikimedia.org/wiki/File:Mask_depicting_the_Bad_Spirit_of_the_Mountai n,_Yupik_Eskimo,_Yukon_Riv er_area,_probably_St._Michael,_late_1800s,_wood,_paint,_f eathers_-_Dallas_Museum_of_Art_-_DSC04535.jpg).

Deficient cortisol production, induce/increased secretion of corticotropin (ACTH). Overproduction of ACTH causing adrenal hyperplasia (CAH). The net effect is a build-up in the adrenal gland of cortisol precursors and androgens.[20] The production of more of adrenal androgens is an additional ordinary aspect of it. This hormone amount that is more than necessary results make greater physical size, hairy body, deep voice and double meaning in external genitalia.[20] Hypersecretion of adrenal androgens during intrauterine life cause masculinization of the female external genitalia. Increased body growth at an accelerated rate and show advanced skeletal maturation. However, because of premature closing of the epiphyses, their ultimate height is below average.[21] Hypospadia 46,XX successfully with reconstruction urology repair,[12,13] whereas hypospadia 46,XY reared male or female can lead to successful long-term outcome for the majority of cases.[22]

II. METHOD

Systematic review (SR) using Science Direct search engine and cross checked by PubMed. Two times collected articles and extracted data independently. The SR and meta-analysis (MA) references are preferable than the other methods. PRISMA design has used to get flowchart and table of references that support hypospadia-CAH (189) caused by CYP21 or CytP450

deficiencies to support hypermethylation which already report in Fragile-syndrome, Parkinson, autism and bipolar cases.[1,3,23] Keywords: hypospadias- CGG repeat (0), -fragile (10), -CYP21 mutation (2). Hypospadias-CYP21 deficiency (1), hypospadia-CytP450 (1), - cytochrome P450 (25), hypospadia-fragile (10). Hypospadia-Parkinson (12): pesticide, -autism (63), - bipolar (23); CAH-Parkinson (11), CAH-autism (58), CAH-bipolar (12). Change the keywords to CAH-CYP21 (72), CAH-CytP450 (0), CAH-Cytochrome P450 (139). The 100 y CAH and associated with syndrome hypermethylation is clinical in high prevalence in wet and warm climate area, will be the Bayesian network and analysis. Included the CytP450 deficiency and excluded the genetic but epigenetic cases.

III. RESULT

Twenty-one references supported FMR1 intermediate and Small CGG expansion associated with neurosteroid direct via hypermethylation, or indirect via Fragile-X syndrome (FMR1)/Parkinson/autism/bipolar which have more CGG repeat / CpG islands and hypermethylation patient or pedigree. This stable and unstable hypermethylation is associated with silence genetic of CYP21 or high production of hormonal androstane group (C19). Four designs of the references are SR/MA, 1 clinical trial, 1 cross-sectional, 5 case-control, 1 cohort, 1 retrospective, 3 distribution, 5 reviews. At least 3126 cases of CGG repeat expansion were studies.

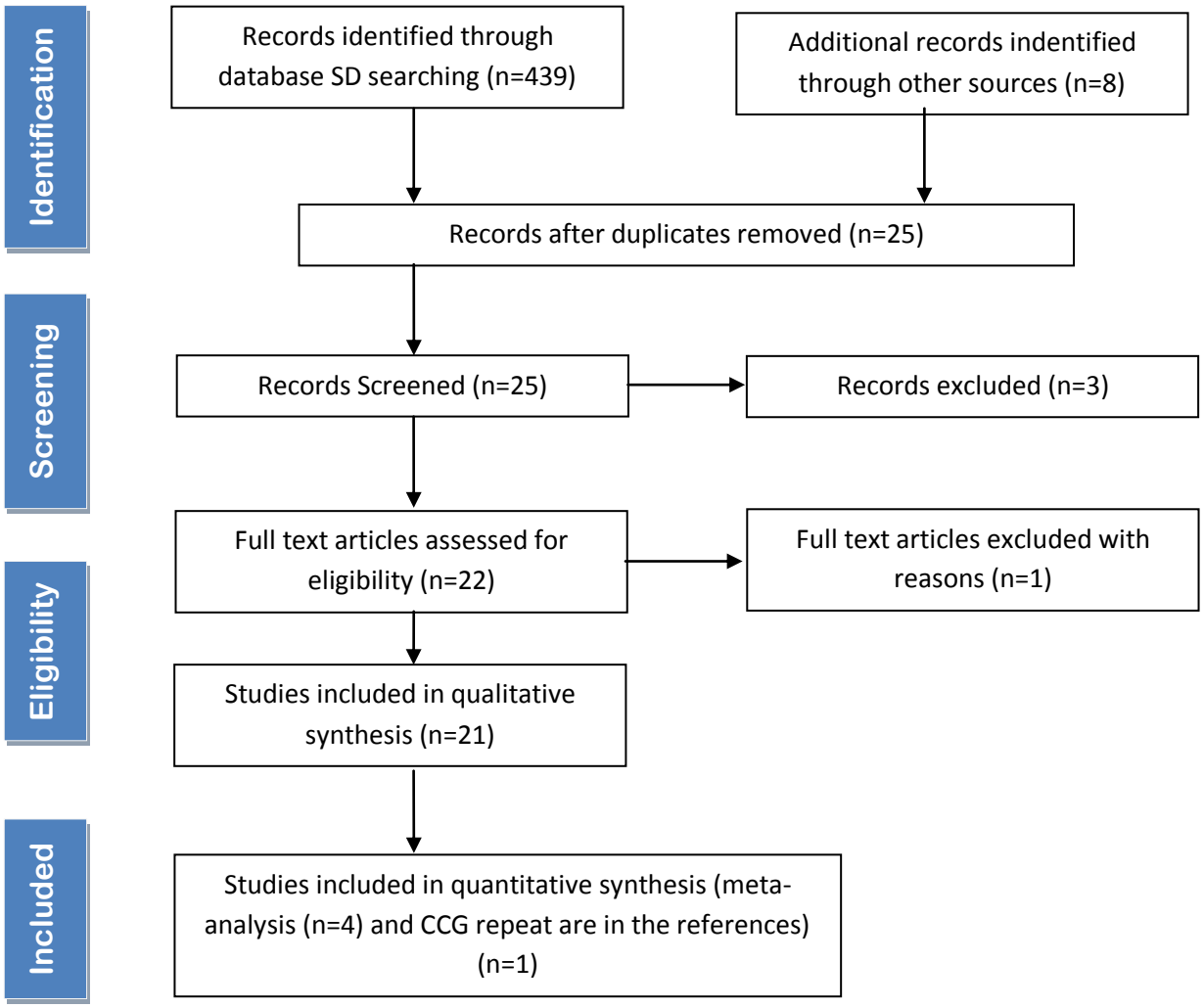

FIGURE 1: Twenty-one references flowchart which support hypospadia-CAH-CYP21 deficiency in association with hypermethylation cases in intermediate/ Small CGG neurosteroid.

TABLE 1
TWENTY-ONE REFERENCES WHICH SUPPORT HYPOSPADIA-CAH-CYP21 IN ASSOCIATION WITH HYPERMETHYLATION DIRECT OR HYPERMETHYLATION CASES ON ANDROGEN BULK

Study, year	Design	Population	Hypospadia/CAH*/CYP21/ Cyt P450	Hypermethylation/ CGG repeat#/CpG island/Journal (J**)
[24]Richards G, 2020	SR/MA	CAH 2D/4D	Hypospadia-CAH	J: Hormones and Behavior
[25]Parsa AA, 2017	Review	CAH	CYP21 varying degree-CAH	J: Steroid Biochem Mol Biol 6p chromosome
[26]Mutalib PKS, 2002	Review	Sport Women	Gender Verification-CAH in Competition	J: Dexa Media. Majalah Kedokteran dan Farmasi
[15]De Jaeger C, 2012	Distribution	Parkinson	Hypospadia	Parkinson: pesticides, sante et longevite
[27]Sharma S, 2013	Review	Parkinson	CAH	Parkinson[#]
[28]May T, 2020	SR & MA	Autism	Sex hormone levels or response	Autism
[29]Knickmeyer R, 2006	Cross sectional	Autism	CAH, Androgens	Autism
[30]Tordjman S, 2018	Review	Autism	Behavioral Syndrome	Reframing Autism 16p11.2 deletion syndrome
[31]Johansson AG, 2012	Case-Control	Bipolar	DHEAS[+] and progesterone	Paranoid, bipolar, mania hypomania[#]
[32]Walsh MJM, 2021	SR	Autism	MRI, fMRI, DTI findings	Autism: Brain-based sex differences
[33]Zhang O , 2016	MA	Bipolar	CAH, Reproductive abnormalities	Valproate in Bipolar, Total and Free Testosterone treated> Non treated Valproate
[34]Joffe H 2006	Clinical Trial	Women Bipolar	Hyperandrogenism	Valproate treated Women with Bipolar disorder
[35]Cesta CE, 2016	Cohort	Nationwide Swedish	POS[++]	Psychiatric disorders, patients and family need mental health care
[36]Rahman Q, 2003	Retrospecti ve	Human sexual orientation	Born Gay Childhood Gender nonconformity	Psychobiology Neurobiological female homosexuality
[37]Okten A, 2002	Case-Control	2D-4D CAH	CYT-21 deficiency, androgen prenatal	J: Early human development
[38]Ingudomnuku l E, 2007	Case-Control	Women ASC	Elevated testosterone	Women Autism Spectrum Conditions (ASC)
[39]Ruta L, 2011	Case-Control	Adult ASC	Increased and rostenedione (precursor of testosterone and estrogen)	Adult with ASC
[40]Rivet TT, 2011	Review	ASD	Gender differention	Autism spectrum disorders (ASD)
[41]Henningsson S, 2009	Case-Control	ASD	Androgen receptor gene -, CAG repeat Prenatal brain expose by Androgen	Autism spectrum disorder (ASD)
[42]Goldman S, 2013	Distribution	Autism	A biosocial ASD	Sex, gender and the diagnosis of autism
[43]Wang C, 2017	Distribution	Human	CAH-CytP45021A2 variant	J: J of biol chem

CAH: Congenital Adrenal Hyperplasia **J: Journal [#]fragile-X/parkinson/autism/bipolar-neurosteroid [+]DHEAS: Dehydroepiandrosterone sulfate (androgen) [++]POS: Polycystic Ovary Syndrome

IV. DISCUSSION

Hypospadia associated with CAH could be seen in table 1). Over 90% CAH individuals are caused by the mutating of the gene on chromosome 6p21.3 which produce 21-hydroxylase.[18,19] The CAH peoples extend over a number of subjects with illness or condition that disrupt due to the systematic function of cortisol precursors hydroxylation. The missing enzyme could be 21-hydroxylase, 17-hydroxilase, 18-hydroxilase, etc. CYP21 is the mainly steroid missing enzyme gene due to mutation, hypermethylation on CpG islands, epigenetic non coding RNAs. The CYP21 mutation or deficiency is in broad spectrum. Similar to blocking, or deficiency in CGG repeat in autism, could be an autism spectrum conditions (ASC) and autism spectrum disorders (ASD).

4.1 Genetic, epigenetic or Pesticide

When a serious autosomal recessive (AR) disorder, which results in affected both alleles, has a high occurrence in a big population, the explanation must lie in either a very high mutation rate or in heterozygote advantage. Yupik Eskimos has been reported resistance or advantage in Influenza B in CAH disorder, an AR genetic disorder.[19]

The sexual orientation based on 46XX for female and 46XY for male, maybe also from the anatomical of urological structure,[12,13,14] and androgen-brain level.[37,41,42] Sexual orientation is the psychological hypospadias-bipolarism is associated with epigenetic hypermethylation. Androgen, disturbance of epigenetic inheritance arranging of gene silencing or enzyme down regulating during development is associated with hypospadias.[2] The human androgen synthesis disruption in CAH associated with cytochrome P450 mutation has been analyzed.[17]

Parkinson, Alzheimer, reproductive tracts, DNA damage, to alter the expression of the gene at the stage of non-coding RNAs, histone deacetylase, DNA methylation system, suggesting their role in epigenetics, and epigenetic cases of Parkinson is reported cause by pesticide.[16] Geographically, in Brazil, Martin-Bell-Renpenning Syndrome; in South American Countries, Escalante's Syndrome; has the clinical figures as this neurosteroid destruction,[1,3] and anatomical face and ear, also 100 years CAH in Swedia[7] and the important of mental health care POS and family in Sweden Cohort Nationwide.[35] Brazil, South America incl. Argentina, South East Asia i.e.: Thailand, Indonesia has a wet and warm climate, also Sweden in North Europe, has rich of lakes and located in peninsula surrounding with water. The CGG repeat expansion – hypermethylation means silence off the gene for protein enzyme incl. hydroxylase or monooxygenase (cytP450), one of the specific isoforms are CYP21. Cytochrome P450-term since the enzyme was find when it was recognized that composing of microsomes that had been chemically reduced and then substituted to carbon monoxide, display a definite peak at 450 nm. The distinctive P450s are then autocratically allocated to Arabic numeral, e.g., CYP21, with the gene encoding CYP21 is *CYP21*, in italic.[20]

4.2 Hormonal level in CYP21 mutation/deficiency

Hypospadia and neurosteroid production is due to CYP21 mutation or CYP21 deficiency.[6] Hypospadia–enzyme hydroxylase 21 deficiency give Congenital Adrenal Hyperplasia has high level of androgen hormonal production. The CGG repeat spectrum-hypermethylation is associated with off gene which produce protein enzyme such as CYP21. Hypospadias – enzyme hydroxylase 21 deficiency which cause CAH (hormonal) is associated with CYP21-CAH neurosteroid production,[25] whereas over 90% CAH is caused by CYP21 deficiency.[18,19]

Made from cholesterol, the cholesterol side-chain split is associated to basic steroid hormone structures. The commons sterol closed chain are recognized by the letters A to D known as cyclopentanoperhydrophenanthrene nucleus. The carbon elements are numbered 1 to 21, beginning from the A ring. A brief record of facts, the estrane classification has 18 carbons (C18). Cortisol (C21H305) and progesterone has 21 carbons known as pregnane group, Testosterone has C19 (androstane group).[20,44] With the 21-hydroxylase, pregnane group go the pathways to become cortisol and aldosterone. With deficiency of 21-hydroxylase (CYP21 gene mutation or methylation), C21 produce androstenedione (C19) a precursor of testosterone (C19) and estrogen (C18). The 21-carbon steroid involves sequential hydroxylation in this adrenal steroidogenesis.

4.3 Cyp21 deficiency and T Cell finds with Science Direct (16)

CAH-severe infection (T cell), and autoimmune-CAH are not the core of this research, but could be a greater amount in many broad spectra of CAH in clinical and population cases. Interactions between sex steroids (androgens, estrogens, progestogens)-corticosteroids and 25- hydroxycholecalciferol, and the immune system may reveal novel approaches as putative treatments of immune-driven disease.[45]

The term activated immunity describe the epigenetic and metabolic medium-term rearranging of the peripheral tissues or in the bone marrow stem cell niche strong related with antigen presentation to T cells, in innate immune cells.[46] Single-walled carbon nanotubes (SWCNTs) inhibit heat shock protein 90 (HSP90) instruction in human keratinocytes and lung fibroblasts,

influencing T cell proliferations.[47] Like in Yupik-Eskimo, who have genetics of resistance to Influenza B in CAH disorder infectious disease,[19] chicken MHC gene has the gene and their contribution in immune responses.[48]

4.4 Hypermethylation and Hypospadia

Geographical Brazil, South America incl. Argentina, SEA incl. Thailand and Indonesia, have a wet and warm climate, has a high prevalence of hypermethylation associated with CGG repeat cases.[3] FMR1 gene 10 y 10-month girl with a history of precocious puberty and a family history of Fragile-X syndrome,[11] is associated with CGG repeat expansion, CpG islands and hypermethylation. A mutual relationship of CpG island and CGG methylation show a knock out of the FMR1 genes, extensively known full mutation have 200-4000 CGG repeat in FMR1 gene are reported as Fragile-X Syndrome subject. A 55-200 CGG repeat size known as permutation is associated with psychiatric disruption, weaken of cognitive proficiencies.

Hypermethylation of CGG repeat represent of the methylated CpG islands.[3] Upstream CGG repeat in FMR1 gene, known as the 65-75 CpG, become stable DNA methylation boundaries, in premutation carriers. Cases of hypermethylation as LGBT in high moisturized and warm countries have high prevalence including Sweden Europe with much lakes and the land located surrounding sea water (peninsula). Transexual operation are high in Bangkok.[3] In both gender, homosexuality is correlated with childhood sexual difficult to determinate. The neurobiological underlying support of preferred gender targets and strongly associated of female homosexuality.[36] Hypermethylation associated with CGG repeat has been reported in many cases incl. LGBT, Fragile-X Syndrome, Parkinson, psychiatric diseases.[1,3,23] Repeat CGG associated with Parkinson, bipolar, autism support epigenetic change of DNA methylation is strong related with hypospadias in a case-control study.[2]

V. LIMITATION

The psychosocial and biosocial of psychological, culture, religion, childhood, puberty, adolescence should have been analyzed in cases associated with hypospadias to find the submerge of the ice berg. This study also not totally have done it, however, sentential logic (nominal and ordinal)[49] approach has been used for supporting the Bayesian analysis, and network (interval) scale which is a sentential calculus. The other limitation is that the role of wet climate correlated with tropical health and infectious diseases is not yet known widely especially in the transgenic technology.[50] Large natural incubator like tropical rainforest area with high relative humidity climate condition is not mystic and need to be disseminated especially in accordance with climate change, global warming, carbon tax, carbon trading, and green activity.

VI. CONCLUSION

Hypermethylation, CGG repeat, CpG island is associated with steroid genes silencing, is the cause of hypospadias, psycho, neuro, metabolism in wet and warm countries high prevalence, which hide by culture, religion and high social stigmatization.

CONFLICT OF INTEREST

The author declares nothing.

ACKNOWLEDGEMENT

Akademi Ilmu Pengetahuan Indonesia (AIPI) 12 April 2021, which has been brough up the submerged ice berg by saying sexual development disorders is only as the tip of an ice berg. CRID-TROPHID who ask physics department to participate in infectious diseases and tropical health in fighting these health burden. IMERI catalog which launch XGA10053 High Relative-Humidity as the basic condition which should be disseminated to industrial countries. IJSER who published carbon nano tube in industry 4.0 should be support with sociology 5.0 in wet and warm climate area.

FUNDING

CRID-TROPHID which support the funding to complete the practice measurement instrument to high relative humidity for all.

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Beneficial Effects of Nitrogen-Fixing Bacteria on the Growth and the Yield of Corn Cultivated at an Giang Province

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Abstract**—** *A net house and a field experiment were conducted in An Phu district, An Giang Province to assess the effectiveness of bacterial strains applied to the NK7328 corn. Bacterial cultures of Bacillus aryabhattai ADR3 and Klebsiella pneumoniae DNR5, which were isolated from rehabilitated corn soils in the Mekong delta Vietnam, were used in this study.The experimented soil type was silty clay loam, pH 5.64, and total N (0.063%). The recommended fertilizer dose was NPK 180 kg N+135 kg P_2O_5+90 kg K_2O/ha and corresponding to 100% N+100% P_2O_5+100% K_2O. In the treatments, the percentage of nitrogen (% N) increased gradually from 0% N, 25% N, 75% N, 100% N. There were 15 treatments that were growing in pots in the greenhouse, and 20 treatments that were in the randomize complete block designed in the fields experiment. Each treatment had 4 replications.The results of the experiments showed that corns, which inoculated with nitrogen-fixing bacteria, and applied 75% N, helped increasing plant height, stem diameter, leaf color, number of leaves, ear corn height, fresh shoot weight, fresh root weight, dry weight of the plant, amount of N, 1000-seed weight, and the higher yield compared to the corns which applied 100% N and without inoculation. Inoculated corn with Klebsiella pneumoniae DNR5 strains gave a higher yield than inoculated corn with Bacillus aryabhattai ADR3. Thus, inoculating corn with bacterial strains, 25% N could be saved.*

Keywords**— Bacillus aryabhattai ADR3, fertilizer, hybrid corn NK7328, Klebsiella pneumoniae DNR5, Klebsiella pneumoniae HN1, nitrogen-fixing bacteria.**

I. INTRODUCTION

Nitrogen is a very important nutrient source for plants. Supplying nitrogen to plants is very necessary to meet the growth and the development needs of the plants, and partially compensate for the amount of nitrogen that the plants have been absorbed. To increase productivity, farmers often used a lot of chemical nitrogen fertilizers in their fields. That causes many harmful effects on the soil, changing the physicochemical properties of the soil, reducing soil fertility, losing ecological balance, causing pollution due to nitrate loss, and adverse effects on the ecosystem. Application microbial fertilizers in crops can help improving the microenvironment of rhizosphere soil and alleviating the damage of saline stress.

That free nitrogen-fixing bacteria convert nitrogen into NH_3 under the normal physiological conditions due to the activity of the enzyme nitrogenase [1]. Plants absorb nitrogen to synthesize plant protein. Over the past 10 years, several papers mentioned the effects of plant growth promotion of plant-associated bacteria on corn were published. Test pot and field experiments have been conducted in many countries. According to the study of Piromyou et al. (2011) of *Pseudomonas* sp. SUT19 and *Brevibacillus* sp. SUT 47 promoted the germination and the growth of corn plants grown in Leonard jars. In the field experiment, strains Pseudomonas sp. SUT 19 and Brevibacillus sp. SUT 47 mixed with compost could promote the best growth of corn among all treatments and could be applied as PGPR inoculum for forage corn [2]. Seven trains, which belonged to genera *Klebsiella*, *Enterobacter*, and *Pantoea* isolated from sugarcane showed abilities of nitrogen fixation and the production of IAA. These bacterial strains were tested for their abilities to promote plant growth on potted corn plants at 40 days old [3].

The study of Puneet (1998) showed that the *Azotobacter* sp. strain stimulated seed germinating, rooting and the yield of wheat and corn and increased by 10-15% compared to the control [4]. When applying *Azospirillum lipoferum* strain to corn, the results showed that it reduced 50% of the amount of N used for plants, even the corn yield was still guaranteed [5]. Bacterial *Klebsiella pneumoniae* bacteria fix atmospheric nitrogen to supply wheat [6].

Corn (*Zea may* L.) is an important food crop in the global economy. Corn needs a huge amount of fertilizer for its growth, but this cost is high. In addition, the excessive use of inorganic fertilizer will cause pollution, affect the health of humans and animal [7]. A recent trend in agricultural production is how to improve soil fertility, reduce the number of chemical fertilizers, increase bio-fertilizers and to reduce production costs, reduce environmental pollution, contribute to creating safe products, and developed sustainable ecological agriculture. In previous studies, a collection of bacteria-associated with corn, which had the abilities of nitrogen fixation and IAA production, were selected and stored in the microbiology laboratory of Can Tho University, Vietnam. In this study, two strains of well-characterized bacteria, which were selected from the collection, were continued to be evaluated for their abilities of plant growth-promoting on the host plant as corn. Bio-fertilizer, which was composed of bacterial strains and peat, was applied to the experimented corn at the greenhouse and in the field.

II. MATERIALS AND METHODS

2.1 Description of Study Area

A field experiment was conducted at Vinh Truong village, An Phu District, An Giang Province in Vietnam during spring-summer cropping season 2018 and 2019 (September to January). The study area was 10^{o}78'72''East and 105^{o}11'74'' North with a mild climate, and an average annual temperature of 27.93°C, sunshine duration of 213.90 hours, rainfall of 142.7 mm and humidity of 82.25% per month occurring from September to January, Statistical Yearbook of An Giang [8]

2.2 Soil characteristics

Alluvial soil used in the greenhouse experiment was collected at the depth from 0-20 cm, before sowing seeds maize field of Mr. Loi's family, An Phu district, An Giang province. Eighty soil samples were taken in the field (zigzag style).Each soil collection site corresponds to 1 point in the experimental plot, 10 kg were collected and the total weight of 800 kg of soil was collected for the experiment in the greenhouse. The soil was transported to the lab and air-dried, sheltered, naturally in a dry place. Then the soil was mixed evenly into a uniform mass, minced and screened through a mesh (2x2 mm size).500 g sample of mixed soil were put it in a glass jar. Three replication were used. These samples jars were kept at 18-25°C in the plastics box and transferred to the laboratory and were used to do soil physicochemical analysis immediately in Can Tho University.

The pH and EC of the soil were determined in distilled water using a soil/liquid ratio of 1:2.5. After stirring for 30 minutes the pH value was read using a glass electrode pH meter. Total Nitrogen was determined by the Kjeldahl method [9]. Useful nitrogen NH_4^+ and NO_3^- in soil has determined by colorimetric method at 650 nm for NH_4^+ and 540 nm for NO_3^-. Total phosphorus was measured by extraction method HCl: HNO_3 with the ratio 3:2. Phosphorus dissolved available in the soil: measured by method of Olsen. Mechanical analysis soil textured sand, silt and clay by method hydrometer of Gavlak. Soil sample was analysed by Department of Soil Sciences, College of Agriculture, Can Tho University. Some physical and mechanical properties were showed in Table 1.

TABLE 1
PHYSICAL AND CHEMICAL PROPERTIES OF SOILS OR THE EXPERIMENTAL SITE

Soil depth (cm)	pH	EC (mS/cm)	N			P		Soil texture		
			Total N (%)	NH_4^+ mg/kg	NO_3^- mg/kg	Total P_2O_5 (%)	dissolved P mg/kg	Sand (%)	Heavy (%)	Clay (%)
0-20	5.64	5.64	0.063	2.74	36.20	0.16	89.50	18.86	62.68	18.46

Origin: Soil analysis Lab. Department of Soil Sciences, college of Agriculture, Can Tho University

2.3 Bacterial strains, mixed microbial fertilizers, experimental corn

Bacterial cultures of *Bacillus aryabhattai* ADR3 and *Klebsiella pneumoniae* DNR5, which were isolated from rehabilitated roots of corn in the Mekong delta Vietnam, were used in this study. All the isolates were previously identified based on 16S rRNA gene sequence similarities and were also characterized based on morphological and biochemical properties. *Klebsiella pneumonia* HN1) from Biotechnology R&D Institute, Can Tho University. Bacterial cultures were produced in Nfb broth for 4 days, reaching to $>10^9$ CFU/mL. Carrier material peat was dried, ground by pestle and porcelain mortar, sieved through rails with the size of 2x2 mm, and then disinfected at 121°C for 20 minutes. A mixture of microbial fertilizers including a carrier with bacteria culture. VK1 biofertilizer was a peat carrier mixture with *Bacillus aryabhattai* ADR3. VK2 fertilizer

was a peat carrier mixture with *Klebsiella pneumoniae* DNR5. VKDC fertilizer was a peat carrier mixture with *Klebsiella pneumonia* HN1. Hybrid corn NK7328 seed was used in all treatments of the experiment.

2.4 Treatments and Experimental Design

2.4.1 Experimental of corn grown in a greenhouse

Greenhouses plot size was 9 m x 9 m = 81 m^2 covered by insect nets. The experiment contained 60 pots. The cross-sectional area on the top is 30 cm, height 25 cm, bottom 24 cm of each pot. Each pot was cleaned and disinfected with 75° alcohol, containing 12 kg of dry ground soil. The evaluation of nitrogen fixation ability of bacterial strains was done in a completely randomized design, with 4 replications at 15 treatments. Row to row distance was 75 cm. Corn kernels were disinfected with 75° alcohol, soaked for 2 minutes, removed alcohol, soaked in H_2O_2 (3%) for 3 minutes, then washed 4 times with sterilized distilled water. Continue soaring in distilled water for 12 hours, incubating for 3 hours. Mix 10 g of microbial fertilizer with corn kernels, incubated for 1 hour. Then sow 3 seeds into each pot according to the respective treatments, 7 days after sowing (DAS), kept 1 plant/pot. The temperature difference between the experiment in the greenhouse and the experiment in the field is about 1-2°C.

There were 15 treatments for growing pots in greenhouses as follows 0% N (control without nitrogen, biofertilizer) [Treatment 1], 0% N+VK1 (VK1) [Treatment 2], 0% N+VK2 (VK2) [Treatment 3], 25% N (45 kgN) [Treatment 4], 25% N+VK1(45 kgN+VK1) [Treatment 5], 25% N+VK2 (45 kgN+VK2) [Treatment 6], 50% N (90 kgN) [Treatment 7], 50% N+VK1 (90 kgN+VK1) [Treatment 8], 50% N+VK2 (90 kgN+VK2) [Treatment 9], 75% N (135 kgN) [Treatment 10], 75% N+VK1 (135 kgN+VK1) [Treatment 11], 75% N+VK2 (135 kgN+VK2) [Treatment 12], 100% N (180 kgN) [Treatment 13], 100% N+VK1 (180 kgN+VK1) [Treatment 14], 100% N+VK2 (180 kgN+VK2) [Treatment 15]. Treatments 1, 2 and 3 were without NPK, Other all treatments were supplied (from 4 to 15) 135 kgP_2O_5/ha and 90 kgK_2O/ha.

The recommended fertilizers were NPK: 180 kg N+135 kg P_2O_5+90 kg K_2O/ha (equivalent to 100% N+ 100% P_2O_5+100% K_2O). Treatments were fertilized with the percentage of nitrogen (% N) increasing gradually from 0% N, 25% N, 75% N, 100% N. The fertilizer schedule for each treatment was different. There were 4 times of fertilization including the first time the first time [10 days after sowing (DAS) with 30% N+30% P_2O+20% K_2O], the second time 20 DAS with 30% N+20% P_2O+30% K_2O, 3rd time (40 DAS with 40% N+40% P_2O+30% K_2O), 4th time (60 DAS with 10% P_2O+20% K_2O). All treatments were sprayed with the same amount of water at the spray location. Pest protection followed the guidelines of the Department of Plant Protection, An Giang province. Use according to Syngenta's NK7328 corn pest control solution. Depending on the growth stage of corn, all treatments were similar in caring, spraying insecticides, and controlling corn diseases.

2.4.2 Experimental of corn grown in a field

The experiment was arranged with a randomized complete block design with four replications. The experimental area had a total area of 0.35 ha. There were 80 plots and each plot was 21 m^2 (7 m x3 m) square meters, rows to rows of 70 cm and in rows of 25 cm. The number of plants was about 57,000 plants/ha. The ground was plowed to a depth of about 40 cm. The horizontal surface was 3 m and 30 cm high (measured from the surface to the bottom of the ditch). The experiment was four blocks which a horizontal dike 60 cm and a 70 cm ditch surrounding the treatments. The experimental area was protected by two rows of planted corn, with a 1.2 m width row. Mixing 120 g of microbial fertilizer with 1.5 kg of germinated corn kernels (disinfected surface as described in the greenhouse experiment) for each type of fertilizer, incubating for 1 hour, then sowing every 3 seeds in 1 hole, at 7 DAS only 1 plant was kept.

There were 20 treatments for growing in the field as follows 0% N (control without nitrogen, biofertilizer) [Treatment 1], 0% N+VK1 (VK1) [treatment 2], 0% N+VK2 (VK2) [Treatment 3], 0% N+VSDC (VSDC) [Treatment 4], 25% N (45 kgN) [Treatment 5], 25% N+VK1 (45 kgN+VK1) [Treatment 6], 25% N+VK2 (45 kgN+VK2) [Treatment 7], 25% N+VKDC (45 kgN+VKDC) [Treatment 8], 50% N (90 kgN) [Treatment 9], 50% N+VK1 (90 kgN+VK1) [Treatment 10], 50% N+VK2 (90 kgN+VK2) [Treatment 11], 50% N+VKDC (90 kgN+VKDC) [Treatment 12], 75% N (135 kgN) [Treatment 13], 75% N+VK1 (135 kgN+VK1) [Treatment 14], 75% N+VK2 (135 kgN+VK2) [Treatment 15], 75% N+VKDC (135 kgN+VKDC) [Treatment 16], 100% N (180 kgN) [Treatment 17], 100% N+VK1 (180 kgN+VK1) [Treatment 18], 100% N+VK2 (180 kgN+VK2) [Treatment 19], 100% N+VKDC (180 kgN+VKDC) [Treatment 20]. Treatments 1, 2, 3 and 4 were without NPK.

Other treatments were supplied (from 5 to 20) 135 kgP_2O_5/ha and 90 kg K_2O/ha. Weeds were removedat 9, 19, 39, and 59 DAS. The experiment was watered by rainy or flooded fertilizer application as mentioned in section 2.4.1

2.5 Yield component and grain yield

Each treatment was four rows with 54 corn plants included rows 1, 4 (protection rows), and 2, 3 (lines for monitoring indicators). Twelve corn plants were marked with a zigzag line in 2 rows inside the tracking plot. Ripe corn harvested at 105 DAS. The monitored indicators of growth and yield included plant height, stem diameter, leaf color, number of leaves, ear corn height, dry biomass, total N, length of corn ear, a diameter of corn ear, number of row/ corn, number of seed/ row, the weight of one thousand grains, actual yield [10]. Corns were collected at 60 DAS and 105 DAS. Leaf color (Spad index): Chlorophyll meter Minolta Spad 502 Plus were used. Spad readings were taken on three young fully expanded leaves (above silking) and index leaf (first leaf below the ear) according to the recommendation of Costa et al. [11]. Four Spad measurements were taken per leaf, on either side of the midvein, at mid-silking near the midpoint of the leaf blade about 20 cm from the stalk and an average of absolute Spad values were recorded. Twenty plants were measured for each plot. Sample plants were dried at 55°C, approximately 5 days until weight was constant. Dry matters were weighed to record the biomass of each part as leaf, stem, root, grains, biological yield, and total nitrogen analysis. Samples were analysed at the Genetics laboratory of Dinh Thanh agricultural research center.

Actual yield: At maturity 42 corn plants per plot were collected. The grain yields were dried to 14% moisture content. Grains per plot were weighed and then converted to its hectare equivalent.

$$Actual\ yield\ \left(\frac{t}{ha}\right) = \frac{P_1}{S_0} \times \frac{P_2(100 - A_0)}{P_3(100 - 14)} \times \frac{1000\ m^2}{1000}$$

Calculation of yield is based on the formula (t/ha)

P_1: Fresh fruit weight of 42 plants in a plot

A_0: Grain moisture when weighing sample grain.

S_0: The planted area of 42 plants, at a density of 57,000 trees/ha (7.37 m^2).

P_2: Grain weight of 10 corn samples (weighed when measuring grain moisture"A_0").

P_3: Fresh corn weight of 10 corn samples.

$$\frac{(100 - A_0}{(100 - 14)} = \text{Conversion factor of NTT at 14\% humidity}$$

1000: is the number to convert from kg to tons. 10,000 is the number to convert from m^2 to hectare (ha).

2.6 Data analyses

The data was saved and calculated with Microsoft Office Excel 2013 and statistically tested by one-factor analysis of variance. All analyses conducted using the Statgraphics centurion xv software. The experimental results were from plant height, stems diameter, index of leaf chlorophyll, number of leaves, dry matter weight, 1000 seeds weight and yield corn considered significantly different at $P<0.05$, Total N considered significantly different at $P<0.01$.

III. RESULTS AND DISCUSSION

3.1 Evaluation of the effect of nitrogen fixing bacteria on the growth of corn under greenhouse conditions

3.1.1 The Growth of corn in the greenhouse at 60 DAS

The results showed that the average height of corn plants in the VK1 or VK2 treatments was different from the average height of corn in the non-VK1, VK2 treatments at the same level of nitrogen (Table 2). At 50% N, 75% N with the VK1 and VK2 treatments, the height of corns was as similar as the height plants in the treatment that applied only 100% N. Similarly, the treatments at level 75% N+VK1 had a significantly greater difference in root internode diameter, chlorophyll index, and number of leaves than ones only applied nitrogen fertilizer at the same level. The treatments at 75% N+VK1 were not significantly different from the treatment with only 100% NPK strain without bacterial (Figure 1).

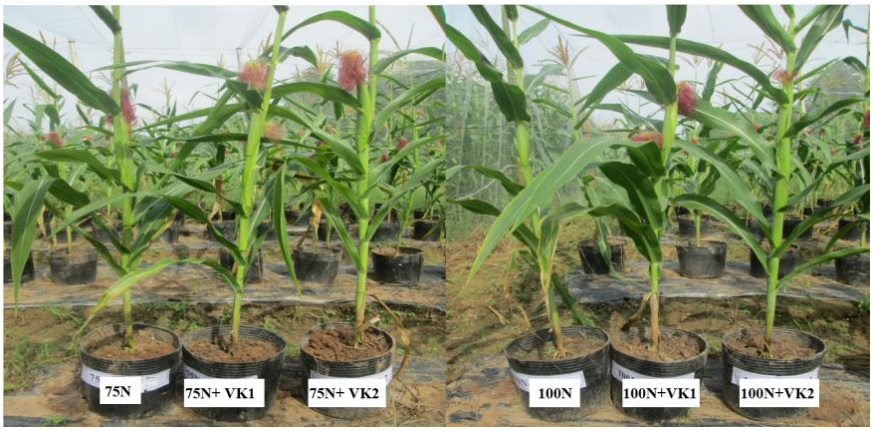

FIGURE 1: Treatments of 75% N and 100% N were grown pots in a greenhouse at 60 DAS

TABLE 2
SOME AGRICULTURAL PARAMETERS OF CORN AT 60 DAS AFTER SOWING AT GREENHOUSE

Treatment	Plant height (cm)	Stem Diameter (cm)	Leaf color (Spad index)	Number of leaves
0% N	105.50 g	1.68 g	22.78 h	17.50 f
0% N+VK1	116.45 f	2.04 f	26.23 g	18.25 e
0% N+VK2	114.10 fg	1.92 f	25.38 g	18.00 ef
25% N	148.18 e	2.17 e	28.98 f	18.50 de
25% N+VK1	160.96 d	2.59 c	31.10 ef	19.00 cd
25% N+VK2	161.40 d	2.42 cd	30.25 ef	19.25 bc
50% N	166.85 cd	2.66 bc	31.80 e	19.00 cd
50% N+VK1	176.55 ab	2.76 b	40.25 bc	19.25 bc
50% N+VK2	175.98 abc	2.78 b	39.85 c	19.25 bc
75% N	171.13 bc	2.71 b	35.73 d	19.25 bc
75% N+VK1	182.48 a	3.16 a	42.43 ab	20.00 a
75% N+VK2	180.28 ab	3.14 a	42.10 abc	19.75 ab
100% N	183.64 a	3.17 a	43.08 a	19.75 ab
100% N+VK1	182.55 a	3.15 a	42.93 a	19.75 ab
100% N+VK2	184.70 a	3.12 a	43.45 a	19.75 ab
F	**71.09***	**134.77***	**79.63***	**10.96***
C/V (%)	**4.02**	**3.24**	**4.71**	**2.31**

Means within a column followed by the same letter/s are not significantly different at p < 0.05

According to research by Adoko, bacterial fertilizer contained *Pseudomonas putida* showed significant improvements in height (49.49%), stem diameter (32.70%), leaf area (66.10%) on ferrallitic soil with the application of biostimulant compared to the control [12]. *Bacillus aryabhattai* S10 reported increase in water contents (19% in mungbean and 32% in corn), chlorophyll "a" and chlorophyll "b" contents in mungbean and corn crops as compared to uninoculated control [13].

3.1.2 Agronomic index and biological yield of corn at harvest stage

At the treatment fertilized 75% N with VK1 or VK2, the plant height was not significant difference as compared to the 100% N treatment without any bacterial strains. Similarly, first ear corn height and fresh stem leaf weight of corn, only fertilized with nitrogen at 0% N, 25% N, 50% N, 75% N, had lower values and statistically significant differences compared to corn fertilized with VK1 or VK2 strains and inorganic nitrogen 0%-75%. Corn with VK1 or VK2 strains fertilized with 75% N had the same first ear corn height, fresh stem weight, and root weight as the positive control treatment with only 100% NPK and no bacterial strains. The fresh root weight of corn plants, which had significantly different at 25% N, 50% N, 75% N was lower than ones of with nitrogen fertilizer combined with bacterial strain VK1 or VK2 (Table 3).

The biomass of corn's dry matter in the treatments with the VK1 or VK2 strain was significantly different from ones in the treatments that applied only inorganic fertilizer at the same amount of nitrogen fertilizer without the bacterial strain. This result was similar to the result in the experiment of Montañez and Sicardi (2013), which had the ability to fix nitrogen of 4 strains of bacteria on the potted corn [14]. The results showed that the dry matter weight of the root increased to 42-53% and the dry matter weight of the leaves increased to 22%-50% compared to the control at the 90 DAS-old corn stage. When adding the endogenous bacteria *Lactococcus lactis* and *Klebsiella* sp. to corn plants 25% of the amount of NPK fertilizer

could be saved and help to increase the dry biomass of rhizomes as equivalent to the corn which fertilized 100% NPK without bacteria strains [15].

TABLE 3
EFFECTS OF VK1 AND VK2 FERTILIZERS ON PLANT HEIGHT, EAR CORN HEIGHT, FRESH STEM AND LEAF, ROOT WEIGHT AND DRY BIOMASS CORN AT THE STAGE OF HARVEST

Treatment	Plant height (cm)	Ear corn height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry Biomass (g)
0% N	106.50 h	33.28 g	99.63 g	67.10 j	45.82 j
0% N+VK1	116.45 g	40.28 f	108.18 f	71.25 ij	49.31 ij
0% N+VK2	114.10 gh	39.13 f	113.17 f	74.61 i	55.77 i
25% N	148.18 f	61.35 e	173.43 e	108.58 h	148.15 h
25% N+VK1	160.96 e	72.18 d	199.53 d	131.13 f	171.81 f
25% N+VK2	161.40 e	71.63 d	204.23 d	119.18 g	163.63 g
50% N	166.85 de	72.58 d	203.35 d	128.25 f	172.72 f
50% N+VK1	176.55 bc	77.55 c	261.00 b	179.46 cd	260.93 d
50% N+VK2	175.98 bc	79.08 bc	250.25 c	176.25 d	248.18 e
75% N	171.13 cd	75.85 cd	245.45 c	169.56 e	247.44 e
75% N+VK1	182.48 ab	82.65 ab	269.55 a	181.31 bc	272.43 c
75% N+VK2	180.28 ab	82.98 ab	268.88 ab	185.71 ab	277.36 bc
100% N	183.64 ab	86.10 a	275.43 a	183.76 abc	286.28 a
100% N+VK1	182.55 ab	83.08 ab	273.25 a	181.16 bc	283.89 ab
100% N+VK2	184.70 a	87.38 a	271.85 a	187.75 a	285.02 ab
F	**97.98***	**108.63***	**534.01***	**795.15***	**1051.73***
C/V (%)	**3.41**	**4.94**	**2.59**	**2.27**	**2.81**

Means within a column followed by the same letter/s are not significantly different at p < 0.05

3.1.3 Components of yield and theoretical yield of corn kernels

Corn, fertilized with 50% N, 75% N without bacterial strain, had its length shorter than the one which fertilized the same amount of nitrogen and with the VK1 or VK2 (Figure 2). The length and the stalk diameter of the corn with 75% N fertilization and with VK1 or VK2 strains were not statistically different from those of 100% NPK (Table 4).

The number of rows of seeds/corns and the number of seeds/rows of corn fertilized with 50% N with VK1 strain and 50% N with VK2 strain were not statistically different from those of corn fertilized with only 100% NPK and without bacterial strain.

FIGURE 2: Effects of VK1 and VK2 fertilizers on the length of corn ear in greenhouse

TABLE 4
EFFECTS OF VK1 AND VK2 FERTILIZERS ON LENGTH OF CORN EAR, DIAMETER OF CORN EAR, NUMBER OF ROW SEAD, WEIGHT OF ONE 100 GRAINS, GRAIN YIELD AT HARVEST

Treatments	Length of corn ear (cm)	Diameter of corn ear (cm)	Number of row/ corn	Number of seed/ row	Weight of 100 grains (g)	Grain yield/ pot (g)
0% N	11.43 g	2.24 f	6.00 d	9.50 f	17.67 h	10.37 h
0% N+VK1	12.48 f	2.78 e	9.50 c	12.00 e	19.50 g	23.33 g
0% N+VK2	13.28e	2.82e	9.00 c	11.50 e	19.57 g	22.29 g
25% N	13.43 e	3.14 d	12.50 ab	20.25 d	21.24 f	53.48 f
25% N+VK1	15.23 d	3.29 c	13.00 b	24.25 c	23.91 de	72.29 e
25% N+VK2	15.40 d	3.25 cd	13.00 b	23.75 c	23.76 de	73.67 e
50% N	14.90 d	3.17 d	13.00 b	23.25 c	23.21 e	70.26 e
50% N+VK1	17.95 b	3.67 b	13.50 ab	28.75 ab	26.31 abc	106.17 bc
50% N+VK2	17.80 b	3.58 b	13.50 ab	28.50 ab	25.55 bc	100.06 cd
75% N	16.95 c	3.64 b	13.50 ab	27.00 b	25.17 cd	91.68 d
75% N+VK1	19.05 a	3.87 a	15.00 a	29.50 a	27.14 a	121.18 a
75% N+VK2	19.23 a	3.92 a	15.00 a	28.75 ab	27.15 a	113.36 ab
100% N	18.95 a	3.91 a	15.00 a	29.25 a	26.86 ab	116.86 ab
100% N+VK1	19.08 a	3.90 a	15.00 a	28.75 ab	27.55 a	120.54 a
100% N+VK2	19.13 a	3.92 a	15.00 a	29.27 a	27.32 a	116.56 a
F	**126.56***	**160.12***	**24.56***	**118.16***	**42.69***	**95.21***
C/V (%)	**2.97**	**2.37**	**8.34**	**5.53**	**4.13**	**9.74**

Means within a column followed by the same letter/s are not significantly different at p < 0.05

The weight of 100 seeds had an average value ranging from 17.67 g to 27.55 g. In the control treatment 0% N (Control), the weight of 100 seeds was lowest and significantly different from other treatments. In the treatment with 75% N+VK1 or 75% N+VK2 fertilizer, the weight of 100 seeds was significantly different and respectively higher at 1.08 times than corn which only fertilized 75% N and was not significantly different from the treatment with 100% N. The mean corn kernel yield had an average value ranging from 10.37 g (0% N) to 121.18 g (100% N+VK1). Corn yield in control treatments without fertilizer and without strain (0% N) was completely lower than those of the treatments with fertilizer and lower than the treatment with bacterial strains. At the same level of nitrogen fertilization of 0% N, 25% N, 50% N, 75% N with VK1 or VK2 of bacterial strain had a higher grain yield than the treatment with only nitrogen fertilizer. The treatment 75% N fertilization with bacterial strains VK1 or VK2 had a high yield, respectively 1.32 or 1.24 times higher than the treatment with only 75% N nitrogen fertilizer. Treatments 75% N+VK1 or 75% N+VK2 fertilizer were not significantly different and were equivalent in yield of the treatment with 100% NPK and without bacteria. This result proved that when applying 75% N+VK1 or 75% N+VK2 helped the plant to grow better and the grain yield/pot was similar to ones with only 100% NPK nitrogen fertilizer without bacterial strain.

This result of plant height, the length, the diameter of corn, the number of seeds/corn, and 1000-grain weight similarly increased in the treatments with the nitrogen-fixing bacteria *Gluconacetobacter diazotrophicus* with 75% N compared to when the treatment was fertilized only 100% N and without bacterial strain [16]. Inoculation with rhizobacteria could be efficiently improved the growth and grain yield of corn, reduced fertilizer costs and reduced the emission of the greenhouse gas, N_2O as well as reduced leaching of NO_3^-, N to groundwater even [17].

Six potential corn endophytic bacterial isolates were positive influenced on corn plants in a pot-house experiment under glasshouse conditions. Endophytic bacterial as *Pantoea dispersa*, *Pantoea* sp., *Klebsiella variicola*, *Lactococcus lactis*, *Bacillus cereus*, and *Staphylococcus hominis* inoculated corn, made increased the growth and yield of corn and save about 25% NPK of chemical fertilizers. *Lactococcus lactis* inoculation influenced maximum, followed by *Klebsiella variicola* isolates [18]. The study results of Ahmad revealed that inoculation of *Bacillus aryabhattai* S10 made increasing of nodules number, and nodules fresh and dry weight of mungbean compared to the control. This inoculation of bacterial strain also increased nitrogen (N) concentration up to 142%, phosphorus (P) concentration up to 90%, and potassium (K) concentration up to 71% in shoots of mungbean and corn crops. In conclusion, the tested strain *Bacillus aryabhattai* S10 had the potential to use as such promising bio-inoculants that maximize plant growth and nutritional status of the crop in sustainable crop production [13].

3.2 Evaluating the effectiveness of nitrogen-fixing bacteria on corn grown in the field

3.2.1 Growth of corn in the field at 60 DAS

The study results showed that the plant height, stem internode diameter, and chlorophyll index of corn leaves, which applied with 75%VN+VK1 or 75% N+VK2 fertilize, were significantly different than those of the treatment with only 75% N without strain, and were not significantly different from the 100% NPK fertilization treatment. Similarly, corn in the fertilized nitrogen treatments with VK1 or VK2 fertilizes had the number of leaves as same as those in the 100% NPK treatment without bacteria strain. In the treatment 50% N+VK2, the chlorophyll index and leaf were higher than those of the other treatments (Table 5). Thus, corn at 60 DAS required a large amount of additional nitrogen fertilizer to grow and develop. Corn with VK1 or VK2 strain helped reduce 25% N but also increased the growth of corn as same as the ones fertilized with 100% N.

TABLE 5
SOME AGRICULTURAL PARAMETERS OF CORN AT 60 DAS AFTER SOWING AT FIELD

Treatment	Plant height (cm)	Stem Diameter (cm)	Leaf color (Spad index)	Number of leaves
0% N	96.94 g	1.71 g	21.29 f	17.48 f
0% N+VK1	98.77 g	1.87 f	21.61 f	17.75 ef
0% N+VK2	99.02 g	1.86 f	21.37 f	18.02 ef
0% N+VKDC	98.77 g	1.90 f	22.02 f	18.20 e
25% N	141.25 f	2.11 e	29.53 e	17.18 d
25% N+VK1	144.94 e	2.18 e	29.36 e	19.24 d
25% N+VK2	143.10 ef	2.16 e	29.68 e	19.23 d
25% N+VKDC	144.43 e	2.18 e	29.3 e	19.20 d
50% N	168.99 d	2.57 d	31.70 d	19.16 d
50% N+VK1	179.83 c	2.70 bc	31.80 d	19.35 cd
50% N+VK2	184.34 b	2.76 b	33.74 c	20.18 a
50% N+VKDC	182.23 bc	2.70 b	31.12 d	19.38 bcd
75% N	183.44 b	2.63 cd	37.37 b	19.65 a-d
75% N+VK1	197.24 a	2.92 a	40.02 a	20.08 a
75% N+VK2	195.88 a	2.95 a	40.44 a	20.11 a
75% N+VKDC	196.46 a	2.90 a	40.05 a	20.00 a
100% N	195.39 a	2.94 a	40.84 a	20.10 a
100% N+VK1	195.22 a	2.95 a	41.04 a	19.98 abc
100% N+VK2	196.71 a	2.92 a	41.25 a	20.15 a
100% N+VKDC	197.45 a	2.96 a	41.36 a	20.13 a
F	**1922.45***	**214.66***	**224.22***	**14.34***
C/V(%)	**6.93**	**2.44**	**2.98**	**2.32**

Means within a column followed by the same letter/s are not significantly different at p < 0.05

Plant growth-promoting bacteria (PGPB), which was inhabiting the phyllosphere, improved the growth and yield of plants by producing natural growth regulators. There was a beneficial effect of most bacterial treatments, especially on the chlorophyll content and chlorophyll content index of inoculated plants [19]. Bacterial strain *Azotobacter* significantly increased plant height, a number of leaves corn [20]. *Azospirillum brasilense* supported development of corn, increased plant height and stem diameter [21]. This result was in agreement with the results of Amogou et al. when they applied bacterial strain *Bacillus panthothenicus* and got an increasing of 49.65% in stem diameter at the plants treated, and *Pseudomonas putida*+ 50% NPK was 32.08% was greater than those of the non-inoculated control [22]. Mucilage of Sierra Mixe corn harbors native diazotrophs and inoculated *Bacillus unamae* confirmed a significant enrichment of 15N in chlorophyll (converted to pheophytin for analysis) of these roots compared to the negative controls [23].

3.2.2 Agronomic index and biological yield of corn grown in the field at harvest stage

In our study, the values from 97.41 cm (0% N) to 201.26 cm (100% N+VKDC) were obtained from plant lengths (Table 6). Plant height of corn with 50% N, 75% N, and inoculated nitrogen bacteria (VK1 or VK2 or VKDC) had higher values and statistically significant differences compared to those applied fertilized at the same amount of 50% N and 75% N. In particular, the treatments 50% N and 75% N, which inoculated with this bacterial fertilizer, had no statistically significant difference in height compared to the treatment of 100% N. The Ear corn height had a contribution to the easy transfer of nutrients to the fruit to increase the size of the fruit. Corn plants, which had the first ear corn height higher, produced larger

fruits. The treatments 50% N, 75% N with VK1 or VK2 or VKDC gave higher values and statistically significant differences compared to those which was fertilized at the same 50% N and 70% N. In addition, treatments 50% N+VK2 had the ear corn height of corn highly different from the treatment with 50% N+VK1 strain at the same 50% N fertilizer level. The results also showed that corns, which only were fertilized with 75% N with bio- fertilize, had the same height as of 100% NPK.

TABLE 6
EFFECTS OF VK1 AND VK2 FERTILIZERS ON PLANT HEIGHT, EAR CORN HEIGHT, DRY AERIAL BIOMASS AND TOTAL N OF CORN AT THE STAGE OF HARVEST

Treatment	Plant height (cm)	Ear corn height (cm)	Dry Aerial Biomass (t/ha)	Total N (%)		
				Leave	Stem	Grain
0% N	97.41 f	48.49 h	15.95 h	0.4 k	0.22 j	0.58 e
0% N+VK1	100.47 f	50.17 g	19.73 g	0.64 i	0.34 hi	0.79 d
0% N+VK2	100.72 f	50.13 g	19.82 g	0.64 i	0.37 gh	0.78 d
0% N+VKDC	100.46 f	50.28 g	19.79 g	0.55 j	0.35 hi	0.81 d
25% N	142.99 e	67.82 f	21.87 f	0.95 h	0.29 i	1.10 c
25% N+VK1	146.93 e	68.48 f	25.21 e	1.47 fg	0.34 hi	1.08 c
25% N+VK2	145.08 e	68.50 f	25.87 de	1.57 e	0.39 fgh	1.09 c
25% N+VKDC	146.45 e	68.58 f	25.25 e	1.44 g	0.42 efg	1.07 c
50% N	170.97 d	77.66 e	24.46 e	1.55 ef	0.46 b-e	1.28 b
50% N+VK1	182.69 c	81.65 d	29.73 c	1.71 bcd	0.47 a-e	1.28 b
50% N+VK2	187.18 bc	84.72 c	30.20 c	1.69 cd	0.45 cde	1.30 b
50% N+VKDC	191.85 b	82.56 d	29.00 c	1.65 d	0.43 def	1.31 b
75% N	186.71bc	85.25 c	27.24 d	1.66 d	0.42 efg	1.47 a
75% N+VK1	200.79 a	96.49 ab	36.17 ab	1.77 abc	0.49 abc	1.49 a
75% N+VK2	199.39 a	97.04 a	37.01 a	1.78 ab	0.52 a	1.48 a
75% N+VKDC	199.96 a	95.63 b	35.78 ab	1.79 ab	0.49 abc	1.46 a
100% N	199.27 a	96.55 ab	34.95 b	1.79 ab	0.51 ab	1.48 a
100% N+VK1	199.09 a	96.46 ab	36.10 ab	1.80 a	0.50 abc	1.49 a
100% N+VK2	200.54 a	97.10 a	36.61 a	1.80 a	0.49 abc	1.47 a
100% N+VKDC	201.26 a	96.19 ab	35.49 ab	1.79 ab	0.49 a-d	1.46 a
F	**341.41***	**1508.53***	**136.06***	**493.03****	**27.40****	**369.73****
C/V (%)	**2.57**	**1.18**	**4.12**	**3.10**	**7.44**	**2.46**

Means within a column followed by the same letter/s are not significantly different at Means within a column followed by the same letter/s are not significantly different at 5% (*) p< 0.05 or 1% (**) p< 0.01

The dry aerial biomass varied from 15.95 t/ha (0% N) to 37.01 t/ha (75% N+VK2). In the treatment 0% N, corn's dry leaf weight was very small and trendily increased when the plant was applied more % N. In the treatments that fertilized only 0% N, corn had the lowest biomass. In the treatments 75% N+VK1 or 75% N+VK2, the corn had the same biomass as those in the treatment with only 100% NPK without bacterial strain. Control corn had lower nitrogen deficiency content in leaves, stems, and seeds than those of other treatments. The treatment 50% N+VK1 in the leaves and stems had a high total nitrogen content and was not statistically different from the treatment 100% NPK. Similarly, the treatment 75% N with bacterial fertilizer had no statistical difference compared to the treatment of 100% NPK.

The experimental results of Lopez-Ortega showed that the endogenous bacteria *Klebsiella variicola* increased the biomass of corn's shoots and roots up to 39% compared to the control without bacterial strain, and at once accumulated 10% more phosphate in the plant [24]. Inoculation with *Azotobacter* significantly increased ear height, number of ears per m^2, ear length of corn [20]. Inoculation with *Azospirillum brasilense* had a positive effect on the characteristics of yield and productivity of corn, independent of growing season and hybrid used. The agronomic characteristics of corn seeds, which inoculated with *Azospirillum brasilense*, were grown on black oat and ryegrass straw and managed under different doses of nitrogen (0, 50 and 100 kg/ha). In two agricultural seasons (2012/2013 and 2013/2014) under drought conditions, *Azospirillum brasilense* to supported the corn development increased plant height and ear height [21]. The foliar spray of corn treatments included *Bacillus subtilis+Stenotrophomonas maltophilia*, *Bacillus megaterium*, and *Enterobacter hormaechei* significantly increased the shoot dry weight by 9.53, 8.73 and 6.00% compared to the control [19]. The same results were found in the experiment of Amogou, bacteria where bacterial strains *Serratia marcescens+50%* NPK increased the best results of height (41.09%), fresh underground biomass (217.5%), dry aboveground biomass (213.34%) and dry underground biomass (93.82%) compared to

the control [22]. The co-inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 performed that there was the highest increase in N concentration (18%), and protein contents (19%) in the shoot of corn over uninoculated control [13].

3.2.3 Components of yield and the yield of corn kernels in the field experiment

Corns applied 50% N or 75% N and VK1 or VK2, had their stem length equivalent with those which only were fertilized at the same level of nitrogen (50% N or 75% N) (Table 7). In particular, the treatment with the 50% N+VK2 showed that the length of the corn was not significantly different from those which were applied 100% NPK (only 100% NPK and without bacterial strain) (Figure 3)

FIGURE 3: Effect of bacterial fertilizers VK1, VK2, and VKDC on increasing the length of corn in the field

The number of rows varied from 9.55 to 14.55 rows. The number of seeds/ row varied from 13.38 to 33.22 seeds (75% N+VK1). The results in Table 7 showed that when adding the strain to corn VK1 or VK2 and 75% N, the number of seeds/ row had no statistical difference compared to those in which the corn was fertilized 100% NPK.

TABLE 7
EFFECTS OF VK1, VK2 AND VKDC FERTILIZERS ON LENGTH OF CORN EAR, DIAMETER OF CORN EAR, NUMBER OF ROW, NUMBER OF SEADS/ROW, WEIGHT OF ONE THOUSAND GRAINS, GRAIN YIELD AT HARVEST

Treatments	Length of corn ear (cm)	Diameter of corn ear (cm)	Number of rows/ corn ear	Number of seeds/ row	Weight of one thousand grains (g)	Grain Yield (t/ha)
0% N	12.28 i	3.12 h	9.55 g	13.38 i	209.17 j	2.41 k
0% N+VK1	13.09 h	3.17 gh	10.33 f	14.43 h	226.67 i	2.83 j
0% N+VK2	13.60 g	3.15 h	10.28 f	14.34 h	230.08 hi	3.28 hi
0% N+VKDC	13.48 gh	3.16 h	10.20 f	14.52 h	227.04 hi	3.07 ij
25% N	14.30 f	3.25 fg	11.15 e	21.19 g	232.21 gh	4.88 e
25% N+VK1	14.36 f	3.31 ef	11.60 d	23.20 f	242.51 de	4.58 fg
25% N+VK2	14.39 f	3.42 d	11.93 d	23.87 e	236.34 fg	4.82 ef
25% N+VKDC	14.33 f	3.37 de	11.95 d	24.54 d	236.68 ef	4.44 g
50% N	14.36 f	3.39 de	11.60 d	24.38 de	246.85 d	3.36 h
50% N+VK1	17.91 d	3.92 c	12.85 c	30.40 c	291.94 bc	5.69 c
50% N+VK2	18.05 bcd	3.90 c	13.40 b	30.22 c	293.35 abc	5.94 c
50% N+VKDC	17.19 e	3.90 c	13.35 b	30.12 c	289.34 c	5.35 d
75% N	17.93 cd	3.91 c	12.85 c	31.76 b	289.89 c	5.71 c
75% N+VK1	18.30 a-d	4.14 b	14.25 a	33.22 a	297.20 a	8.06 b
75% N+VK2	18.625 a	4.20 ab	14.55 a	33.12 a	296.88 ab	8.35 a
75% N+VKDC	18.35 ab	4.18 ab	14.35 a	32.93 a	295.99 ab	8.14 ab
100% N	19.38 ab	4.15 ab	14.30 a	33.10 a	296.18 ab	8.11 ab
100% N+VK1	18.39 ab	4.13 b	14.35 a	33.12 a	297.98 a	7.99 b
100% N+VK2	18.31 abc	4.20 ab	14.30 a	32.95 a	296.75 ab	8.21 ab
100% N+VKDC	18.40 ab	4.25 a	14.40 a	33.03 a	295.78 ab	8.07 b
F	**276.15***	**206.84***	**163.07***	**1089.01***	**311.14***	**464.71***
C/V (%)	**1.71**	**3.9**	**2.09**	**1.84**	**4.51**	**3.43**

Means within a column followed by the same letter/s are not significantly different at p < 0.05

The weight of 1000-seed of corn was also affected by nitrogen-fixed bacteria in biofertilizer. The treatments with VK1 or VK2 or VKDC bacterial strains also gave 1000-seed weights heavier than those in the treatment of inorganic fertilizers applied from 25% N to 75% N. In particular, when applied 50% N+VK1 or 50% N+VK2 the weight of 1000-seed was not significantly different from the treatment with only 100% NPK.

The actual productivity of corn had a variable average value from 2.41 t/ha (0% N) to 8.35 t/ha (75% N+VK2). The applied non-nitrogen treatment (Control) gave the lowest productivity and had significant differences compared to the other treatments. At a similar level of 75% N, corn of treatment 75% N+VK2 gave higher productivity than those of 75% N+VK1 (0.29 t/ha difference). The results also showed that VK2 gave an efficiency in increasing productivity of corn better than VK1. Corn, which applied 75% N and with VK2 fertilizer, gave the actual productivity equivalent to the treatment of 100% NPK (without bacterial strain). The treatment applied 100% N+VK1 or 100% N+VK2 or 100% N+VKDC, increased actual productivity but was not significantly different from the treatments that were only fertilized 100% NPK (without bacterial strain).

Similarly, the results of Montañez and Sicardi (2013) showed that when nitrogen-fixing bacteria inoculated to corn NK900 in the field supported the 1000-seed weight and corn productivity significantly higher compared to the control treatments with only chemical fertilization. On the other hand, when calculating profits, the treatments with bacterial strains helped increase 10.5% profit compared to the control treatment at the same level of chemical fertilization [14]. Inoculation with *Azotobacter* significantly increased kernel per row, 1000 grain weight, grain, and stover yield of corn [20]. The productivity of corn plants increased from 20-70% when the bacterial strain was applied to corn grown in Mexico [25]. It found that a positive effect of *Azotobacter* application on corn grain yield increase at organic field condition. Only inoculation of *Azotobacter* increased corn grain yield upto 35% over non inoculated treatment [26]. The benefit of *Azotobacte*r inoculation was higher when not using chemical fertilizer. A positive additive (15% yield increased) effect of 10 t/ ha FYM with *Azotobacter* inoculation was seen [20]. The experimental results of Oliveira et al (2018), showed that *Azospirillum brasiliense* increased corn yield compared to non-N-fertilized plants. The inoculation with *A. brasiliense*+100 kg/ha N at topdressing provided a similar grain yield as 100 kg/ha N at sowing [27]. Bacterial strain *A. brasilense* had a positive effect on corn productivity different from the treatment with *A. brasilense*+0 kgN increases of 38% at the same only N fertilizer level. The treatment with *A. brasilense*+50 kgN increases of 74% at the same only 50% N and *A. brasilense*+100 kgN increases of 41% at the same only 100% N [21]. The research results of Salvo et al (2018) showed that the bio-fertilizer with *Azospirillum Brasilense* and *Pseudomonas Fluorescens*, which were applied to corn plants, made increased productivity grain yield (11.03 t/ha) respectively compared to the treatment 50% N (10.94 t/ha) and but it was significantly different from the treatment with 100% N and without bacterial strain (12.05 t/ha) [28]. Bacterial strains *Serratia marcescens*+50% NPK gave the best results for grain yield (39.05%) respectively compared to the control [22].. The combined use of *Bacillus* aryabhattai and *Bacillus subtilis* had good effect on the biofortification of corn as well as improvement in growth and yield parameters [29].

IV. CONCLUSION

Bacterial fertilizer *Bacillus aryabhattai* ADR3 with peat or *Klebsiella pneumoniae* DNR5 with peat positively affected the growth, and yield of hybrid corn NK7328. The treatments of 75% N with bio-fertilizers (VK1 or VK2) always had higher values than those at the same level of fertilizer with those applied only 75% N chemical fertilizers. The nitrogen-fixing bacteria strain and fertilizing 75% N contribute to the increase of the number of leaves, plant height, stem diameter, the number of leaves, ear corn height, dry weight of the plant, total N, the weight of 1000 seeds, productivity as same as treatments applied only fertilizing 100% N and without inoculation.

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Erosive Indices of Amparo in São Francisco – Sergipe, Brazil Related to the Phenomena El Niño La Niña

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Abstract— *Erosion is originally from the Latin, which means to corrode and, in a comprehensive way, can be considered a set of natural processes that promote the alteration of landscapes through degradation, wear and transport from one point to another of materials on the earth's surface through agents erosive. The objective is to evaluate the erosive index for the municipality of Amparo de São Francisco – Sergipe, relating them to the El Niño and La Niña phenomena. The calculation of pluvial and erosive anomalies was used, generating their respective graphs for the area under study coupled with the extreme events of large scale El Niño and La Niña to see the erosive contributions of the studied area. Erosion calculations were performed using the formulation developed by França (2021) for the study area. The rainfall erosivity indices of anomalies in the study area, taking into account the El Niño and La Niña periods, is a relevant factor for decision makers on the most adequate soil management practices, aiming at the sustainability of exploration for projects farming and fertile soil management.*

Keywords— Soil withdrawal, rainfall erosive potential, Soil conservation and loss.

I. INTRODUCTION

One of the impacts caused by water erosion is the exhaustion of soil lacking due to the loss of nutrients, organic matter, siltation and contamination of water tables, through the displacement of fertilizers and pesticides, causing direct changes in flora and fauna (BERTONI et al., 2012; PIRES et al., 2013). According to Pires et al. (2013) erosion is evaluated as a process of natural origin with the purpose of landscape formation and soil renewal.

Erosivity has been expressed as the potentiality of the erosive agent, wind and/or water, to generate erosion. As for the rainfall erosive capacity procedure, the EI30 (kinetic energy of impact of "E" drops by the maximum rainfall intensity in 30 minutes) was created and the “R” factor of the Universal Soil Loss Equation was suggested as a rainfall index (WISCHMEIER et al., 1978). FOSTER et al. (1981) dimensioned the units for the International System of Measurements, expressing in MJ.mm/h year ago. With the help of rainfall correlation and erosivity, the rainfall erosive potential of a location with the same climate type and which does not have rainfall data records is estimated (OLIVEIRA et al., 2012; TRINDADE et al., 2016).

In the northeastern semiarid region, precipitation spreads irregularly with long periods of drought. Temperatures register higher averages causing high evaporative and evapotranspiration rates (CLEMENTE, 2021; MARENGO, 2008). Precipitation in this region is a result of atmospheric dynamics, and the influence of local and regional factors, such as relief and geographic position (MARENGO et al., 2011).

Medeiros et al. (2016a) analyzed the oscillations of El Niño and La Niña and their influence on the number of rainy days in the municipality of Bom Jesus do Piauí. For the Northeast, the periods of El Niño are associated with the scarcity of rain and La Niña, in general, with abundant rainfall, while in the South and Southeast regions conditions are observed with opposite events. The El Niño south oscillation (ENOS) considerably influences the climate in places where it operates, with long periods of drought and total rainfall above historical normal’s (ROMERO, 2013).

Medeiros (2018) showed that there is a lack of studies on the correlation of rainfall with large-scale climatic phenomena, such as El Niño and La Niña. The author analyzed the influence of rainfall variability and the number of rainy days in the city of Recife - PE, and their relationship with the phenomena El Niño and La Niña. According to this author in the dry four-month period, which corresponds to October, November, December, and January, there is no interference from the El Niño and La Niña episodes in the increases and decreases in the days with rain occurrences, which are directly linked to local factors such as breeze, convective movements and line of instability. The phenomena El Niño and La Niña have little influence on the days with rain occurring in Recife - PE, because in the months with the greatest intensity of these episodes, the trend curves showed no increase or decrease. Coherent results were found in the studies by Medeiros et al. (2016) for the municipality of Bom Jesus do Piauí.

França et al. (2018) calculated the climatological water balance for the municipalities of São Bento do Una and Serra Talhada and investigated the influence of the phenomena El Niño (2012, 2016) and La Niña (2008, 2011) on the distribution of rainfall through analysis of the water balance extract. They found that the El Niño episode influenced the rainfall rates of the cities studied. In the La Niña episode, the distribution of these indices was irregular, reflecting on the water balance.

Medeiros (2014) analyzed the occurrences of extreme precipitation events in Campina Grande, with daily data covering the years 1970–2010. The extreme events analyzed were those with the highest daily precipitation intensity. The results showed that there was a change in the behavior of precipitation occurrences from the 70s onwards. There was an intensification in the maximum precipitation with a greater number of events with precipitation values greater than 80 mm. There was, in general, no direct relationship between the increase in precipitation and occurrences with ENSO events. Extreme events were evident between the months of the rainy season with 88% of occurrences, and 12% of evidence in the dry season. Medeiros et al, (2012), analyzing the climatology of precipitation in the city of Bananeiras - PB, in the period 1930-2011, as a contribution to Agroindustry, found that rain gauges are essential to agro-industrial sustainability even with activities from external events

The objective is to evaluate the erosive index for the municipality of Amparo de São Francisco – Sergipe, relating them to the El Niño and La Niña phenomena.

II. MATERIAL AND METHODS

Amparo de São Francisco is limited to the east and south by Telha, to the west by Canhoba and the state of Alagoas to the north, it is positioned at 10°08'04" south and 36°55'46" west, with an altitude of 51 meters (Figure 1)

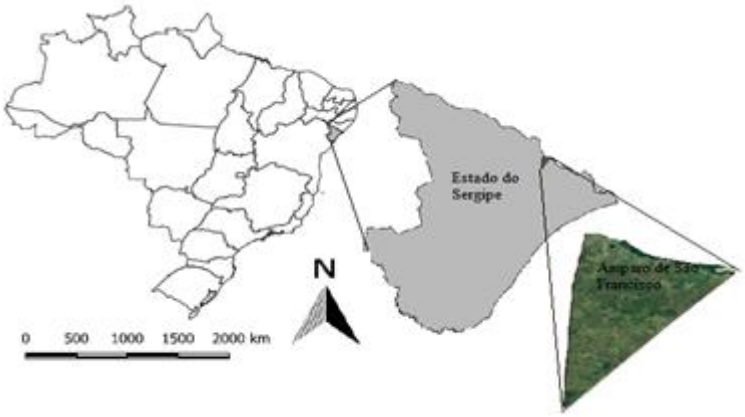

FIGURE 1: Positioning of the city of Amparo de São Francisco within the state.
Source: França (2022).

Monthly and annual rainfall data provided by the Superintendence for the Development of the Northeast (SUDENE, 1990) and the Agricultural Development Company of Sergipe (EMDAGRO - SE, 2020) between 1963 and 2019 were used.

Amparo de São Francisco is located in a region characterized by two well-defined seasons, a rainy period ranging from February to August and a dry period, flowing from September to January. According to the climate classification (KÖPPEN 1928; KÖPPEN et al., 1931; ALVARES et al., 2014), the study area has an "As" type climate (hot and humid Tropical rainy). Annual precipitation of 1138.2 mm; maximum temperature of 31.5 °C; average temperature of 25.9 °C; minimum temperature 20.9°C; annual relative humidity of 80.2% and total insolation of 2341.9 hours. (FRANÇA, 2021).

It is interesting to note that the rainy season is marked by intense and frequent rainfall in a short period of time, favoring the emergence of erosion, since due to the frequency of rain in a short period, the water cannot infiltrate into the soil and ends up carrying out solid particles.

The equation for erosive calculations in the municipality of Amparo de São Francisco – Sergipe was developed by França et al. (2021)/

$$EI_{30} = 0{,}3908(P)^{1{,}651}$$

Where

EI30- factor R (MJ mm ha^{-1} h^{-1} yr^{-1}) for the studied region;

P - Monthly or annual precipitation (mm).

The calculation of pluvial and erosive anomalies was used, generating their respective graphs for the area under study coupled with the extreme events of large scale El Niño and La Niña to see the erosive contributions of the studied area.

Table 1 shows the classification and intensity of the El Niño and La Niña phenomena, for Amparo de São Francisco – Sergipe.

TABLE 1

CLASSIFICATION AND INTENSITY OF EL NIÑO LA NIÑA PHENOMENA BETWEEN THE YEARS 1963 TO 2019

years	classification	Intensity	years	classification	Intensity
1963			1992	El Niño	Forte
1964	El Niño	Fraco	1993	El Niño	Forte
1965	El Niño	Forte	1994	El Niño	Moderado
1966	El Niño	Forte	1995	El Niño	Moderado
1967	La Niña	Fraca	1996	La Niña	Fraca
1968	El Niño	Moderado	1997	El Niño	Forte
1970	El Niño	Moderado	1998	La Niña	Moderda
1971	La Niña	Moderada	1999	La Niña	Moderada
1972	El Niño	Forte	2000	La Niña	Moderda
1973	El Niño	Forte	2001	La Niña	Moderdo
1974	La Niña	Forte	2002	El Niño	Moderado
1975	La Niña	Forte	2003	El Niño	Moderado
1976	La Niña	Forte	2004	El Niño	Forte
1977	El Niño	Fraco	2005	El Niño	Forte
1978	El Niño	Fraco	2006	El Niño	Forte
1979	El Niño	Fraco	2007	El Niño	Forte
1980	El Niño	Fraco	2008	La Niña	Forte
1981			2009	El Niño	Fraco
1982	El Niño	Forte	2010	El Niño	Fraco
1983	El Niño	Forte	2011	La Niña	Moderada
1984	La Niña	Fraca	2012	El Niño	Moderado
1985	La Niña	Fraca	2013	El Niño	Forte
1986	El Niño	Moderado	2014	La Niña	Neutra
1987	El Niño	Moderado	2015	El Niño	Forte
1988	El Niño	Moderado	2016	El Niño	Forte
1989	La Niña	Forte	2017	La Niña	Moderada
1990	El Niño	Forte	2018	El Niño	Moderado
1991	El Niño	Forte	2019	El Niño	Forte

Source: CPTEC/INPE.

III. RESULTS AND DISCUSSIONS

Figure 2 shows the variability of rainfall and erosive anomalies in the La Niña period for the protection of São Francisco – Sergipe. Remember that all Figures were created by pluvial and fluvial anomalies in the study area, taking into account the La Niña periods.

The year of 1964, except for the month of April, registered erosion superior to the pluvial indices, the months of January, February and from May to December the erosive indices were inferior to the pluvial ones, therefore the year of 1964 the rains were of irregular significance and of low distribution rainwater.

In 1965, rainfall and erosive anomaly rates were negative from February to June. In the months of August to December, the rainfall indices were few causes of erosion, that is, the rains were not well distributed and did not cause erosivity as expected.

In 1967, the rainfall distribution of greater intensity and in a short period of time caused anomalous erosivity in the months of April, May and July.

FIGURE 2: Rainfall and erosive variability in the La Niña period for Amparo de São Francisco – Sergipe.

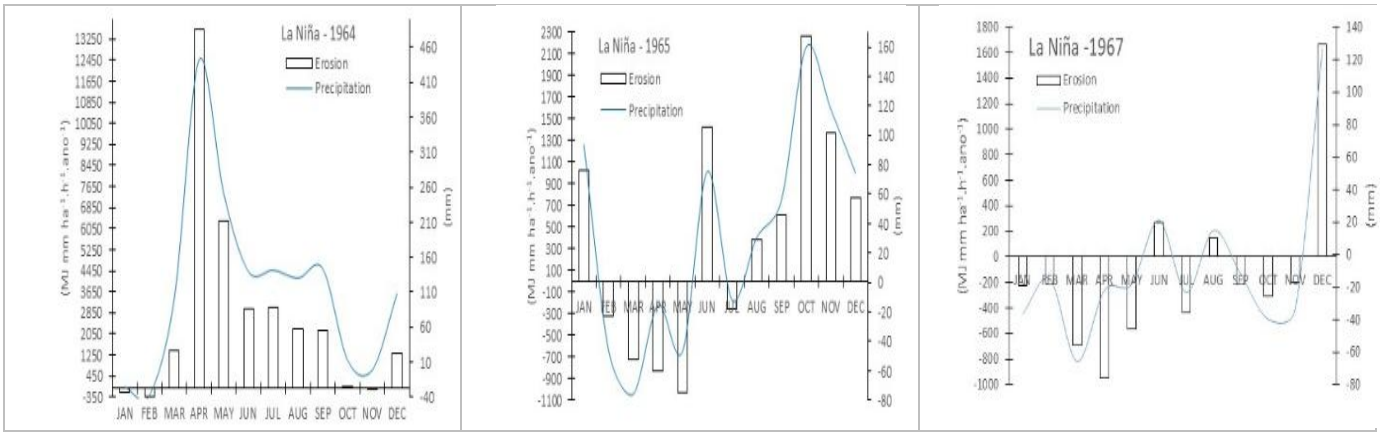

FIGURE 2 (A)
Source: França (2022).

The year 1968 registered negative anomalies for both elements under study, except for the month of May. Rainfall in the study year was below the climatological normal and its distributions were more irregular than normal.

The year 1970 registered negative anomalies in rainfall and erosivity, except in March and November. The months from April to July stand out, where both elements under study had their greatest variability due to rainfall irregularities. These irregularities are discussed in the studies (MARENGO et al., 2011; MARENGO et al., 2008 and MENEZES et al., 2011).

With positive anomalies in rainfall and less irregular distribution throughout the year, it recorded erosive indices above its normais pattern, as can be seen in 1971.

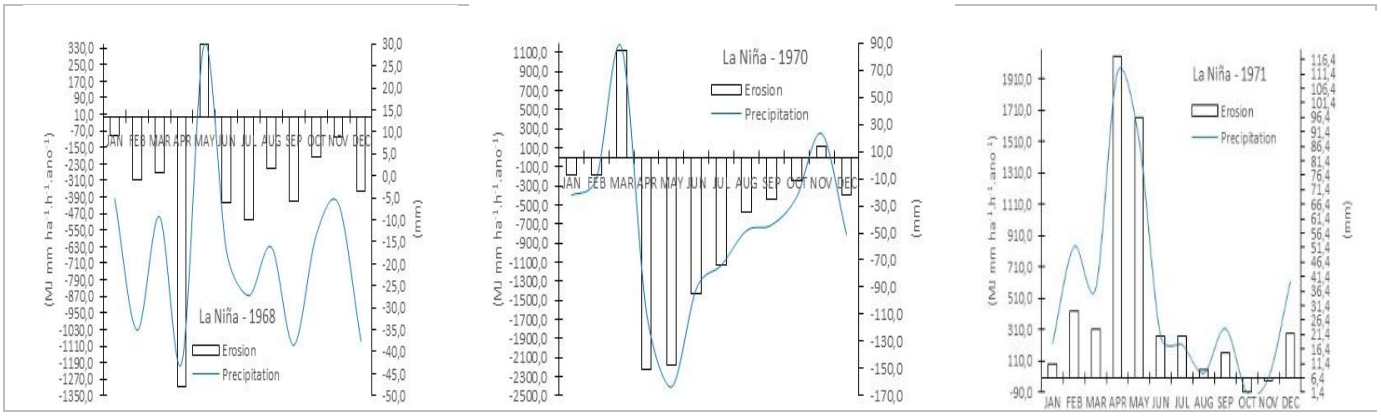

FIGURE 2 (B)
Source: França (2022).

The years 1973 and 1974 show positive rainfall and erosive anomalies ranging from 30 (MJ mm/ha h year) to 4450 (MJ mm/ha h year) and rainfall flowing from 13 mm to 193 mm while fluctuations 1974 erosions occurred between -200 (MJ mm/ha h year) to 1800 (MJ mm/ha h year) and rainfall with anomalies from -30 mm to 100 mm, stand out even though in 1974 in the month August, there were negative anomalous rates of erosion and rainfall.

The erosive and rainfall variability in 1975 registered positive anomalies from January to May and negative anomalies from June to December. Expected, that is, above normal.

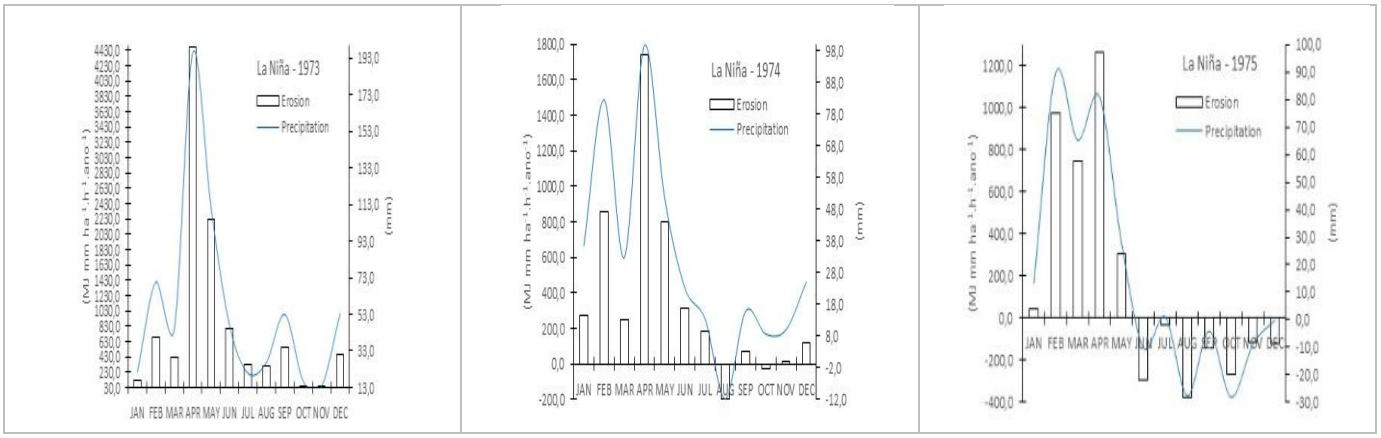

FIGURE 2 (C)
Source: França (2022).

The irregularities in the pluvial indices registered in the year 1976 also cause irregularities in the erosive indices, the months of April, May, June, August, September stand out with high incidences of negative anomalies for the elements rain and erosion.

With positive anomalies for precipitation and erosion from January to May and September. In the months of June to August and October and November there are negative anomalies of rain and erosion. The high rates of positive anomalies for rain were caused by atmospheric variability aided by the regional and local systems for the year 1983.

The anomalous erosive and pluvial indices for the year 1984 were all negative, where the rains that occurred were not necessary for many activities in the study region. The months from April to July stand out with the highest erosive and rainfall indices, while in the months from August to January these elements registered the lowest values. These fluctuations in rainfall are similar to the study by France (2021).

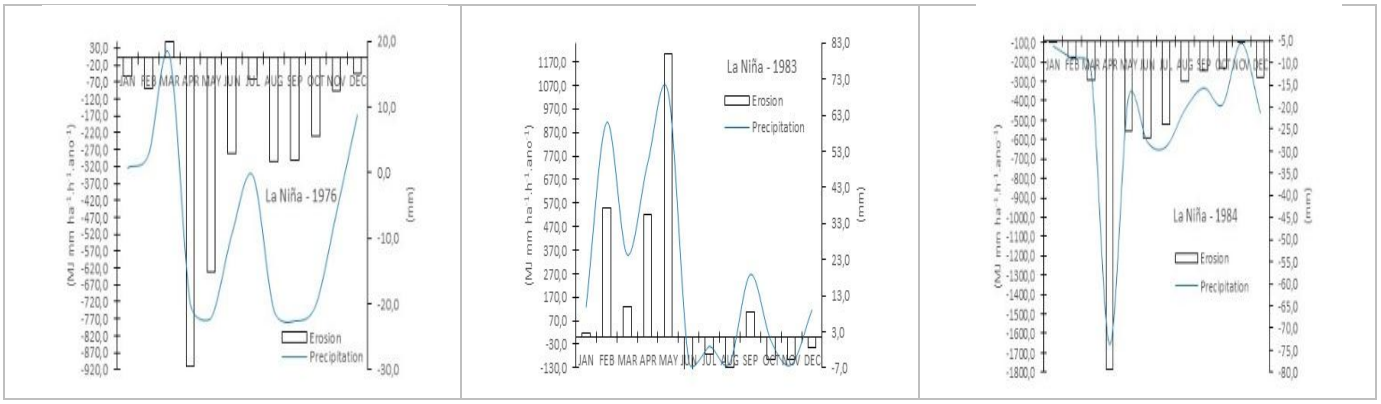

FIGURE 2 (D)
Source: França (2022).

The year 1985 was characterized by negative rainfall and erosion anomalies. The month with the highest rainfall and erosivity was April and the lowest rainfall and erosive index was November. In 1989, attention is drawn to the months from June to December, which registered positive rainfall and negative erosivity, showing that the rain did not have the necessary intensity to cause runoff and drag the soil, on the other hand, the month of March the highest indexes of these elements were registered.

The erosive and pluvial variability of 1995 show us irregularities where the month of April registered the biggest indices of pluvial and erosive anomalies, as well as the month of June the biggest positive values of the elements in studies. Such fluctuations in these years are similar to the study by França (2021).

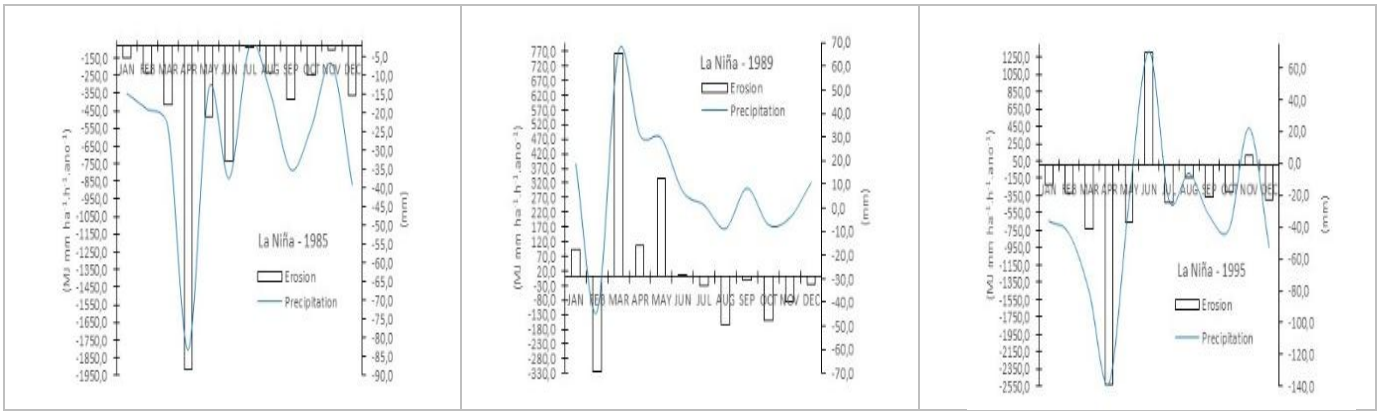

FIGURE 2 (E)
Source: França (2022).

The year 1996 registered positive rainfall and erosion anomalies in the months of April and August, the erosion anomalies were 400 (MJ mm/ha h year) and 900 (MJ mm/ha h year) while the anomaly indices rainfall was 400 mm and 600 mm in the months of May and November, the smallest anomalies of the elements under study are observed. The other months had negative anomalies, being September the one with the highest intensities and January the one with the lowest fluctuations.

With negative fluctuations observed from February to December (1998) in both elements of studies. It is noteworthy that the pluvial anomalies were more intense than the erosive ones, the month of January registered positive anomalies. This study is similar to the study by França et al (2021).

The positive anomalies occurred between September and November were caused by isolated synoptic systems aided by local factors causing anomalous erosion rates between 250 to 400 (MJ mm/ha h year), in the other months of that year (1999) there were negative erosion anomalies and precipitation.

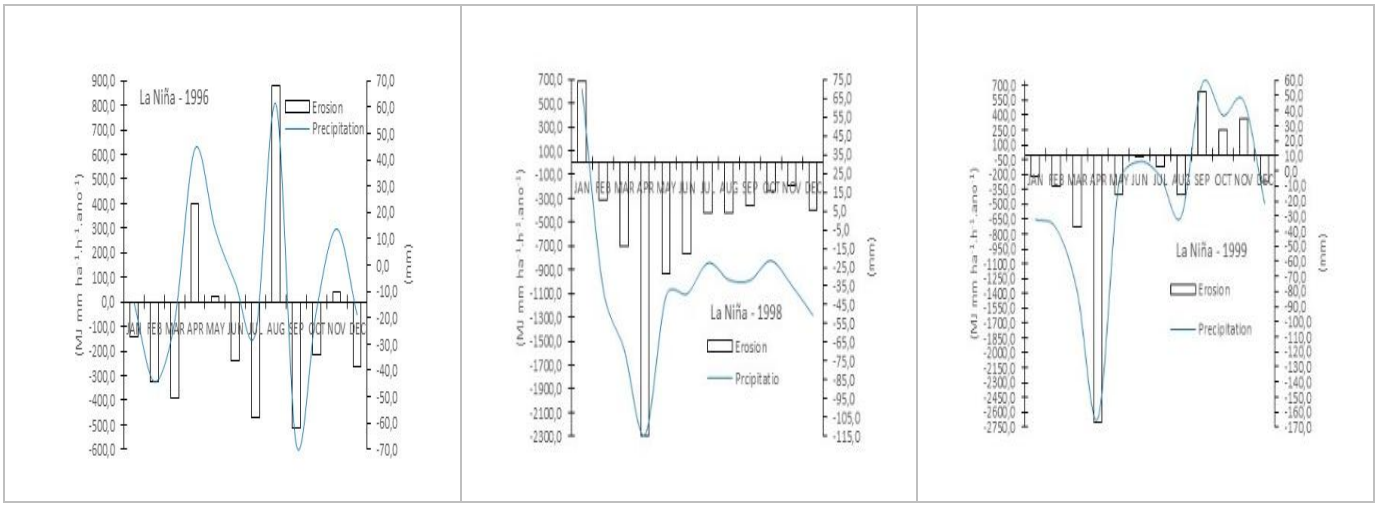

FIGURE 2 (F)
Source: França (2022).

The year 2000 was characterized by erosive anomalies and negative rainfall with fluctuations flowing between -50 (MJ mm/ha h year) to -1800 (MJ mm/ha h year), the rains recorded were not necessary to meet the water needs of the place of study. The highest erosivity and negative rainfall occurred in May and the lowest erosivity and anomalous rainfall occurred in January, February, April, June, September and December.

With irregular variability of pluvial and erosive anomalies seen for the year 2001 and with the months of June (larger), July, August (smaller), October and December positive anomalies the other months were registered negative anomalies with the months of April and May (major) and January to March (smaller).

The months of February (smaller) March (larger), May and August (2007) recorded positive anomalous indices, in the other months the anomalies of the elements in studies were negative, these variability show similarities in the studies of (SHAMSHAD et al., 2008; CARVALHO et al., 2014).

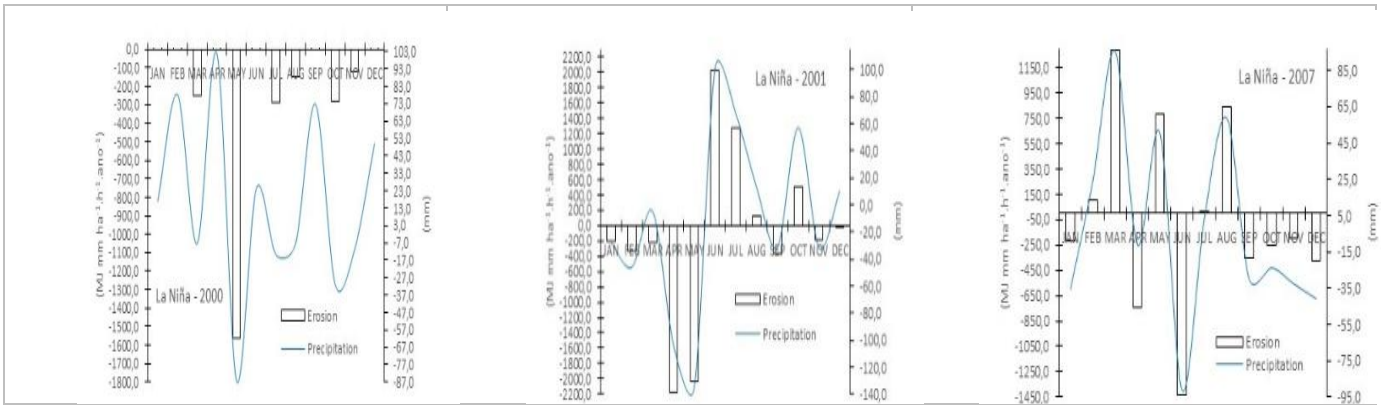

FIGURE 2 (G)
Source: França (2022).

The year 2008 registered a positive anomaly of erosivity and rainfall in the months of February and March, the positive erosive index ranged from 110 (MJ mm/ha h year) to 1500 (MJ mm/ha h year) and rainfall from 25 mm to 115 mm. In the other months, the anomalies were negative and their greatest fluctuations were registered in the months of April -2500 (MJ mm/ha h year) and -115 mm, in the month of June it was observed -1000 (MJ mm/ha h year) and -55 mm.

2010 was characterized by irregular erosive and pluvial anomalies, in negative anomalies, the highest rainfall and erosive variability was recorded in May and the lowest occurrence in January. The biggest erosive and pluvial occurrence was registered in June and the smallest occurrence in the month of July. In the year of 2010 the biggest negative fluctuation of the elements in studies was for the month of May and the smallest in January.

The biggest negative oscillation of erosion and rain occurred in the month of June and the smallest in the month of September. The month with the highest recorded high erosive and rainfall intensity, these rainfall and erosive irregularities show similarity with the studies by França et al, (2021) and Marengo et al, (2008).

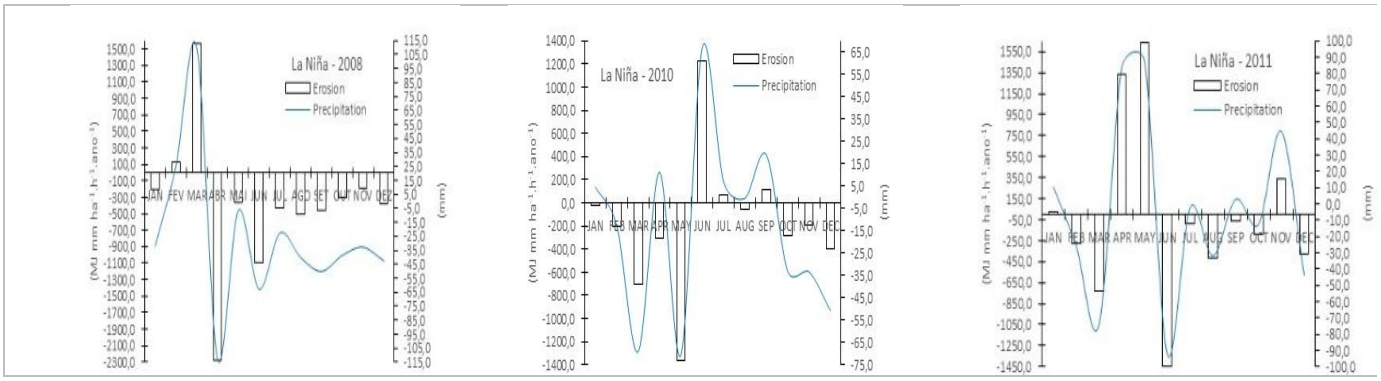

FIGURE 2 (H)
Source: França (2022).

In 2012, negative erosive anomalies were recorded, flowing from 0.0 MJ mm / ha h year to -2800 MJ mm / ha h year and negative rainfall anomalies ranging from 10 mm to -190 mm, these rainfall variations were due to isolated systems microscale and local effects that aided moderate erosivity in the study area.

The rainfall anomaly registered negative values in 10 months (2013) with the exception of July and October, which were positive, the anomaly oscillations flowed between -70 mm to 110 mm these oscillations are in accordance with the results of the studies of (MARENGO 2008; MARENGO et al., 2012; FRANCE 2021).

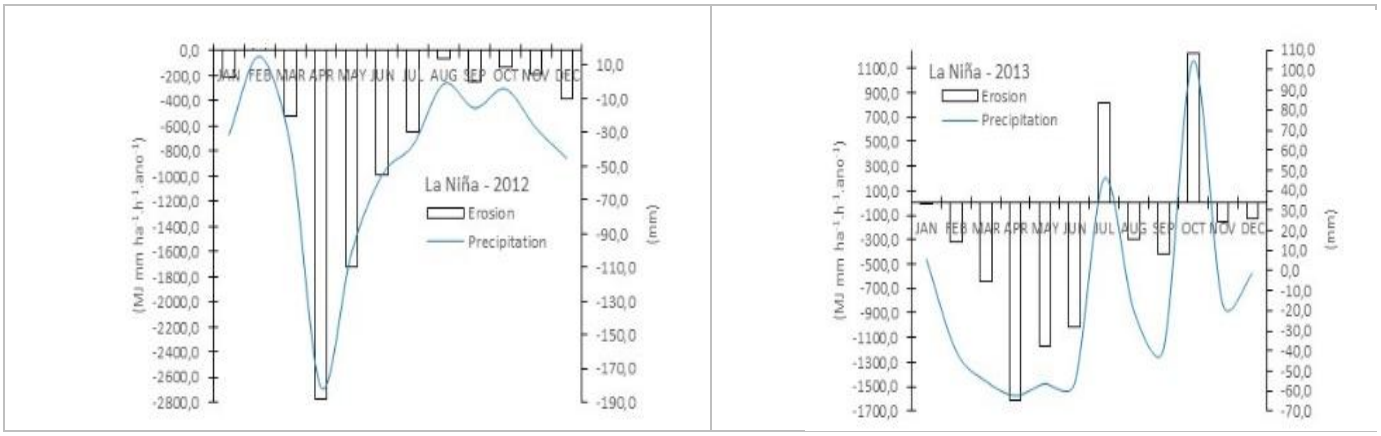

FIGURE 2 (I)
Source: França (2022).

Although monthly rainfall fluctuations and their specialties will explain a large part of the variations in erosivity (BAZZANO et al., 2010), it should be noted that these are not the only factors influencing erosive processes, where we cite the contribution of geology, pedology , relief and land use (SILVA et al., 2014).

According to the IPCC (2014), changes in the variability of rainfall distribution over the years in Northeast Brazil are largely due to large-scale climatological events, such as El Niño and La Niña, which cause heating and cooling of waters in the region. Equatorial Pacific Ocean, influencing the climate, regionally and globally.

Figure 3 shows the variability of rainfall and erosive anomalies in the El Niño period for the protection of San Francisco – Sergipe. Remember that all Figures were created by pluvial and fluvial anomalies in the study area, taking into account the El Niño periods.

With negative anomalies of medium rainfall and erosive intensity recorded in January, September and October, positive anomalies occurred in the other months of 1963. In 1966 there were negative anomalies from June to January with low to very low intensity, the months February, the largest predominated positive anomalies. The year 1969 presents irregularities in its pluviais and erosive anomalies and intensities. Erosive indices are similar to the studies by França et al (2021).

FIGURE 3: Rainfall and erosive variability in El Niño period for Amparo de São Francisco – Sergipe.

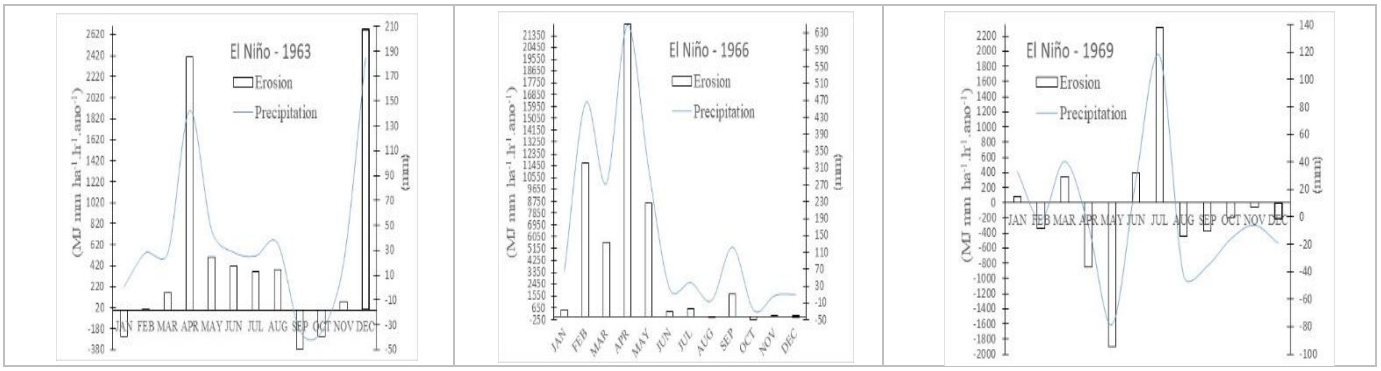

FIGURE 3 (A)
Source: França (2022).

The anomalous behavior of erosivity and rainfall in 1972 were all positive with fluctuations from 10 mm to 14 mm and from 50 (MJ mm /ha h year) to 8000 (MJ mm /ha h year). The year 1977 records rainfall irregularity and erosion in every month of the year, anomalous rainfall indices flowed from -60 mm to 140 mm and erosive indices -700 (MJ mm /ha h year) to 9700 (MJ mm /ha h year). With negative fluctuations for the months of January, February, April, June and October to December, the other months of the year the anomalous erosive and rainfall fluctuations (1978) were positive, such fluctuations corroborate França et al. (2021).

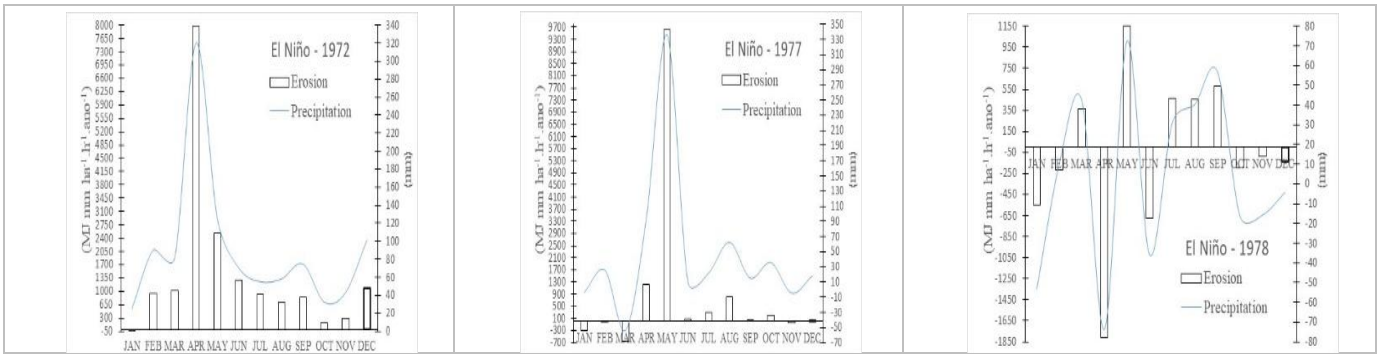

FIGURE 3 (B)
Source: França (2022).

In 1979 there were pluvial and negative anomalies flowing from -60 mm to 30 mm, the erosive anomalies were all positive and their oscillations flowed from 0 (MJ mm /ha h year) to 8000 (MJ mm /ha h year). These oscillations are similar to those found by França et al (2021).

With positive erosive fluctuations flowing between 0 (MJ mm /ha h year) to 2200 (MJ mm /ha h year) while the anomalies registered with negative and positive fluctuations ranging from -200 mm to 120 mm according to the year 1980. For 1981, erosive anomalies ranged from 100 to 4100 (MJ mm /ha h year) and rainfall anomalies were positive with variability from 10 mm to 110 mm.

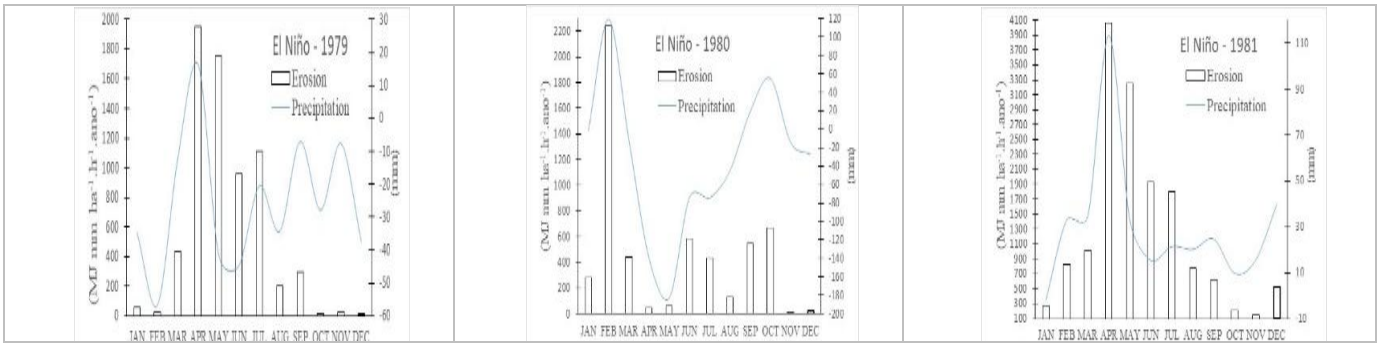

FIGURE 3 (C)
Source: França (2022).

Negative and positive anomaly indices were recorded for erosivity with fluctuations ranging from -220 (MJ mm /ha h year) to 2500 (MJ mm /ha h year), while rainfall anomalies were centered from 10 mm to 150 mm in 1982. The rainfall anomalies in 1986 ranged from -120 mm to 90 mm and erosive anomalies. The year 1987 registered positive erosive anomalies flowing from 0 (MJ mm /ha h year) to 1900 (MJ mm /ha h year) and rainfall anomalies flowed between -160 mm to 60 mm, such oscillations corroborate the results found by França et al, (2021) in their studies for the municipal area of Amparo de São Francisco.

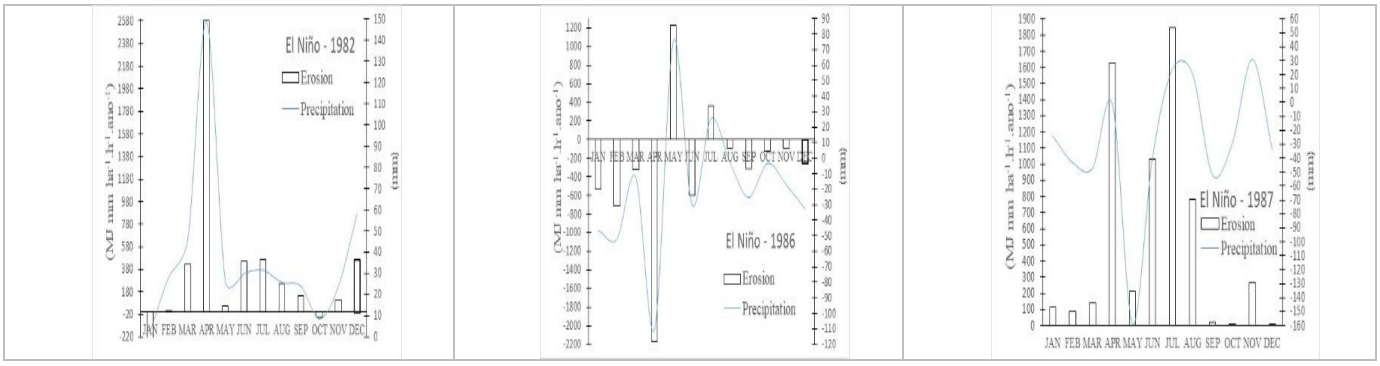

FIGURE 3 (D)
Source: França (2022).

The anomalous variability of erosion ranged from -2750 (MJ mm /ha h year) to 1650 (MJ mm /ha h year) in 1988, the greatest positive anomaly occurred in June with 1650 (MJ mm /ha h year) and in the negative anomaly it was registered in the

month of May with -2750 (MJ mm /ha h year). Positive rainfall anomalies were registered in the months of June, November and December in the other months, the respective anomalies were negative.

In 1990, two months of low erosive and rainfall anomalies were recorded in the months of January and October, in the other months the anomalies studied were positive and showed strong irregularities, and these irregularities are in accordance with the studies by Marengo et al, (2007); Marengo et al (2008).

With moderate to strong rainfall irregularity and erosive variability recorded in 1991, these fluctuations are expressed in the study by França et al, (2021) and corroborate the results discussed.

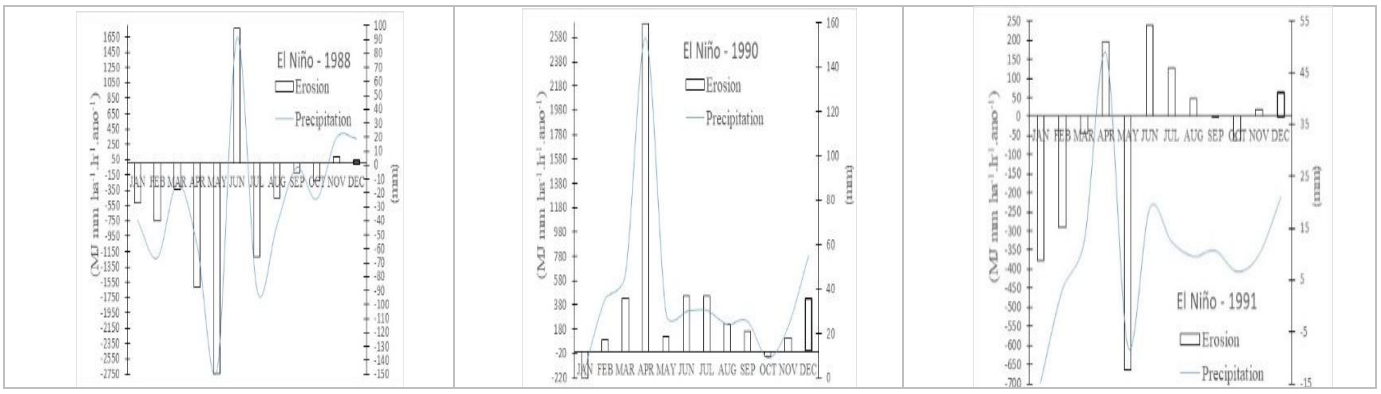

FIGURE 3 (E)
Source: França (2022).

The anomalous erosive and pluvial behaviors of 1992 registered the month of August with low positive index and the other months with moderate to strong oscillations of the elements under study. For the years 1993 and 1994, predominant negative anomalies were observed with moderate intensities and isolated months of positive anomalies for both years studied.

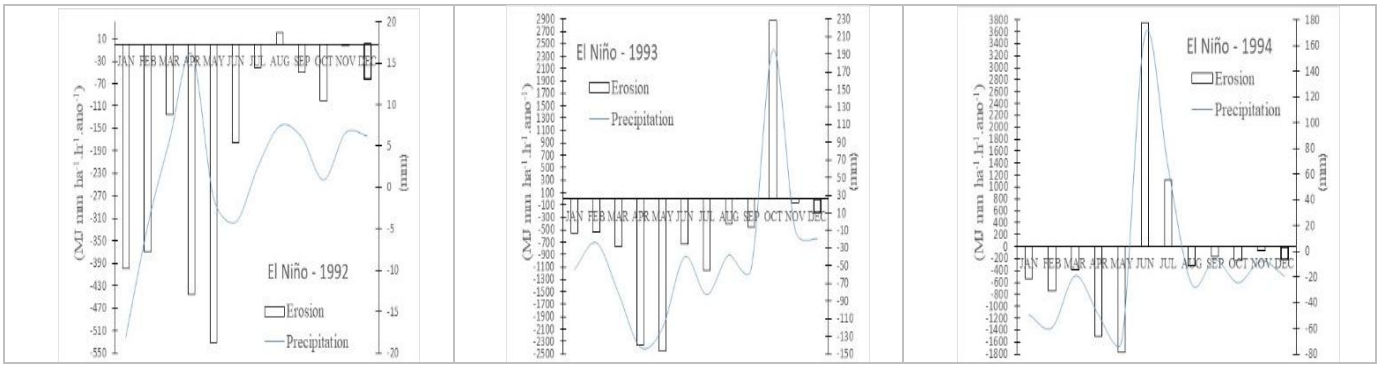

FIGURE 3 (F)
Source: França (2022).

The years 1997, 1998 and 2002 record large anomalous irregularities of rainfall and erosion, most of them being negative anomalies with moderate to strong intensity, these variations have similarities with the study by França et al, (2021).

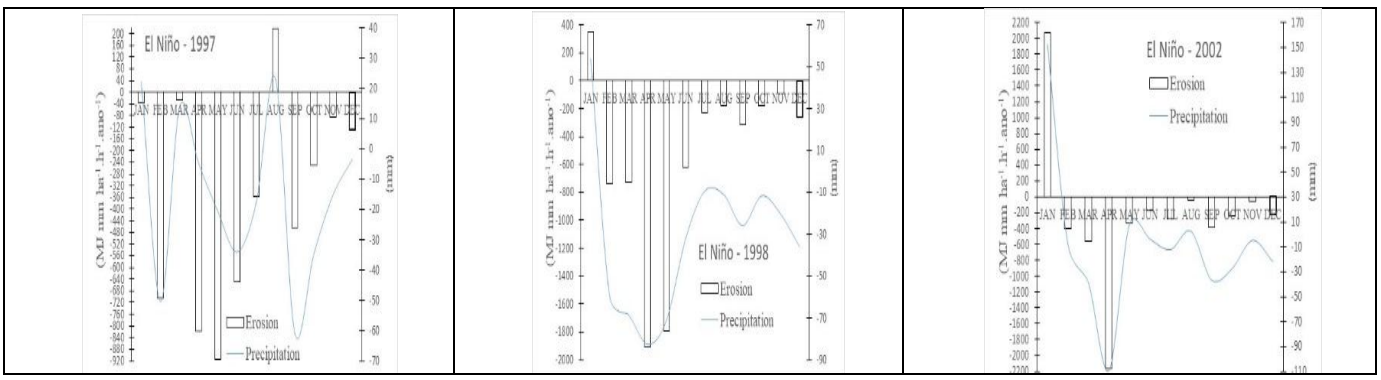

FIGURE 3 (G)
Source: França (2022).

With negative anomalies registered in the months: January, February, April to September and December. The months of April and May had the biggest anomalies and the smallest negative anomaly in December. The months of March, October and November had positive anomalies of weak to moderate intensities for the year 2003.

With a very strong intensity of positive erosion and rainfall anomalies occurring in January, and low intensity in August and September, in the other months of 2004, both elements under discussion were negative anomalies. These erosive fluctuations are similar to the studies by França et al, (2021) and rainfall variations corroborate the results of Marengo et al, (2007) and Marengo et al (2008).

The year of 2005 registered negative pluvial and erosive anomalies in ten months of the year and positive anomalies in the months of May and June, the erosive anomalies were registered between -1500 (MJ mm /ha h year) to 2000 (MJ mm /ha h year), while rainfall anomalies ranged from -80 mm to 120 mm.

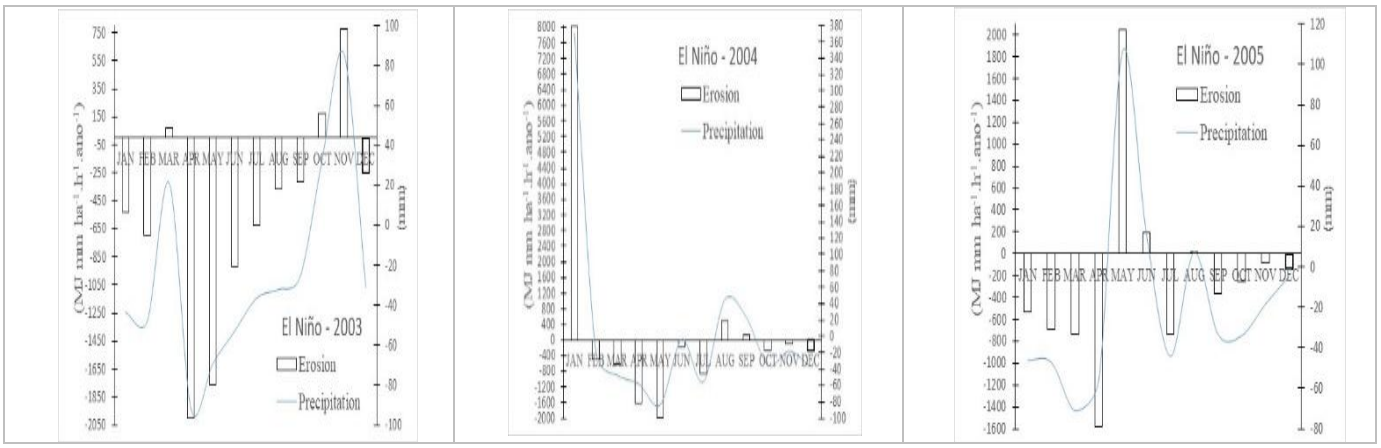

FIGURE 3 (H)
Source: França (2022).

In the figure of the years 2006, 2009 and 2014 the anomalous oscillations of erosivity and positive rain registered four months, two months and two months respectively and in the other months the anomalies were negative, both intensities were from moderate to strong.

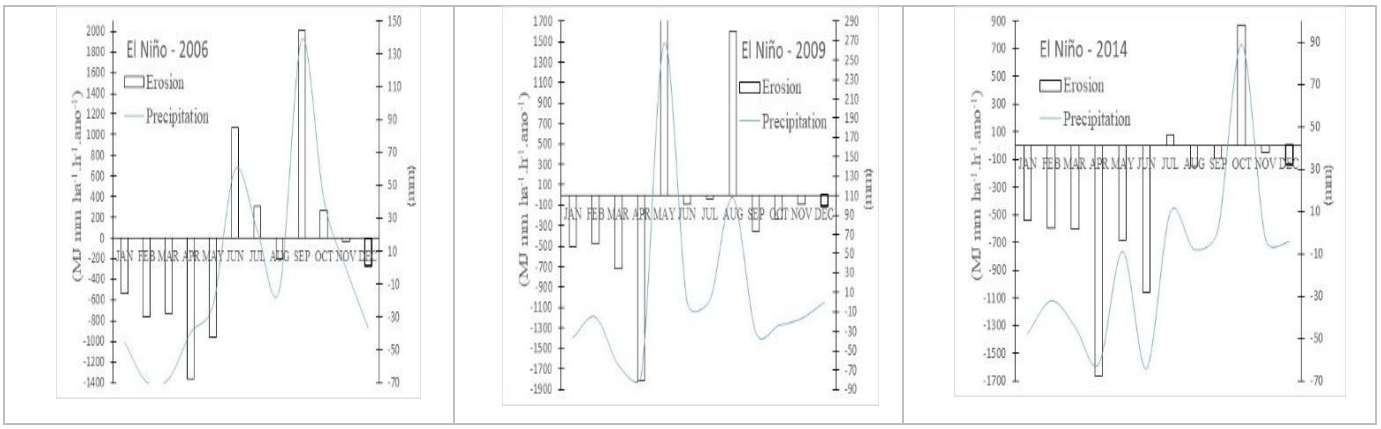

FIGURE 3 (H)
Source: França (2022).

The year 2015 registered eleven months of negative anomalies for erosion and rainfall with fluctuations ranging from -2200 (MJ mm /ha h year) to -100 (MJ mm /ha h year) and rainfall anomalies oscillating from -20 mm to -100 mm. The month of July registers positive anomalies.

With negative rainfall and erosion anomalies between February and December. Its biggest anomalies occurred in the months of June and July and the smallest anomalies were registered in November. January being the only month that there were positive anomalies of the elements studied for the year 2016.

Negative anomalies predominated between November and April and positive anomalies flowed from May to October in 2017. These erosive variables are similar to the studies by França et al, (2021) and rainfall variations corroborate the results of Marengo et al, (2007) and Marengo et al (2008).

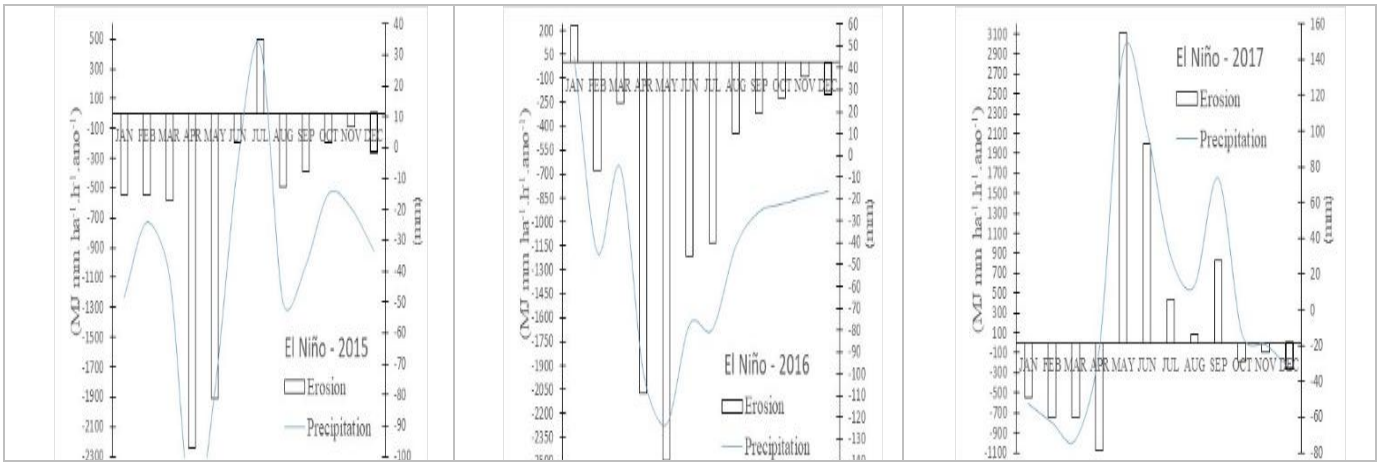

FIGURE 3 (I)
Source: França (2022).

With negative rainfall and erosive rates between March and September 2018, with fluctuations from 1300 to -2100 (MJ mm /ha h year) in erosivity and with oscillation from -115 mm to 10 mm for the rainfall anomaly. The months from October to March registered positive rainfall anomalies and negative erosive indices, showing that rainfall anomalies were not necessary to cause erosion.

The year 2019 registered negative anomaly rates for the elements in studies, except for the month of March, which were positive.

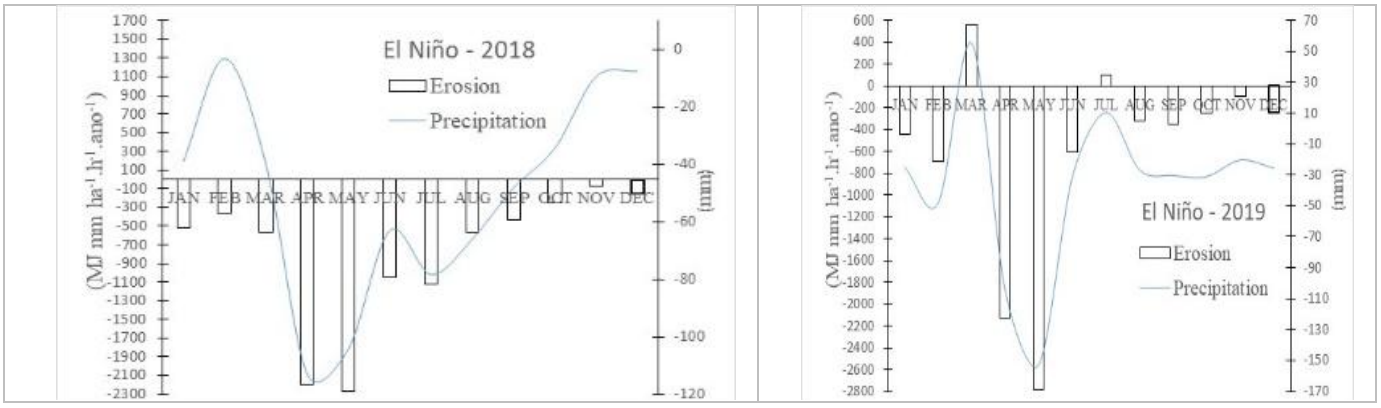

FIGURE 3 (J)
Source: França (2022).

IV. CONCLUSIONS

The anomalous variability of erosion and rainfall against large-scale El Niño and La Niña phenomena did not show large isolated activities in years of their occurrence, since in isolated years both events presented equal intensities in the elements under study.

The erosivity and rainfall indices of anomalies in the study area, taking into account the El Niño and La Niña periods, is a relevant factor for decision makers on the most adequate soil management practices, aiming at the sustainability of exploration for agricultural projects and fertile soil management.

The information provided in the study should serve as a support for the area's conservation planning, where it will be possible to follow strategies to recover and prevent damage to environmental resources and increase the property's productive capacity, contributing to socioeconomic development.

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Determination of Irrigation Time by Utilizing Plant Water Stress Index (CWSI) Values of II. Crop Sesame Genotype in Siirt Conditions

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Abstract— *This research was carried out to determine the plant water stress index (CWSI) by using infrared thermometer (IRT) data calculated as a result of leaf crown temperature measurements of the second crop sesame plant grown in semi-arid climate conditions in Siirt, and to determine the relationships between irrigation time and seed yield of sesame plant and CWSI by using these index values. In this study, the irrigation program was established to reintroduce 100 % (I_{100}), 70% (I_{70}), %35 (I_{35}) of the decreased water through the effective root depth of 90 cm every 7 days. Thus, a full irrigation (I_{100}) and irrigation with 2 different levels of stress (I_{70} and I_{35}) were created. In the research, a total of 575.00 and 576.66 mm of irrigation water was given to I100 (control) irrigation in the first and following years, respectively. The water consumption of the above-mentioned subject was determined as 606.00 mm in the first year and 646.33 mm in the second year of the study. The yield per hectare in the first year of the research on the aforementioned irrigation was 1110.34 kg; In its second year, it was determined as 1319.00 kg. In the first and second years of the study, the lower limit (LL) values for the absence of water stress needed to determine the plant water stress index were Tc-Ta=0.373-2.42 VPD and Tc-Ta=0.74-2.52 VPD, respectively; The upper limit (UL) values, where the plant is completely water stressed, were determined as 2.04 °C and 1.77 °C, respectively. From the infrared thermometer measurements at the time of irrigation, the threshold plant water stress index value, where sesame seed yield starts to decrease, was calculated as 0.31. On the other hand, it was determined that there is a negative linear proportionality between sesame seed yield and plant water stress index values.*

Keywords— Sesame, Crop water stress index, Irrigation time.

I. INTRODUCTION

Jackson et al. (1982), explained how to make leaf crown temperature (vegetation temperature) measurements with infrared thermometer device, which is accepted as an indicator of plant water stress index, and what should be consideredIt is stated that the temperature of the plant leaf crown can be measured with a portable infrared thermometer and the device should be held at an angle of 30 °C from the ground at the time of reading and it should be covered with 80% vegetation in order to make accurate measurements. The internal water status of plants; It has been reported that neither soil water content nor atmospheric demand can be determined as accurately as the plant water stress index (CWSI) (Reginato and Howe, 1985). Therefore, methods aiming to determine the internal water status of plants are used by many researchers in making irrigation plans (Reginato and Howe, 1985; Yazar, 1993). In a study, it was determined that the slope and intersection of the lower limit threshold without water stress increased until the vegetation reached 70 %. It was found that the slope of the lower boundary line without water stress and the correlation coefficient obtained from measurements under conditions where the crown temperature was greater than 27.4 °C were greater than that obtained from daily measurements (Wanjura et al., 1990). Gençoğlan (1996), in his study to prepare an irrigation program by using the CWSI determined from infrared thermometer (IRT) and porometer observations in Çukurova conditions, found that the grain yield started to decrease and the threshold CWSI value determined from infrared observations before irrigation was 0.19 and the threshold value determined from porometer observations was 0.26 reported that there will be no yield loss in irrigated corn under these conditions. Fischer (2001) reported that the leaf crown temperature (cover temperature) of the plant is an important criterion determining the difference from the ambient air temperature and that the leaf crown temperature is lower than the air temperature. The plant

water stress index was developed from the relationship between the crown temperature and the air temperature difference versus the vapor pressure gap of the air. When the plant reaches a certain water stress index value, it should be watered. This threshold value varies from plant to plant, climate and cultivation techniques (Çolak et al., 2012). Gençel (2009), in his study to prepare an irrigation program by making use of the CWSI values. It was determined that the grain yield started to decrease, the threshold CWSI value determined from infrared observations before irrigation decreased to 0.35-0.40 (just before irrigation) in the most frequently irrigated I40 subject and to 0, which was the lowest value approximately two days after irrigation. Tanriverdi (2010) reported that Water Stress Index (WSI), or WSI and Water Deficiency Index (ADI), are useful tools that can be used to optimize irrigation time. It has been reported that the water stress present in the plant can be determined quantitatively by using some measurement and observational criteria. It can be said that these parameters are the difference between the plant crown and the air temperature and the vapor pressure gap of the air (Jackson, 1981). Moroni et al. (2012) emphasized that the fastest and most accurate method for measuring water stress is the canopy (leaf-crown) temperature (CWSI). In general, the decrease in soil moisture before irrigation has an effect on increasing the plant crown temperature values, and the CWSI values are higher with the decreasing moisture in the soil (Kırnak and Gençoğlan, 2001). It has been reported that the CWSI value determined by using the lower (LL) and upper limit (UL) lines, which are found theoretically and experimentally, varies between zero and one (Gençoğlan and Yazar, 1999). Çamoğlu et al. (2011) stated that leaf water content values can be used in the instant determination of plant water stress. Some researchers stated that the leaf crown temperature determined as a result of IRT measurements and the plant water stress index calculated by using these values can be used in the preparation of irrigation plans (Clawson and Blad, 1982). On the other hand, Nielsen and Gardner (1987) reported that the irrigation time can be determined by using the plant water stress index values, but the amount of irrigation water to be applied cannot be determined with the aforementioned method. The aim of this study is to determine the CWSI by using the leaf crown temperature values measured in the second crop sesame plant grown in Siirt conditions in 2016 and 2017, to determine the stress threshold line (watering time of sesame) where the decrease in yield begins, and to determine the relationship between grain yield and plant water stress index.

II. MATERIAL AND METHOD

Hatipoğlu sesame genotype was used as plant material in the research. The experiment was set up according to the randomized blocks design and was carried out in triplicate. The distance between rows was adjusted to be 70 cm, 4 rows were planted in each plot, and the plot dimensions were arranged as 6 m x 2.8 m. In the study, hand sowing was done on 1 July 2016 in the first year and on 18 June 2017 in the second year. Before planting, di-ammonium phosphate and urea fertilizers were applied homogeneously to each plot, with 8 kg of phosphorus and 4 kg of nitrogen per decare over pure matter (Arslan, 2003). In the harvest of sesame plants, one row from the parcel edges and 50 cm from the beginning and end of the parcel were taken as edge effect, and the parcel seed yield was determined on the remaining two rows (Arslan, 2014). The harvest date was made on 07 November 2016 in the first year and on 29 October 2017 in the second year. The research was carried out in Siirt University, Faculty of Agriculture, during the growing season of the second crop sesame plant in 2016 and 2017. In the study, different irrigation levels were applied with 100% (I_{100}, control subject) of the water consumed in a soil profile of 90 cm every seven days, full irrigation, and 70% (I_{70}) and 35% (I_{35}) of full irrigation applied. It was created from limited irrigation issues. Thus, 1 full irrigation (I_{100}) and 2 different levels of limited irrigation (I_{70}, I_{35}) were formed from a total of 3 irrigation subjects. Irrigation applications were made by drip irrigation method. In the year the research was conducted, 7 irrigation applications were carried out and the soil water content change was followed by the gravimetric method. Data on climate are given in Table 1. The region is mostly under the influence of dry and hot tropical air masses settled in the Basra low pressure center during the summer season. The highest temperature during the day is above 40 ºC. In the winter season, the region is under the influence of the fronts coming from the Central Mediterranean. The front activities that cause these precipitations continue until April (Atalay and Mortan, 2013). In the study area, it is observed that the average temperatures do not fall below 26 ºC in the summer period (June, July, August) and 2.7 ºC in the winter period (December, January, February). In the area subject to the research, the long-term relative humidity average is highest in January with 70.2 %, and the lowest in August with 26.9 %. The water holding capacities of soils at field capacity (33 kPa) and wilting point (1500 kPa) were determined according to the bulk weight Blake and Hartge (1986) (in intact soil samples). The soil of the trial field has a low electrical conductivity, lime rate does not pose a problem for plant cultivation, does not have salinity problems, and has a clayey textured soil structure. In the analysis of irrigation water, the method specified by (Tüzüner, 1990) was used to determine the electrical conductivity and pH values, and anions and cations. Irrigation water quality class used in the study area; As a result of the samples taken, it was determined as C2S1. The electrical conductivity of the irrigation water was determined as 0.34 dS m-1 and pH 7.20. The irrigation water used in the experiment does not pose a problem in terms of irrigation of the sesame plant.

TABLE 1
CLIMATE DATA FOR MANY YEARS AND RESEARCH YEARS (2016-2017)

Years	Months	Maximum temperature average (°C)	Average temperature (°C)	Minimum temperature average (°C)	Average humidity (%)	Average wind speed (m s^{-1})	Average sunny days (h)	Total rainfall (mm)
Everage 1962 - 2018	May	25.2	19.4	9.0	49.3	1.0	9.1	36.9
	June	27.2	26.0	17.8	34.9	1.1	11.6	11.5
	July	35.1	30.5	23.4	30.3	1.1	12.3	0.6
	August	34.5	30.3	27.0	29.5	1.0	11.4	2.7
	September	30.0	25.1	14.7	37.4	1.0	10.1	7.0
	October	24.5	17.9	12.7	42.0	1.0	7.2	50.9
2019	May	26.62	19.29	14.52	50.87	1.0	8.7	39.6
	June	26.09	28.16	20.0	35.50	1.1	11.5	10.6
	July	34.13	31.45	24.35	32.69	1.0	12.4	0.1
	August	33.92	31.19	24.23	32.95	1.0	11.3	0.4
	September	31.23	25.43	21.5	39.90	1.1	10.0	9.2
	October	24.3	16.8	11.5	42.3	1.1	7.0	55.1
2020	May	24.69	21.29	14.59	51.77	1.0	9.3	37.7
	June	28.19	28.41	20.25	34.40	1.1	12.0	9.3
	July	36.24	33.19	25.35	29.69	1.0	12.5	0.1
	August	35.92	32.45	24.73	29.95	1.0	11.5	0.0
	September	32.23	27.43	21.65	36.79	1.1	10.0	12.2
	October	21.1	19.7	12.0	44.2	1.0	7.3	69.20

The moisture content of the soil profile at a depth of 90 cm was determined by gravimetric method before each irrigation. In finding the amount of irrigation water to be given to the subjects, the amount of irrigation water that will bring the missing moisture in the soil depth of 90 cm to the field capacity for full irrigation (I_{100}) was used. The moisture content (%) determined for each layer was converted to moisture content in depth using the equation below

$$d = \frac{PwxAsxD}{10} \quad (1)$$

In the equation d; water content of soil moisture in depth (mm), Pw; moisture content (%), As determined for each layer; bulk weight of soil (g cm^{-3}) and D; layer depth (cm). The total water (d_T) amount for the 90 cm soil profile was found by summing the water depth calculated for each layer.

$$d_T = d_{(0-30)} + d_{(30-60)} + d_{(60-90)} \quad (2)$$

The volume of water to be given to the parcels was calculated from Equation 3 by multiplying the total water amount, the parcel area, the percentage of curtailment (1, 0.70, 0.35) and the percentage of cover.

$$V = d_T x A x U_o x P \quad (3)$$

V in equality; the volume of water (L) and A to be given to the plots; parcel (m^2), Uo; cut percentage (%) and P: cover percentage (%) Cover percentage was calculated by dividing the plant crown width by the plant row spacing. Its true value is fixed at 0.30 until the ÖY is 30 %, and then at 80% until it reaches 80% thereafter. The lateral lines of the drip irrigation system to be used in the experiment have an outer diameter of 16 mm, and the drippers to be used are internally permeable and with constant flow. 1 lateral is laid on each plant row. After calculating the amount of water to be given to the plots, the irrigation water to be applied was given by passing through the meters.

CWSI measurements were started with infrared thermometers, before and after irrigation, from the date when the plants covered the soil surface by approximately 80 %, and the measurements continued until the date of physiological maturity. Crown temperature (Tc) measurements were made between 12:^{00} and 14:^{00} in conditions where the weather was completely clear or the clouds did not block the sun. The average crown temperature of that parcel was found by taking the average of 12 measurements in total, in the direction of the diagonals of the parcels (from 4 corners) and with 3 replications from each of them. At the beginning and end of the plant crown temperature measurements, wet and dry thermometer values were read with a digital psychrometer.

In the determination of CWSI, The empirical method developed by. (1981) was used. According to the method developed by Idso et al (1981), CWSI was calculated with the following equation.

$$CWSI = \frac{[(T_c - T_a) - LL}{UL - LL} \quad (4)$$

Eq. Tc; crown temperature (°C), Ta; air temperature (°C), LL; lower limit of plants free of water stress, UL; refers to the upper limit values at which the plants are completely under stress. The lower limit (LL) at which plants experience no water stress, Idso et al. (1982) and obtained as a result of the regression analysis between the crown-air temperature difference and the vapor pressure gap (VPD, kPa) were calculated with the help of the following equation.

$$T_c\text{-}T_a = a\text{-}b * VPD \quad (5)$$

In the equation, as is the cross-sectional value of the line (°C); b shows the slope of the line (°C kPa^{-1}). Vapor pressure gap Howell et al. (1992) using the basic psychrometer equations. These equations are given below.

$$e_w = 0.61078 \exp\left[\frac{17.27 T_w}{237.3 + T_w}\right] \quad (6)$$

$$e_a = e_w - [(AP)(T_a - T_w)] \quad (7)$$

In equations; e_w, saturated vapor pressure (kPa) at wet bulb temperature; e_a, actual vapor pressure at air temperature (kPa), Tw, wet bulb temperature (°C); A is the psychrometric constant (kPa°C^{-1}); P is the barometric pressure, (kPa).

The psychometric constant (A) was calculated from the equation given below.

$$A = [0.00066(1 + 0.00115 T_w)]$$

The saturated vapor pressure was determined from the equation given below.

$$e_a * (T_a) = 0.61078 \exp\left[\frac{17.27 T_a}{237.3 + T_a}\right] \quad (8)$$

The vapor pressure gap (VPD) is found by taking the difference between the saturated vapor pressure at the dry bulb temperature and the actual vapor pressure at the same temperature.

$$VPD = [(e_a * (T_a) - e_a)]$$

In the equation: $ea * (T_a)$, is the saturated vapor pressure (kPa) calculated at dry bulb temperature, The upper limit (UL) where plants are assumed to experience complete water stress, Idso et al. (1982) with the help of the methods suggested.

$$T_c\text{-}T_a = a\text{-}b\ VPG \quad (9)$$

$$VPG = [(e_a * (T_a) - e_a * (T_a + a))] \quad (10)$$

where: a and b; The regression coefficients in the lower bound (LL) equation without water stress, VPG, are the negative atmospheric vapor pressure slope required for zero crown-air vapor pressure training.

The findings obtained from the study were subjected to analysis of variance and statistically significant applications according to the results of analysis of variance were compared with the LSD test. Correlation analysis was performed to determine the relationship between the features. (Der and Everitt, 2002).

III. RESULTS AND DISCUSSION

Seasonal water consumption (ETa) of the sesame genotype was 606.00 mm and 646.33 mm, respectively, during the research years, throughout the soil effective root depth of 90 cm in I100 irrigation, where water stress was determined at a minimum level; In the case of I35 irrigation, where water stress is applied excessively, it was determined as 171.00 mm and 212.00 mm

in the first and second years of the study, respectively. In order to determine the plant water stress index in the sesame genotype in the years of the study, the lower limit (LL) formulas to define the situation where the genotype potentially transpiration and the upper limit (UL) equations, which define the situation where the plant is under extreme water stress, are given in Figure 1.

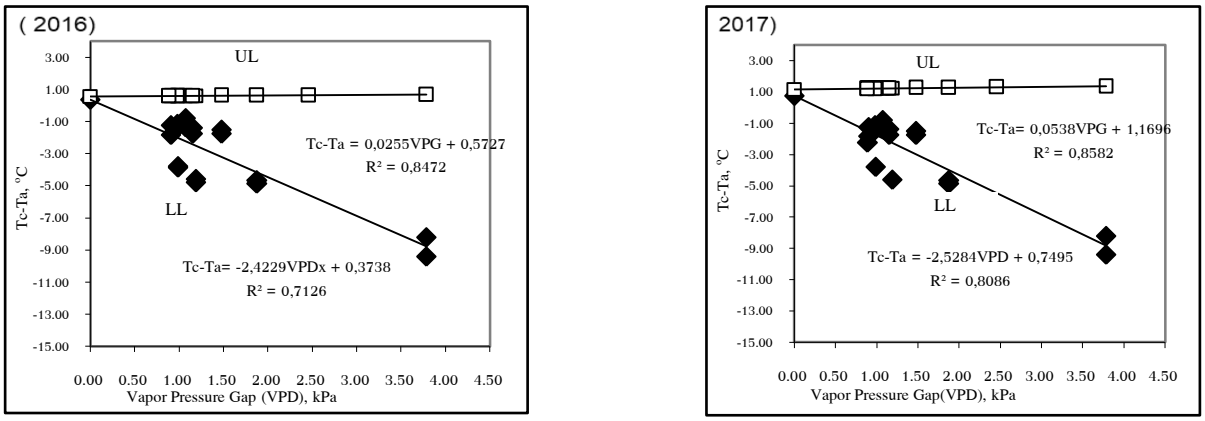


FIGURE 1: The lower and upper bound relationship values of sesame plant during the research years

As can be seen in Figure 1, the condition of no water stress in the plant for the first year of the study, that is, the lower limit (LL) equation assumed to potentially evapotranspiration Tc - Ta = 0.373 - 2.422 VPD (R2 = 0.71) and Tc - Ta = for the second year of the study 0.74 - 2.52 VPD (R2 = 0.80). In the equation, the intersection values of the LL line are determined as positive. Idso et al. (1982) reported that the intersection value could not be less than 0 and that this value occurred due to the formation of a positive water vapor flow from the leaves to the atmosphere, even if the VPD was reduced to zero by saturating the atmosphere. In the light of these data, according to the LL equation, it can be concluded that a positive water vapor flux towards the atmosphere occurs during the whole growing season, as mentioned in previous studies on this subject (Köksal, 1995; Gençel, 2009). The upper limit (UL) equation, which is assumed to be completely under water stress, was determined as Tc - Ta = 0.572 + 0.025 VPG for the first year of the study, and Tc - Ta = 1.169 + 0.0538 VPG for the second year of the study. The differences between the crown temperature and the air temperature in UL were determined as 1.169-0.572 ºC for the first and second years of the study, with the slope being neglected in the UL formula due to its minimum level. Although the CWSI values measured at different levels of irrigation were mostly between 0 (no water stress) and 1 (maximum stress state), some of these values were negative. It can be said that the negative CWSI values obtained are due to the fact that the measurement values used in the calculation are lower than the LL line shown in Figure 1. The average CWSI values determined in irrigation subjects during the research years are given in Table 2. As can be seen from Table 2, the average CWSI values determined in irrigation subjects were obtained as 0.62 for the highest I35 irrigation in the first year and 0.21 for the lowest I_{100} irrigation, respectively. In the second year of the study, the highest I_0 was 0.60 in irrigation; the lowest I_{100} irrigation was determined as 0.20. In other irrigation issues, it changed between these two values. It is seen that the CWSI values determined in all irrigation subjects in the first year are slightly higher than the CWSI values obtained in the second year. This can be related to the fact that the temperature values in the first year, especially in July and August, when the second crop sesame plant grows, are higher than the second year. The CWSI values determined according to the days measured during the growing season are given in Figure 2 for the first and second years of the study, respectively. As can be seen from the aforementioned figures, CWSI values, which show an increase depending on the severity (excess) and duration of the applied water restriction, generally showed a tendency towards maximum before irrigation and decreasing after irrigation. Köksal (1995), CWSI values of 0.13-0.43 in the subject that receives the most water in Çukurova runners; He found that it varies between 0.42-073 in the subject that receives the least amount of water. Ödemiş and Baştuğ (1999) reported that a time period is needed for the CWSI value to decrease after irrigation and this period varies between 4-5 days. The researchers emphasized that as the amount of water in the soil decreases, the plant suffers from water stress and therefore there is an increase in the CWSI value. Simsek et al. (2005) stated that the CWSI value changes depending on the amount of water applied and in general, high CWSI values cause yield loss in plants. Gençel (2009) found the CWSI value of 0.35-0.40 for the most frequently irrigated I_{40}, 0 to 0.55 for I_{60} irrigation, which is similar to the irrigation program applied by the producers, and 1.0 for I_{80}, which represents extremely stressful conditions in the plant. In short, depending on the amount of water stress applied, it was observed that there was a change in the CWSI values between the irrigation subjects as expected,

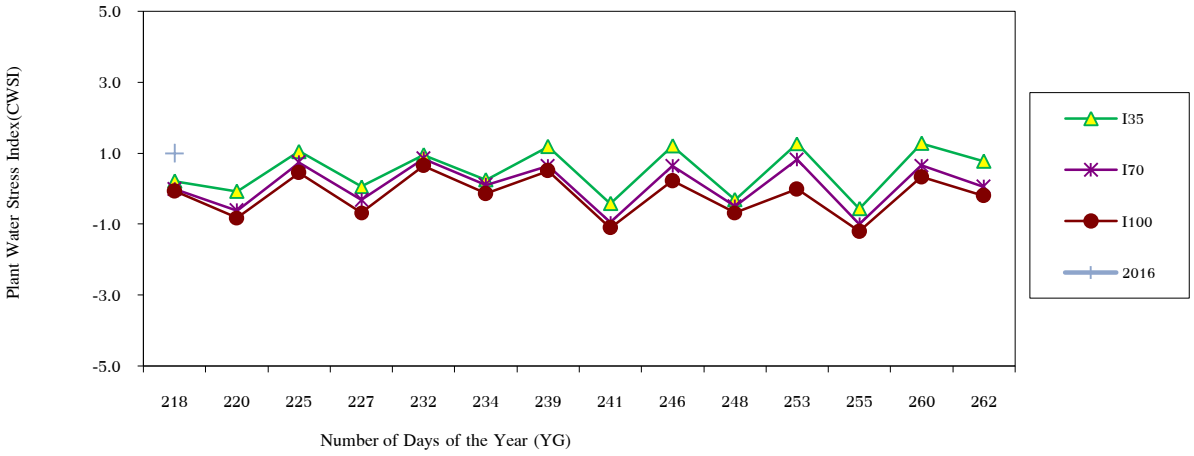
as expected. The CWSI threshold values, where the efficiency determined from the IRT observations began to decrease, were found to be 0.32 for the first year of the study, 0.31 for the second year of the study, and approximately 0.31 when the results of both years were evaluated together. Köksal (1995) found the threshold value as 0.33 for grain yield and 0.32 for dry matter in the second crop corn plant. Gençoğlan and Yazar (1999) determined the threshold CWSI value determined from infrared observations as 0.19 and the threshold value determined from porometer observations as 0.26 in Çukurova conditions. Reginato (1983) stated that little or no effects of water stress on plants are observed in conditions where the CWSI value is less than or equal to 0.2. There is a similarity between the threshold CWSI values obtained in this study and those obtained by Gençoğlan and Yazar (1999), whereas there is a partial difference between the threshold CWSI values obtained by Köksal (1995). We can attribute this situation to the fact that the responses of genotypes obtained through breeding in recent years to the decrease in moisture in the soil are different from each other. From this, it can be said that the CWSI (thus the threshold at which the yield starts to decrease) may vary depending on the irrigation program, the soil type in which the experiment is carried out, the climatic values of the trial years, the cultivar that forms the plant material in the trial and the cultivar cultivated as the main or second crop.

TABLE 2

PLANT WATER STRESS INDEX, IRRIGATION WATER AND PLANT WATER CONSUMPTION VALUES DURING THE RESEARCH YEARS

Irigation considerations	Yield (kg ha^{-1}	Plant water stress index	Irrigation water (mm)	Plant water consumption (mm)	Water Usage Efficiency (kg da^{-1} mm)
2016 (first year)					
I100	1110.34 a	0.21 c	575.00	606.00	1.82 c
I70	857.05 b	0.43 b	403.00	435.00	1.96 b
I35	654.54 c	0.62 a	141.00	171.00	3.82 a
LSD	181.82	0.015			0.32
2017 (Second year)					
I100	1319.0 a	0.20 c	576.66	646.33	2.04 b
I70	910.0 b	0.42 b	404.50	474.00	1.92 c
I35	711.0 c	0.60 a	141.33	212.00	3.35 a
LSD	83.10	0.013			0.20

: p < 0.05 and **; p < 0.01. ns;not importent



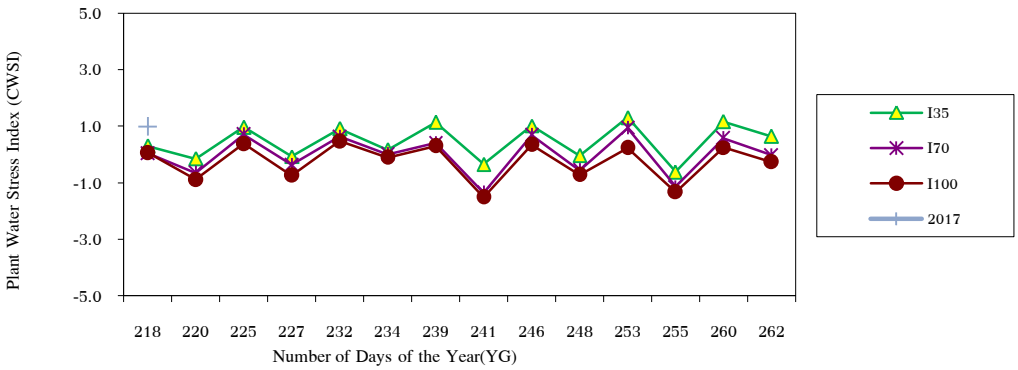


FIGURE 2: Plant water stress index values determined in irrigation subjects during the research years

3.1 Correlation analysis

Correlation coefficients (r) of the relationships between the plant water stress index (CWSI) of sesame plant and yield, water use efficiency and chlorophyll content are given in Table 3 for 2016 and 2017, respectively. When the correlation coefficients of 2016 were examined, it was determined that there were statistically significant relationships ($p \leq 0.01$) between all these features. There is a decreasing (negative) relationship as high as r = -0.85 between CWSI and yield. There is a high (positive) correlation of r = 0.74 between CWSI and water use efficiency. There is a decreasing (negative) relationship as high as r = -0.97 between CWSI and chlorophyll content. In other words, it can be said that as CWSI increases, there is a decrease in yield, an increase in water use efficiency and a decrease in chlorophyll content. When the correlation coefficients of 2017 were examined, it was determined that there were statistically significant relationships ($p \leq 0.01$) between all these features, similar to the first year. There is a high decreasing (negative) relationship such as r = -0.94 between CWSI and yield. There is an increasing (positive) relationship as high as r = 0.50 between CWSI and water use efficiency. There is a decreasing (negative) relationship as high as r = -0.96 between CWSI and chlorophyll content. In other words, it can be said that as CWSI increases, there is a decrease in yield, an increase in water use efficiency and a decrease in chlorophyll content.

TABLE 3
RELATIONSHIPS BETWEEN PLANT WATER STRESS INDEX AND OTHER PARAMETERS

a (2016)	Yield	WUE	CWSI	CC
Yield	1.00	-0.45*	-0.85**	0.86**
WUE	-0.45*	1.00	0.74**	-0.64**
CWSI	-0.85**	0.74**	1.00	-0.97**
CC	0.86**	-0.64**	-0.97**	1.00
b (2017)	**Yield**	**WUE**	**CWSI**	**CC**
Yield	1.00	-0.25ns	-0.94**	0.97**
WUE	-0.25ns	1.00	0.50**	-0.44*
CWSI	-0.94**	0.50**	1.00	-0.96**
CC	0.97**	-0.44*	-0.96**	1.00

: p < 0.05 and **; p < 0.01. ns;not important. WUE; Water usage efficiency. CWSI; plant water stress index. CC; chlorophyll content

IV. CONCLUSION AND SUGGESTIONS

This work; A field study was conducted in semi-arid climate conditions in order to determine the plant water stress index of the sesame genotype grown in water-free and water-stressed conditions and to determine the threshold value at which the economic yield reduction will start from the residual sesame plant by making use of these data. As a result of the findings obtained from the research, II. When the plant water stress index threshold value of the crop sesame plant is 0.31, it can be decided that the irrigation time has come, and when irrigation is done when it is 0.31, there will be no statistically significant loss in yield, and it has been determined that 30% water reduction can be made in conditions where irrigation water is limited. It has been determined that if the CWSI is higher than the value mentioned above, there may be a significant decrease in yield. In the light of the data obtained from the study, yield estimation can be made by making use of the linear relationships between the sesame grain yield and the plant water stress index obtained by using the leaf crown temperature measurements made at the irrigation time.

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Influence of Particle Size on the Bioadsorbent Behavior of Orange Peel

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Abstract— *The physical treatment of citrus peels comprises five stages: collecting, washing, drying, grinding and sieving. It is very important to have a grinding system that allows to regulate the pre-established particle size for future applications of the shell. For this, it is necessary to complement the grinding with a good sieving.*

The optimal particle size for continuous applications through fluidized beds has been found to be between 500-1000 µm. Although the 200-500 µm range has applications in batch processes, particle sizes less than 200 µm are usually not used as they remain in suspension and only precipitate after a considerable period of time.

This work presents a modification of the chemical treatment of orange peel that aims to take advantage of the material that passes through a standard 200µm sieve and reuse it as a bioadsorbent. The results obtained with Cu (II), Mg (II) and Na (I) at different concentrations are consistent with the use of bioadsorbents in metal removal.

Keywords— bioadsorption; reuse; Orange peel; ultrasonic radiation, cation exchange.

I. INTRODUCTION

Currently, society is progressively committing itself to achieving a sustainable economy based on the exploitation of renewable resources by promoting waste recycling and the design of products obtained in ecologically efficient processes. From this point of view, biomass, mainly of plant origin, seems the best option to replace a high percentage of fossil fuels in their energy and chemical applications (Cherubini 2010).

However, according to the Food and Agriculture Organization of the United Nations (FAO), around 30% of global food production is lost during harvest, processing and final consumption, assuming this amount more than 1,300 million tons per year (Thi Phuong 2015).

Agri-food waste is potential raw material for various applications, which is why it is intended to develop high-value products such as cosmetics, fuels, medicines, essential oils, pectin, feed, activated carbon, pollutant adsorbents, fuels, energy, among others (Ortiz et al., 2020; Deba-Rementeria et al., 2021;). These wastes include fruit waste that is generally disposed of in landfills, composted, used as feed, incinerated or solidified; processes that may involve a high demand for energy or generate damage to the environment, which is why it is essential to reduce them and use them as an alternative source of renewable energy (Lin et al., 2021).

Of the wide variety of fruits and vegetables, whose processing generates waste suitable to be used as raw material, it is worth highlighting citrus fruits that generate large amounts of waste in the form of skin, pulp and seeds in the production process of juice and other derived foods (Mirabella et al. 2014).

Citrus fruits have various applications in the food, cosmetic and perfume industry thanks to their taste and aroma (Nateghpouret al., 2021). In the food industry approximately 26% of citrus fruits are used to make juice. During the processing of citrus fruits, the peels are the main by-product and a potential burden on the environment without additional treatment (Wang et al., 2015). Between 50-60% of the mass of the fruit remains after processing (peel, seeds and membrane residues), it is estimated that, annually, citrus waste created by food processing industries exceeds 54 million tons all over the world (Teigiserova et al., 2021).

Among the variety of edible citrus fruits, it should be noted that the most abundant in the world is the orange (Citrus sinensis L.), which represents about 60% of the world total (Erukainure et al, 2016). Part of the waste generated is used to feed livestock. However, it is in such a quantity that large amounts end up being deposited in landfills causing serious environmental and economic problems (Tripodo et al., 2004).

The waste from the manufacture of orange juice is made up of pulp, seeds and skin. The skin is made up of an orange outer layer (flavedo) and a fluffy white inner layer (albedo). The pulp is very moist and rich in monosaccharides (glucose and fructose) and disaccharides (sucrose). The inner layer of albedo is rich in pectin, while the outer layer of flavedo contains a large amount of essential oils, limonene being the main component, and flavonoids (Davies1994). Its composition varies depending on the crop, the time of year and the region and technology used in the production of juice. Despite these possible variations, the orange residue is always very humid, with a variable water content between 80 and 84% (Rezzadori et al,. 2012).

Depending on the particle size, the orange peel has different water adsorption capacity. Table 1 shows the results obtained with 5g samples of orange peel of different particle size and in Figure 1 the appearance of the orange peel after being in contact with water.

TABLE 1
WATER ADSORPTION IN 5 GRAM ORANGE PEEL SAMPLES OF DIFFERENT PARTICLE SIZES AT ROOM TEMPERATURE

Orange	Particle size	mL H_20 Medium	mL / g
	1mm> X> 500 µm	37.8 ± 0.35	7.6
	500 µm> X> 250 µm	28.2 ± 0.5	5.6
	X <250 µm	23.1 ± 0.8	4.6

It can be seen that the adsorption decreased as the particle size decreased. This behavior was attributed to the fact that the possibility of caking increases when there is more contact between the particles (Pietsch 2002). The powdery particles become wet, sticky and compact, finally reaching the liquefaction phase (figure 1).

On the other hand, from the extraction of albedo, citric pectin, a thickener commonly used in the food industry, is obtained by acid hydrolyzing. However, in most processes, obtaining pectins is linked to obtaining essential oil (Cerón-Salazar 2011).

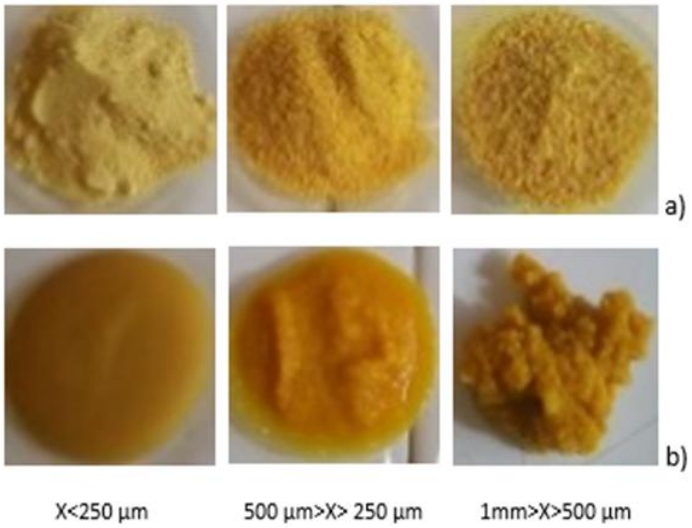

FIGURE 1: Appearance of the orange peel vs particle size a) dry b) saturated with water (Garcia Raurich et al, 2020a)

The industrial production of citrus pectin has the following stages: in the first stage, the peel must be washed to remove as much soluble solids and impurities as these components make the purification process difficult. Then, the shells are subjected

to a drying process, which inactivates the pectin esterase enzyme and lowers the moisture content, increasing the stabilization of the shell for storage and reducing the cost of transportation (Marti et al, 2014).

Subsequently, the dry matter is suspended in hot water with the necessary amount of a strong acid, starting the hydrolysis process. During this process, starting from the macromolecular structure formed by cellulose, hemicellulose and pectins, hemicellulose starts its degradation to glucose, galactose and fructose; cellulose to glucose and pectin to pectin monomer through a depolymerisation process (Chen et al, 2015). After a while, the resulting solution is removed from the insoluble solids by filtration. Next, it is mixed with alcohol, producing the recomposition of the pectin polymer and its corresponding precipitation. The precipitate is removed and purified by washing it with more alcohol. Finally, it is dried and ground (Claus, 2002). The material resulting from the pectin extraction is a poor food supplement for animals due to its low protein content and high sugar content (Siles et al, 2016).

On the other hand, the solid fraction presents the optimal conditions for its subsequent treatment as a bioadsorbent (Masmoudi et al, 2008). The resulting solid is treated in an alkaline medium. In this way, the saponification of the non-soluble pectin in an acid medium is achieved, as well as the solubilization of the soluble fraction of hemicellulose in an alkaline medium (Grace et al., 1996).

After chemical treatment, the resulting product has the characteristics of a cation exchanger. The optimal size for the removal of heavy metals in continuous processes has been established between 500-1000 µm (Garcia Raurich et al, 2020b).

The objective of this work has been to reuse the fraction less than 200 µm obtained in the grinding of the orange peel. For this, various modifications in the chemical treatment have been studied.

II. MATERIALS AND METHODS

The orange peel with a particle size <200 µm was subjected to different variants of the chemical treatment.

The reference treatment was carried out using HCl as the acid reagent. To do this, 50 g of the orange peel were mixed with 700 mL of deionized water in a sealed container and homogenized by stirring on a Movil-Rod rotary shaker. Once the mixture was homogenized, 5 mL of concentrated HCl were added and it was stirred again until further homogenization. Next, the mixture was subjected to an ultrasound treatment (US), using a US Elmasonic bath, model LC 30 H with a fixed frequency of 37.5 kHz and regulation of time and temperature, for a period of 60 minutes.

In order to extract the maximum amount of organic matter, three other modalities of acid attack were tested. In the first, in addition to the 5 mL of HCl, 5 mL of concentrated H_2O_2 (33%) were added. In the second, 5 mL of HCl and 50 mL of Dimethylsulfoxide (DMSO) were added to complete the total volume of 700 mL. Finally, in the third modality, 5 mL of HCl, 5 mL of concentrated H_2O_2 and 50mL of DMSO were added to complete the total volume of 700 mL.

On all occasions, at the end of the treatment, the solid phase was separated from the liquid phase by filtration. In the liquid phase, the presence of pectins was verified by precipitation in a hydroalcoholic medium, while the solid phase was subjected to a treatment with deionized water to eliminate excess HCl. To do this, solid obtained, 700 mL of deionized water were added to it and it was stirred for 30 minutes on a rotary shaker. Then it was filtered and the alkaline attack was carried out.

To carry out the alkaline attack, the resulting solid was introduced into an airtight container with 700 mL of deionized water and the amount of $Ca(OH)_2$ selected (1g vs 5g) previously weighed with a SCALTEC SBA 52 precision balance was added, the contents of the container were homogenized by stirring by means of the rotary shaker and, finally, the mixture was subjected to the influence of US radiation for a period of 60 '. The object of this saponification was to obtain the maximum number of anchor points in the form of carboxylate groups.

In order to obtain the final bioadsorbent, it was necessary to wash the solid phase with deionized water until the excess of calcium was eliminated and, finally, to place it in a Nahita 631/4 laboratory oven for 24 hours at 110ºC.

Experimentally, it was observed that at the end of the neutralization treatments there was a significant loss of solid phase from the initial acid treatment, since the initial size of the orange peel was very small (<200 µm). Table 2 shows the different experimental conditions and the results obtained.

As an alternative, it was decided to carry out the alkaline attack immediately after the acid attack without prior separation of the solid phase from the liquid phase. In Figure 2, it is observed how the solid phase protrudes, in a compact way, from the liquid phase as a consequence of the binding effect of the pectins extracted in the acid attack and that were not separated by filtration.

TABLE 2
FINAL BIOADSORBENT OBTAINED AND PERFORMANCE WITH RESPECT TO THE INITIAL WEIGHT (50g)

Acid treatment	Alkaline treatment	Obtained weight (g)	Performance (%)
5 mL HCl	1 g $Ca(OH)_2$	6.85 ± 1.2	13.70
5 mL HCl	5 g $Ca(OH)_2$	7.37 ± 0.9	14.75
5 mL HCl + 5 mL H_2O_2	1 g $Ca(OH)_2$	10.42 ± 1.8	20.83
5 mL HCl + 5 mL H_2O_2	5 g $Ca(OH)_2$	11.07 ± 1.5	22.14
5 mL HCl + 50 mL DMSO	1 g $Ca(OH)_2$	7.41 ± 1.2	14.82
5 mL HCl + 50 mL DMSO	5 g $Ca(OH)_2$	6.10 ± 1.4	12.20
5 mL HCl + 5 mL H_2O_2 + 50 mL DMSO	1 g $Ca(OH)_2$	10.08 ± 1.0	20.16
5 mL HCl + 5 mL H_2O_2 + 50 mL DMSO	5 g $Ca(OH)_2$	10.25 ± 1.3	20.51

FIGURE 2: Solid phase compacted by the binding effect of pectins

Before proceeding to eliminate the excess Ca(II), incorporated in the form of $Ca(OH)_2$ in the alkaline attack, the compacted solid phase was introduced inside the laboratory oven at 110ºC for a period of 24h. Table 3 shows the bioadsorbent weights obtained after removing the excess Ca(II) by repeated washing with deionized water and drying again at 110ºC for another 24h period. It can be seen that all were significantly higher than those obtained using the standard procedure.

TABLE 3
FINAL BIOADSORBENT OBTAINED AND YIELD WITH RESPECT TO THE INITIAL WEIGHT (50g) USING THE ALTERNATIVE TREATMENT.

Acid treatment + alkaline treatment	Obtained weight (g)	Performance (%)
5 mL HCl + 5 g $Ca(OH)_2$	26.91 ± 2.0	53.82
5 mL HCl + 10 g $Ca(OH)_2$	19.10 ± 1.9	38.20
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	23.82 ± 1.5	46.64
5 mL HCl + 5 mL H_2O_2 + 50 mL DMSO + 5 g $Ca(OH)_2$	17.40 ± 2.2	34.80

Figure 3 shows the IR spectra of the initial shell and of the bioadsorbents obtained by the conventional chemical treatment and the alternative chemical treatment between 1800-1000 cm^{-1}. The peaks that appear centered at 1747 cm^{-1} and at 1638 cm^{-1} that appear in the spectrum of orange peel, before their chemical treatment, are attributable to the carbonyl group (C=O) as indicators of esterified and free carboxylic groups. In fact, the disappearance of the peak at 1747 cm^{-1} in the orange peel spectrum once subjected to a chemical treatment indicates the disappearance of high methoxyl pectins in both the conventional chemical treatment and the alternative chemical treatment.

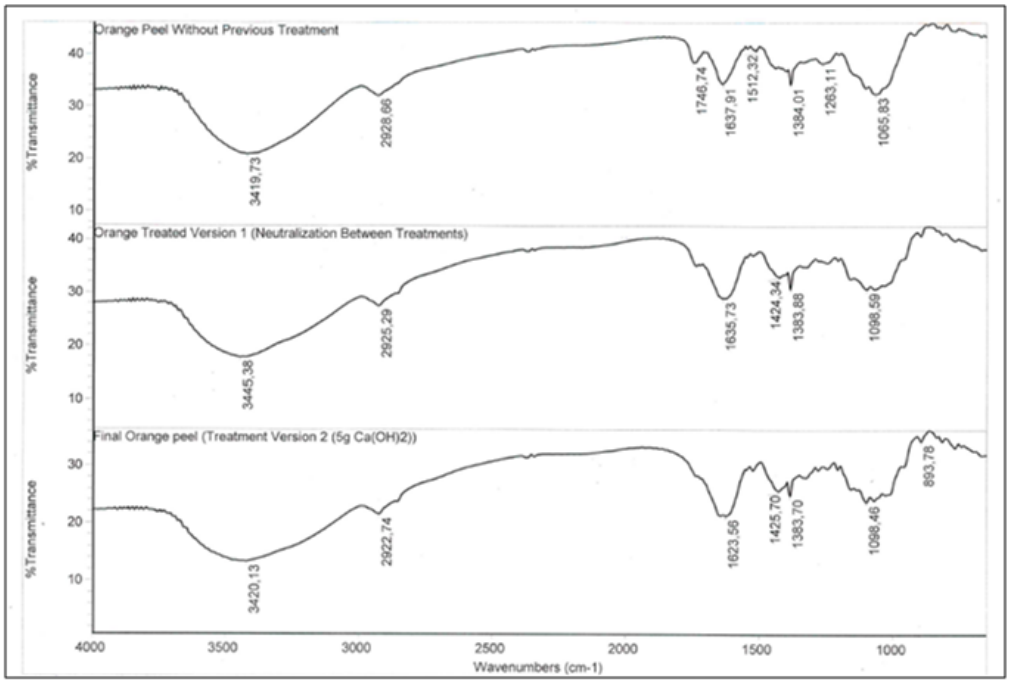

FIGURE 3: Comparison of the IR spectrum of the orange peel before being subjected to the chemical treatment with the IR spectra obtained with both the conventional chemical treatment and the modified chemical treatment.

2.1 Recovery of pectins

Although obtaining pectin from orange peels has been studied extensively (Msebahi et al., 2005), (Liu et al., 2006), (Yeoh et al., 2008), the extraction of pectins by conventional methods is carried out by heat treatment at 90°C for at least one hour in acidic aqueous solutions, so that the pectins that are not sensitive to calcium are extracted. After a while, the resulting solution is removed from the insoluble solid by filtration. Then, it is mixed with 90º alcohol and the pectin precipitates (Claus, 2002). For this, the same volume of alcohol is added as that of the liquid fraction obtained after the acid attack of the orange peel. Figure 4 shows the consistency of a pectin precipitate obtained by adding a volume of ethyl alcohol equal to the volume of the liquid fraction.

FIGURE 4: Appearance of the consistency due to the precipitation of the pectins after adding 100% vol. ethanol

The modification introduced (alkaline attack directly after the acid attack) achieved a higher yield of bioadsorbent, due to the binding effect of the pectins extracted in the acid attack.

The fraction soluble in aqueous medium (water-soluble pectins, excess of Ca(II) and organic matter) was jointly extracted from the bioadsorbent obtained from 50 g of orange peel. To do this, the solid obtained was brought into contact with 500 mL of deionized water and heated at 60°C for 30 minutes with gentle stirring. Next, the volume of the liquid phase was

reduced by means of an IR lamp to a volume of 20 mL. The addition of an equal volume of EtOH caused the pectins to precipitate.

The two experiences with the highest performance described in the table 3, performed with 5mL HCl + 5 g $Ca(OH)_2$ and with 5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$ and compared with one performed with NaOH, the alkali used in the conventional treatment (saponification with NaOH followed by crosslinking with $CaCl_2$).

Table 4 shows that the saponification and crosslinking process carried out in a single stage with the use of $Ca(OH)_2$ is superior to that carried out with NaOH, since the weight of the pectin is significantly lower. The oxidative influence of hydrogen peroxide, which caused a greater release of the pectin retained inside the bioadsorbent, was also detected.

TABLE 4
PERCENTAGE OF PECTIN RECOVERED FROM THE BIOADSORBENT OBTAINED FROM 50 g OF ORANGE PEEL

Acid treatment + alkaline treatment	Pectin weight (g)	Performance (%)
5 mL HCl + 5 g $Ca(OH)_2$	1.76 ± 0.4	3.52
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	3.78 ± 1.2	7.56
5 mL HCl + 5 g NaOH	3.48 ± 0.7	6.96

2.2 Bioadsorption of copper in discontinuous process

To determine the exchange capacity of the bioadsorbent obtained by the alternative procedure, it was ground, sieved and the solid fraction between 500-1000 µm was used.

In three containers of 50 mL capacity, 0.5; 1 and 2 g of bioadsorbent obtained through the simplest attack (5 mL of HCl + 5 g $Ca(OH)_2$) were introduced and hydrated for 24h with deionized water. After this period of time, by filtration, the solid phase was separated from the liquid phase. Then, inside each container, the hydrated solid was put in contact with 20 mL of a 250 ppm Cu(II) solution prepared from $CuSO_4 \cdot 5H_2O$ (supplied by PANREAC S.A.), equivalent to 5 mg of Cu(II).

Hermetically sealed, these containers were shaken for a period of 30' on the rotary shaker. Then, in three test tubes, a 5 mL sample from each container was introduced. Immediately, in each test tube, a spatula tip of KI and 5 mL of CH_2Cl_2 (both reagents supplied by PANREAC S.A.) were added and shaken for 5'. The presence of Cu(II) was only clearly evident in the 250 ppm Cu(II) reference sample, due to the formation of I_2 according to the reaction: $5I^- + 2Cu^{2+} \rightarrow Cu_2I_2 + I_3^-$

Figure 5 shows the different coloration of the organic phase in each of the test tubes. First, on the left of the image, the sample from the 250 ppm Cu(II) solution that was not put in contact with the bioadsorbent. Next, the samples from the containers in which the exchange took place between the Ca(II) contained in the bioadsorbent for the Cu(II) contained in the $CuSO_4 \cdot 5H_2O$ solution.

FIGURE 5: Colouring of the reference sample compared to those from the Ca (II) / Cu (II) cation exchange

This cation exchanger capacity was also shown in the rest of the bioadsorbents obtained in the different experimental conditions collected in the table 3.

To obtain quantitative results, the residual Cu(II) was determined by atomic absorption (AA). For this, a Varian SpectAA 110 spectrophotometer was used.

From the results of Table 5 it appears that, under the predetermined experimental conditions, a large excess of $Ca(OH)_2$ did not contribute to improving the exchange capacity of the bioadsorbent obtained. In addition, the rest of the values of the % of Cu(II) retained made the first treatment, the simplest of all, selected as the optimal treatment of the orange peel with a particle size <200 µm.

TABLE 5
Ca (II) / Cu (II) CATION EXCHANGE CAPACITY FROM 20 mL OF A 250 ppm Cu (II) SOLUTION

Acid treatment + alkaline treatment	BN (g)	ppm Cu (II) residual	ppm Cu (II) retained (*)	% Cu (II) Detained (*)	mg Cu (II) retained (*)	mg Cu (II) / g BN (*)
5 mL HCl + 5 g $Ca(OH)_2$	0.5	6.1 ± 0.9	243.9	97.6	4.88	9.76
	1.0	3.5 ± 0.3	246.5	98.6	4.93	4.93
	2.0	2.2 ± 0.5	247.8	99.1	4.96	2.48
5 mL HCl + 10 g $Ca(OH)_2$	0.5	4.1 ± 0.8	245.9	98.4	4.92	9.84
	1.0	7.0 ± 0.4	243.0	97.2	4.86	4.86
	2.0	10 ± 1.1	240.0	96.0	4.80	2.40
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	0.5	22 ± 3.2	228.0	91.6	4.56	9.16
	1.0	14 ± 1.3	236.0	94.4	4.72	4.72
	2.0	7.5 ± 0.8	242.5	97.0	4.85	2.43
5 mL HCl + 5 mL H_2O_2 + 50 mL DMSO + 5 g $Ca(OH)_2$	0.5	7.5 ± 0.4	242.5	97.0	4.85	9.7
	1.0	4.4 ± 0.6	245.6	98.2	4.91	4.91
	2.0	3.0 ± 0.5	247.0	98.8	4.94	2.47

Initial concentration Cu (II) = 250 ppm ⇔ 5mg of Cu (II) BN: bioadsorbent obtained from orange peel (*) mean value

2.3 Reuse of the bioadsorbent

Samples of 0.5; 1 and 2 g of bioadsorbent that had been subjected to the exchange process. For this, these samples were subjected to a treatment with HCl to achieve the solubilization of Cu(II), previously adsorbed, in the form of $CuCl_2$. In this way, Cu(II) was replaced by H^+. Subsequently, the alkaline attack was carried out with 5 g of $Ca(OH)_2$. Through the process of neutralization and crosslinking, the reintroduction of Ca(II) into the three-dimensional network of the bioadsorbent occurred.

Once the excess Ca(II) had been removed by successive washes with deionized water, the bioadsorbent was dried at 110ºC for 24 hours in a Nahita 631/4 laboratory oven. Once dry, it was ground and the particle size between 500-1000 µm was used. Table 6 shows the results obtained.

TABLE 6
Ca(II) / Cu(II) CATION EXCHANGE CAPACITY FROM 20 mL OF A 250 ppm Cu(II) SOLUTION OF REUSED BIOADSORBENT SAMPLES

Acid treatment + alkaline treatment	BNR (g)	ppm Cu (II) residual	ppm Cu (II) retained (*)	% Cu (II) Detained (*)	mg Cu (II) retained (*)	mg Cu (II) / g BNR (*)
5 mL HCl + 5 g Ca $(OH)_2$	0.47	14.47 ± 1.3	235.53	94.2%	4.71	10.02
	0.85	6.16 ± 0.4	243.84	97.5%	4.88	5.74
	1.70	5.82 ± 0.5	244.18	97.7%	4.88	2.87

Initial concentration Cu (II) = 250 ppm⇔ 5mg of Cu (II) BNR: Bioadsorbent obtained from regenerated orange peel (*) mean value

2.4 Bioadsorption of alkali and alkaline earth metals in batch process

To define the exchange characteristics of the bioadsorbent, the exchange with Mg(II) and Na(I) was determined. Experiences carried out with two types of bioadsorbents were compared, those obtained with the treatments (5 mL HCl + 5 g Ca $(OH)_2$) and (5 mL HCl + 5 mL H_2O_2 + 5 g Ca $(OH)_2$).

First, the samples of each bioadsorbent were hydrated in hermetically closed containers with deionized water for a period of 24 hours. The samples were 0.5; 1 and 4 g. Next, the deionized water was replaced by 80 mL of a 982 ppm Mg(II) solution made from $MgCl_2 \cdot 6H_2O$ (supplied by PANREAC SA), equivalent to 78.56 mg of Mg(II). All samples were kept shaking for 30 minutes.

The determination of the Mg(II) retained by the bioadsorbent was carried out volumetrically (Harris 2006). For this, by means of a double-level volumetric pipet, 10 mL of the $MgCl_2$ solution that had been in contact with the bioadsorbent was extracted and made up to the mark in a 100 mL volumetric flask. Samples of 25 mL were extracted from this solution and their evaluation was carried out. A 0.01M EDTA solution was used as titrating agent and EBT indicator. Both reagents were supplied by PANREAC SA.

In the first place, Mg(II) + Ca(II) was jointly titrated, at pH 10. Then, a second titration was carried out, after precipitation with oxalic acid of the Ca(II) displaced by Mg(II) in the exchange process carried out by the bioadsorbent. Precipitation took place in the form of CaC_2O_4.

The values in table 7 confirmed the greater effectiveness of the simplest treatment since the values of Mg(II) retained and those of Ca(II) displaced from inside the bioadsorbent were higher. These results confirmed that the oxidizing effect of hydrogen peroxide is counterproductive in the exchange process.

TABLE 7
Ca (II) / Mg (II) CATION EXCHANGE CAPACITY FROM 80 mL OF A 982 ppm Mg (II) SOLUTION.

Acid treatment + alkaline treatment	BN (g)	ppm Mg (II) residual	ppm Mg (II) retained (*)	% Mg (II) detained (*)	mg Mg (II) retained (*)	mg Mg (II) / g BN (*)	ppm Ca (II) displaced (*)
5 mL HCl + 5 g $Ca(OH)_2$	0.5	729.6± 10.0	252.4	25.70	20.18	40.35	420.7
	1.0	707.2± 5.0	274.8	27.98	21.97	21.97	458.0
	4.0	524.8± 7.0	457.2	46.56	36.55	9.14	762.0
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	0.5	870.4± 5.0	111.6	11.36	8.92	17.84	186.0
	1.0	857.6± 8.0	124.4	12.67	9.94	9.94	207.3
	4.0	624± 10.0	358	36.46	28.62	7.15	596.7

Initial concentration Mg (II) = 982 ppm ⇔ 78.56mg of Mg (II) BN: bioadsorbent obtained from orange peel (*) mean value

On the other hand, the values of the percentage retained, compared with those obtained in the solution of 250 ppm of Cu(II) show that the effectiveness of bioadsorption decreases when the concentration of the analyte is significantly above 250 ppm, According to (Sthiannopkao 2009).

To corroborate this trend, a solution of 11,000 ppm of NaCl was prepared. The determination of the displaced Ca(II) was carried out volumetrically by means of EDTA. On this occasion, the samples did not contain Mg(II), so the indirect determination of displaced Ca(II) was not necessary. The results obtained are collected in table 8 and show the same behavior as that observed in previous experiences: the introduction of hydrogen peroxide in the treatment of the orange peel does not contribute to obtaining a higher retention percentage of the analyzed analyte. In addition, the performance was much lower compared to the tests with Cu(II) and Mg(II).

TABLE 8
Ca(II) / Na(I) CATION EXCHANGE CAPACITY FROM 80 mL OF A 4329 ppm SOLUTION OF Na(I).

Acid treatment + alkaline treatment	BN (g)	mg Ca (II) displaced	ppm Na (I) retained (*)	ppm Na (I) residual (*)	% Na (I) Detained (*)	mg Na (I) retained (*)	mg Na (I) / g BN (*)
5 mL HCl + 5 g $Ca(OH)_2$	0.5	82.67 ± 2.0	95.07	4233.93	2.16%	7.50	14.99
	1.0	131.73 ± 7.0	151.49	4177.51	3.45%	11.95	11.95
	4.0	270.93 ± 12.0	311.57	4,017.43	7.09%	24.57	6.14
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	0.5	31.47 ± 4.0	36.19	4292.81	0.82%	2.85	5.71
	1.0	44.27 ± 6.0	50.91	4278.09	1.16%	4.01	4.01
	4.0	230.40 ± 15.0	264.96	4,064.04	6.03%	20.89	5.22

Initial concentration Na (I) = 4329 ppm ⇔ 346.32mg of bioadsorbent Na (I) BN: bioadsorbent obtained from orange peel (*) mean value

Finally, the behavior of the bioadsorbent was verified against a complex sample: seawater from the western Mediterranean basin.

Seawater is a solution made up of water and salts, in a proportion of 96.5% water and 3.5% salts. These salts are formed by a great variety of elements and chemical compounds, such as chlorine, sodium, magnesium, calcium, potassium, bromine, strontium, boron and fluorine mainly. Chlorine and sodium are the fundamental constituents of sea water and are found in the form of sodium chloride which is known as common salt and represents 80 percent of salts in solution. After chlorine and sodium, magnesium is the most abundant element in seawater (Anthoni 2000).

Unlike the trace components, the larger ions dissolved in seawater maintain remarkably constant relative concentrations with each other (Custodian1983). Table 9 shows the composition and typical characteristics of seawater (Grasshoff et al. 1999).

TABLE 9
COMPOSITION AND CHARACTERISTICS OF SEA WATER

Parameters	Reference ranges
Dissolved salts, mg / L	30,000 - 45,000
Sulfates, mg / L	2,425 - 3,000
Chlorides, mg / L	17,500 - 21,000
Sodium, mg / L	9,600 - 11,700
Potassium, mg / L	350 - 500
Calcium, mg / L	375 - 525
Magnesium, mg / L	1,025 - 1,400
Temperature ºC	15-35
pH	7.9-8.1

The procedure was analogous to when the exchange of the Ca(II) contained within the bioadsorbent for the Mg (II) of a solution prepared with $MgCl_2 \cdot 6H_2O$ was determined. In the first place, the joint content of Ca(II) + Mg(II) was determined, after the exchange process between the bioadsorbent and the seawater. Subsequently, the previous experience of precipitation of Ca(II) present in seawater in the form of calcium oxalate was repeated, before proceeding to the exchange between the bioadsorbent and seawater.

The Mg(II) content determined after Ca(II) precipitation was 1126.4 ± 25 ppm, while the Ca(II) content was 555 ± 13 ppm.

The experiences carried out with two types of bioadsorbents were compared, those obtained with the treatments (5 mL HCl + 5 g $Ca(OH)_2$) and (5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$) that, previously, had been hydrated in hermetically closed containers with deionized water for a period of 24 hours.

Table 10 shows the experimental values after different bioadsorbent samples of 0.5; 1 and 4 g were put in contact with 80 mL of seawater without previous precipitation of Ca(II).

TABLE 10
Ca(II) / Mg(II) CATION EXCHANGE CAPACITY FROM 80 mL OF SEAWATER WITHOUT PREVIOUS PRECIPITATION OF Ca(II).

Acid treatment + alkaline treatment	BN (g)	ppm Mg (II) residual	ppm Mg (II) retained (*)	% Mg (II) retained (*)	mg Mg (II) retained (*)	mg Mg (II) / g BN (*)	ppm Ca (II) displaced (*)
5 mL HCl + 5 g $Ca(OH)_2$	0.5	1030.4 ± 17.0	96.0	8.52%	7.68	15.36	160.0
	1.0	1020.8 ± 14.0	105.6	9.38	8.45	8.45	176.0
	4.0	627.2 ± 16.0	499.2	44.32%	39.94	9.98	832.0
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	0.5	1040.0 ± 30.0	86.4	7.67%	6.91	13.82	144.0
	1.0	1014.4 ± 23.0	112.0	9.94%	8.96	8.96	186.7
	4.0	812.8 ± 18.0	313.6	27.84%	25.09	6.27	522.7

Initial concentration Mg (II) 0 = 1126.4 ± 25 ppm; Initial concentration Ca (II) 0 = 555 ± 13 ppm (*) middle value

Table 11 contains the results of analogous experiments in which the bioadsorbent samples were put in contact with seawater that, previously, had been treated with excess oxalic acid to precipitate Ca(II) in the form of CaC_2O_4.

TABLE 11
Ca(II) / Mg(II) CATION EXCHANGE CAPACITY FROM 80 mL OF SEAWATER WITH PREVIOUS PRECIPITATION OF Ca(II).

Acid treatment + alkaline treatment	BN (g)	ppm Mg (II) residual	ppm Mg (II) retained (*)	% Mg (II) retained (*)	mg Mg (II) retained (*)	mg Mg (II) / g BN (*)	ppm Ca (II) displaced (*)
5 mL HCl + 5 g $Ca(OH)_2$	0.5	332.8± 12.0	793.6	70.45%	63.49	126.98	1,322.7
	1.0	291.2 ± 19.0	835.2	74.15%	66.82	66.82	1,392.0
	4.0	300.8 ± 10.0	825.6	73.30%	66.05	16.51	1,376.0
5 mL HCl + 5 mL H_2O_2 + 5 g $Ca(OH)_2$	0.5	323.2 ± 10.0	803.2	71.31%	64.26	128.51	1,338.7
	1.0	272.0 ± 15.0	854.4	75.85%	68.35	34.18	1,424.0
	4.0	265.6± 19.0	860.8	76.42%	68.86	17.22	1434.7

Initial concentration Mg (II) 0 = 1126.4 ± 25 ppm (*) middle value

The results obtained confirmed the displacement of Ca(II) inside the bioadsorbent by Mg(II) due to the interaction of excess oxalic acid introduced to precipitate the Ca(II) contained in seawater. In this way, Mg(II) was responsible for maintaining the three-dimensional structure of the bioadsorbent after microprecipitation of Ca(II) in the form of CaC_2O_4 .

III. DISCUSSION

Adsorption is a mass transfer process in which the substances present in a fluid are accumulated on a solid phase and therefore removed from it. The substance that is concentrated on the surface is called adsorbate and the phase that retains it is called adsorbent (Castellan 2000), differentiating between physical and chemical adsorption.

Physical adsorption (physisorption) occurs by both Van der Waals and electrostatic forces between the adsorbate molecules and the atoms that make up the adsorbent surface. Chemical adsorption (chemisorption) is the result of chemical interaction between the solid and the adsorbed substance. It takes place when chemical bonds are formed between adsorbate molecules and specific locations on the adsorbent's surface, also called active sites (Treybal, 1993).

An important consequence of chemisorption is that after the surface has been coated with a single layer of adsorbed molecules, it becomes saturated. Only additional adsorption can occur on the layer present and, in general, it is of a weak type. Because of this, chemisorption is related to the formation of a unimolecular layer, while physisorption can give rise to additional layers (Langmuir 1916).

On the other hand, adsorption falls squarely within ion exchange and is often called ion exchange adsorption, being grouped together as a single treatment in fixed bed processes (Kammerer et al. 2011).

The adsorption process using natural organic materials has been called biosorption (Volesky1990). Bioadsorption or biosorption is a physical-chemical process that includes the phenomena of adsorption of molecules and ions from different materials of natural origin, such as algae, fungi or fruits, which are found in great abundance in nature and the transformation of which biosorbents are not an expensive process (Romera et al, 2007).

The cell walls of bioadsorbent materials contain polysaccharides, proteins and lipids, and therefore numerous functional groups capable of binding heavy metals on their surface. Among the functional groups present, mention may be made of amino, carboxylic, hydroxyl, phosphate and thiol groups which differ in their affinity and specificity with respect to their susceptibility to bind to different metal ions. (Ghimire et al, 2003).

The complexity of the structures of bioadsorbents implies that there are different ways in which contaminants are captured. The mechanisms of bioadsorption are varied. In general, the bioadsorption process is affected by the concentration of surface functional groups (adsorption sites or anchor points); pH (influences the surface charge of the adsorbent and the way in which the species to be adsorbed are found); the surface and pore structure of the adsorbent; the nature of the adsorbate; equilibrium temperature and time (time to saturation of the bioadsorbent) (Ho et al, 2000).

It has been determined that the retention mechanism occurs initially with the migration of the adsorbate from the solution to the surface of the adsorbent, followed by a diffusion process to end in the fixation in the active site (Sivakumar 2010).

Unlike a much more complex phenomenon of bioaccumulation based on active metabolic transport, biosorption by dead biomass is passive and is based mainly on the affinity between the biosorbent and sorbate (Volesky 2007; Gadd 2008; Tejada et al, 2015).

On the other hand, heavy metals are understood to be those whose density is at least five times greater than that of water (> 5 g/cm^3) and with an atomic number value greater than 20 (excluding alkaline and alkaline earth metals). Toxic metals are those whose concentration in the environment can cause damage to the health of people even at low concentrations (Vardhanet al, 2019).

The terms "heavy metals" and "toxic metals" are often used synonymously. However, only a few specific cases belong to both groups. The USEPA (2007) takes into account five heavy metals as the most relevant in terms of their impact on health: Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb) and Mercury (Hg).

There are a large number of inexpensive biosorbents that are represented by lignocellulosic materials, algae, chitin / chitosan, activated sludge, bacterial biomass, fungal biomass, etc. (Castells 2005). Among these, lignocellulosic residues and chitin / chitosan can be applied on a large scale because they have a high availability (Tran et al, 2015).

Lignocellulosic materials (agave bagasse, coconut shell, rice straw, corn cob, rice husk, orange peel, barley straw, etc.) have been used mainly for the removal of metals and organic compounds (Azku 2005). The main components of lignocellulosic residues are: cellulose, hemicellulose and lignin and they have a reasonable adsorption capacity. The selection of a suitable adsorbent, and sometimes a correct chemical modification, can considerably improve the adsorption properties of the material (Abdolali et al, 2014).

Conventional methods for treating wastewater with low concentrations of heavy metals in the ionic state are extremely expensive. For this reason, bioadsorption techniques have gained acceptance due to their effectiveness in eliminating contaminants that are too stable for conventional methods, resulting in high-quality effluents (Basso et al, 2002).

The physical modifications entail a suitable dimensioning of the shell by cutting or grinding, complemented by heat treatments such as reflux, microwave or ultrasonic irradiation. Chemical modifications include treatment with different types of chemical agents, which are used to increase the binding groups in the final bioadsorbent, remove inhibitory groups, and increase its surface area (Patel, 2012).

IV. CONCLUSIONS

It has been proven that:

a) By modifying the chemical treatment, consisting of carrying out the alkaline attack without eliminating the soluble pectins in an acid medium, an easily filterable solid phase is obtained that allows the use of the orange peel of size <200 µm as a bioadsorbent. This modification represents important economic repercussions in an industrial process.

b) The percentage of cation exchange is higher at a lower analyte concentration, which makes bioadsorption a very suitable treatment for the removal of metals in ionic form in effluents with concentrations of around 150 ppm.

c) The exchange capacity is influenced by the presence of an agent that destabilizes the presence of Ca(II) in the bioadsorbent.

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The role of Mild Thrombocytopenia in mild COVID-19 Infection: A Systematic Review

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Abstract—

Introduction: *The COVID-19 pandemic has caused the deaths of more than 9,122,116 people, mostly elderly and/or comorbid such as diabetes and immunosuppressed state using cortisone. While the quarantine measure is necessary, and pancytopenia happens during the hospitalization, earlier thrombocytopenia is often being implemented to manage DHF patients.*

Problem: *Patients with comorbid have thrombocytopenia and have been managed as DHF patients. Meanwhile, the cost of the pandemic is not limited to medical-aspect, and the virus has led to psychological-aspect of patients not being hospitalized in special COVID-19 barracks.*

Aims: *To reveal that thrombocytopenia could be used for COVID-19 diagnosis and as early as possible being managed on COVID-19 treatment management.*

Method: *Systematic Review with PRISMA Design, using Science Direct search machine and Mendeley My Library, with keywords thrombocytopenia and mild COVID-19. Bayesian network and analysis to support in finding references that support the pathogenesis of thrombocytopenia in COVID-19 in the mild stage. Systematic Review and Meta-analysis design are preferable.*

Result: *Flowchart of 15 references, supported thrombocytopenia in mild and the severe COVID-19, while lower thrombocytopenia in critical stage. At least 22 case reports with this mild thrombocytopenia and thrombosis. With over 28,789,321 people infected globally, mild thrombocytopenia in immunocompromised COVID-19 patients could be helped and monitored not to be severe and critical stage as early as possible.*

Conclusion: *Mild thrombocytopenia is useful in fighting mortality in the COVID-19 pandemic for primary care physicians.*

Keywords— Mild Thrombocytopenia; mild COVID-19; comorbid; diabetes; pandemic; pregnant; cortisone, Peripheral blood inflammatory cells (PBICs).

I. INTRODUCTION

Since December 2019, new COVID-19 outbreaks have occurred and spread around the world. It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).. This viral pandemic of COVID-19 led to the deaths of over 9,122,116 peoples, mostly elderly or comorbid as diabetes, and immunosuppressed state, using cortisone, and chloroquine treatment.[1] Corona virus disease 2019 (COVID-19) is a pandemic which started in China in late 2019 and has now spread across the globe in 2020.[2] The clinical characteristics of patients are still unclear. Most patients had fever as the first symptom for admission. Other symptoms included cough, fatigue, hoarseness and diarrhoea. Certain patients did not have fever at the time of consultation, and most were not accompanied by organ damage.[3,4] Currently there is limited knowledge on medical comorbidities and COVID-19.[5] Clinicians are fighting against this new disease and are focusing on various factors that may lead to better survival outcomes. The common symptoms of COVID-19 infection are fever, cough, myalgia and fatigue.[6]

There was analysis of patients with COVID-19 had described various clinical characteristics and their association with the severity of disease, lymphopenia and eosinopenia may serve as predictors of disease severity and disease progression in COVID-19 patients, and enhancing the cellular immunity may contribute to COVID-19 treatment. [7,8] While the quarantine measure is necessary and pancytopenia happen during the hospitalization, earlier thrombocytopenia is often implemented of being managed as DHF patients.[9, 10, 11]. COVID-19 is more a systemic condition than it is a respiratory disease in severe cases, which evidence has revealed thrombocytopenia complications and associated with disease severity and increased mortality.[12]

The underlying changes of peripheral blood inflammatory cells (PBICs)in COVID-19 patients are little known, the risk factor for the underlying changes of PBICs and their predicting role in severe COVID-19 patients remain uncertain. Lymphopenia and eosinopenia may serve as predictors of disease severity and disease progression in COVID-19 patients, and enhancing the cellular immunity may contribute to COVID-19 treatment.[13] Lessons learn that patient with comorbid have thrombocytopenia and managed as DHF patients. [14] The costs of the pandemic are not limited to medical aspect, as the virus has led to psychological aspect not being hospitalized in special COVID-19 barracks.[15,16] Aims of this study is to reveal that thrombocytopenia could be used for COVID-19 diagnosis and as early as possible being managed on COVID-19 treatment management . We conduct a systematic review to evaluate the impact of various morbidities on serious events in COVID -19.

II. METHOD

Systematic Review design by PRISMA using Science Direct and other search engines. Also Using My Library of Mendeley.

Keywords of COVID-19 and thrombocytopenia were chosen. Mild COVID-19 and mild thrombocytopenia are included, severe and critical stage were excluded. With a Bayesian network and analysis also mild lymphopenia is monitored. Systematic Review and Meta-analysis are preferred due to the PRISMA list.

III. RESULT

Fifth-teen references are included which are 5 case reports, 10 reviews and no one is Systematic Reviews or Meta-analysis. Yang,[3] Mahboob,[17] Hussain,[1] Shi,[18] Mangalmurti,[19] Finelli,[4] Toledo,[7] Rapkiewicz,[20] Renu,[21] Schonrich,[13] Zhang,[22] Gouez,[23] Nauka,[24] Song,[25] Yamanoglu,[26] are recorded supporting mild thrombocytopenia in mild COVID-19.

Research novelty:

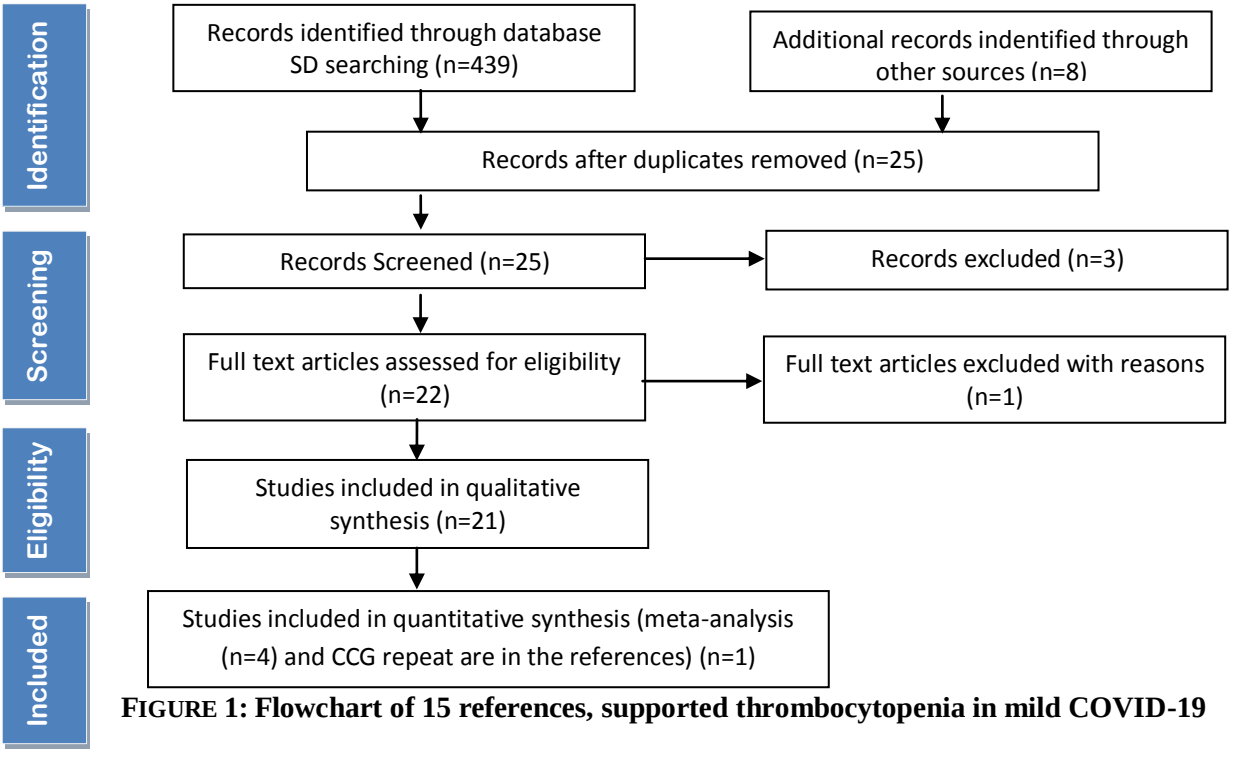

FIGURE 1: Flowchart of 15 references, supported thrombocytopenia in mild COVID-19

TABLE 1
FIFTEEN REFERENCES SUPPORTED THROMBOCYTOPENIA IN MILD COVID-19

Study, year	Design	Population	Thrombocytopenia	Mild/severe COVID-19
Yang, 2020	Review	Wuhan patients	Death	Socio-ecconomic
Mahboob, 2020	Case report	58-year-old woman	Large vessel stroke	Found to be COVID-19 positive
Hussain, 2020	Review	Diabetes, Old, Comorbid	Increased coagulation activity	Range Mild to ARDS
Shi, 2020	Review	COVID-19 patients	Coagulopathy Cytokine storm	Hypercoagulopathy
Mangalmurti, 2020	Basic concept	Cytokine storm	Lead to vascular damage	Worsening clinical outcome
Finelli 2020	Review	Given immunosuppressive with hematologic diseases	Thrombocytopenia, lymphopenia, coagulation abnormalities	Not postpone CPT
Toledo 2020	Review	SArS-Cov-2 infection patients	Hematological test Lymphopenia;	Excluded or confirm the Diagnosis
Rapkiewicz 2020	A case series	7 COVID-19 autopsies	Thrombi in the large pulmonary arteries in MOT	Thrombosis and predominant inflammation
Renu	Review	Comorbid in MOF	Venous thromboembolism	ARDS, ACE2,Cardiovascular, GI dysfunction, CKD, DM, liver, CNS, ocular, conjunctival
Schonrich, 2020	Review	ROS production	Immune system reacts insufficiently	Systemic tissue damage
Zhang 2020	Review	COViD-19 patients	Thrombocytopenia are not rare	Development of T remain to be elucidated
Gouez 2020	Case-report	3 COVID-19 patients on pregnant in China	Mild thrombocytopenia A third of non-pregnant	but to emergency in 1 pregnant case; 2 with PCR-Swab positive
Nauka 2020	Case-report	A 48 y non smoker male, suspect COVID-19	Trombocytopenia	Non critical patients DVP
Song 2020	Review	COVID-19 patients by frontline clinician	Cytokine release syndrome-Rx/ IL-6 inhibitors	Corticosteroid should be limited in comorbid patients
Yamanoglu 2020	Case report	7 series Asymptomatic to severe, result fatal in others	3 thrombocytopenia, and 2 during follow-up	Age and chronic diseases, source of transmission

MOF: Multi-organ function; ROS: Reactive Oxygen Species; NETs: Neutrophil Extracellular Traps; MOT: Multi-organ Tissue; CPT: Convalescence Plasma Therapy

IV. DISCUSSION

4.1 Devilishly radical NETs

Neutrophil extracellular traps (NETs)[13] leads to severe and critical COVID-19. The overwhelming production of reactive oxygen species (ROS) increases the NETs and T cells that are necessary to kill virus-infected cells. Vitamin C or NAC as an antioxidant will cut this vicious cycle.[13]

Coagulopathy on vascular thrombotic events (VTE) contributes to the risk of fatality[28]

Uncontrolled inflammation-mediated endothelial injury and dysregulation of renin-angiotensin system (RAS) are the potential mechanisms in severe and critical stage.[28]

These later fatal thrombotic events covered the thrombocytopenia in mild COVID-19 stage.

4.2 Multi-organ damage and comorbidities

Coronaviruses are associated with comorbidities.[21] Alveolar failure and severe respiratory malfunction are correlated with other damaging organ due to the risk of cardiovascular through ACE2, the dysfunction of gastrointestinal, the dysfunction of liver, the injury of lung, chronic kidney disease, diabetes mellitus, CNS risk, ocular risk, conjunctivitis, and conjunctival hyperemia and venous thromboembolism.[21] At the molecular level, immunopathology and coronaviruses provide information related to comorbidities that explain the coronaviruses replication (Viral Load) with multi-organ damage.[21]

Early diagnosis with thrombocytopenia in mild COVID-19 is useable in fighting multi-organ damage in comorbidities.

4.3 Covid-19 and Diabetes

It is important to understand the common properties of COVID-19 in relation to people with diabetes.[1] The "incubation period" is one of them in the diabetes patient with COVID-19. The general characteristic are from flu-like symptoms to distress syndrome of acute respiratory, failure of multiple organs, and death. Meanwhile, the significant predictor of the morbidity and mortality are comorbidities, diabetes, and elder people. The underlying mechanisms between diabetes and COVID-19 are chronic inflammation, coagulation activity that is increased, impairment of the immune response, potentially damaging pancreas caused by SARS-CoV-2.[1]

The use of chloroquine in diabetes subjects potentially trigger hypoglycemic event. Therefore, research in the future should consider this relationship in combination with its clinical management. [1] Fighting COVID-19 with diabetes at much early stage are the best way in winning the war against high mortality of these cases.

4.4 Cytokine Storm

The increasing of cytokinesis level in the circulation associated with all type of infections and immunity conditions are known as a cytokine storm. The function to protect by cytokines is an 'ideal' responses; a multi factorial which is being able to trigger responses to be pathological that lead to vascular damage, immunopathology, and worsening clinical outcomes.[19]Infection is higher in patients having diabetes mellitus (DM) compared to those who have not. The higher probability of infection is caused by defects in immunity and in humoral innate immunity. Many studies show that in cellular innate immunity the function of chemotaxis, phagocytosis, and killing of diabetic polymorphonuclear cells and diabetic monocytes/macrophages cells decreases than that of control. Control DM improve cellular function. Some bacterial infection becomes more virulent in the high-level glucose. The adherence among microorganisms is higher to diabetic compared to non-diabetic cells.[27]

4.5 Hematologic Setting

The impact COVID-19 pandemic is closely related to clinical practice in the hematologic setting. However, many therapies who administer immunosuppressive effect to patients with hematological diseases might encounter the increased risk of a more severe viral infection. Especially in marginal primary health care, the urgency and priority treatments of individual patient has to be reconsidered. Thrombocytopenia, lymphopenia, and coagulation abnormalities, being useful for the prognostic evaluation of infected patients, have been identified by hematologic laboratories.[4] Proven agents for the treatment of COVID-19 is still promising.[28]

A broad spectrum on the COVID-19 clinical progression have found individuals having asymptomatic after having a severe course while others end up in mortality. While the different clinical course in individuals is influenced by age and clinical diseases, genetic factors and the origin of transmission are considered as an important in the clinical course. The different structure of genetic is reported to be from the same source. Therefore, evaluation are conducted upon the similar but different clinical cases residing in the same nursing home and present to the hospital altogether. [26] As a result, since the significant challenge to be taken care of is performing treatment in intensive care units (ICU), it is essential to recognize early the severe form of triaging of patients supporting by laboratory markers.[29]

Several laboratory parameters severity stage are low lymphocyte count, D-dimers, cardiac troponin, CRP, ferritin, and IL-6. All of which are used in danger delamination to prognosticate hospitalized patients whether or not having severe and fatal COVID-19. Prognosis will be wrong when partly or whole of these parameters are altered.[29] There are also an association of thrombocytopenia and those other laboratory markers, especially thrombocyte and clinical course. Thrombocytopenia in the mild and severe stage of COVID-19 could be a sentinel lab marker especially in the mild diabetic patients (also elder and comorbidities).

On the follow-up experience with the mild Covid-19 diabetic patients, other side effects of steroid therapy, immunosuppressant stuff, evaluated extensively in the COVID-19 pandemic. COVID-19 has also been shown to have mild lower thrombocytopenia in pregnant women.[23]

When patients with critical COVID-19 and corticosteroid replacement are expected to be beneficial in this situation, in mild Covid-19, thrombocytopenia could also use for an indicator between the digging of 'say no to steroid in the population'.

V. LIMITATION

At this study and outcome level, incomplete retrieval of identify research does not identified thrombocyte counting for the early diagnosis in the mild case.[5]

Reporting bias on coagulopathy or thrombocytopenia caused by the coagulopathy such as vascular thrombotic events,[17,18] has covered the potential use of thrombocytopenia for the triage aim and for the patient with diabetes, old age and immunocompromised cases, which is easy got worse in wrong diagnosis and management, especially in the second infection of SAR-Cov-2 which is the immunoreaction is stronger and faster.

Reporting bias of this study is 1) corticosteroid give lymphocytopenia and when it stops, the lymphocyte increase again;[30,31,32] 2) people who are receiving active chemotherapy;[33] 3) the imaging of CT can be sorted into four phases: early phase, progressive phase, severe phase, and dissipative phase is demonstrated,[34] another stage, that is mild, severe and critical,[29,35] then mild, moderate, severe, critical.[36]

Early diagnosis and appropriate medication are able to decrease the mortality rate.[35] One of the risk factors causing severe/critical outcomes is diabetes. [35] At initial diagnosis, the absolute number of lymphocytes could not predict the progression risk from severe to critical condition,[35] but not thrombocytopenia which is not rare in COVID-19 patients.[22]

Zheng finds that in the first time of diagnosis the white blood cell counts is low (30.1 %) and 45.2 % have decreased lymphocytes. Multiple patchy ground-glass shadows outside of the patient lungs were commonly observed, and a single sub-pleural sheet of ground glass shadow with enhanced vascular bundles was also found located under the pleura.[36]

Lymphocyte counts below 0.8×10^9/L cannot be used to predict severe and critical groups from the ordinary group.[35] Family clustering in the later period,[35] should be a screen with thrombocytopenia to triage, much more the incubation period was relatively long, and the incidence was relatively hidden, but the virulence was relatively low.[35]

In COVID-19 patients, the peripheral blood inflammatory cells (PBICs) changes are related to blood routine and lymphocyte subsets. Under clinical classifications, disease-associated phases, and one-month outcomes, the inflammatory cell levels are compared. Based on blood routine and lymphocyte subsets, patients of non-severe type vs. the patients of severe type suffered from significantly decreased counts of lymphocytes, eosinophils, basophils, but increased counts of neutrophils.[37] These PBICs alterations got improved in the recovery phase, but persisted or got worse in the aggravated phase.[37]

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VI. CONCLUSION

In facing the COVID-19 pandemic, most countries feel not early to diagnosis mild COVID-19 on doing fast quarantine, which right possible diagnosis could be done by thrombocytopenia alone or parallel with lymphocytopenia. Thus, Thrombocytopenia might become a sentinel of COVID-19, and it serves attention during mild COVID-19 triage.

CONFLICT OF INTEREST

Nothing

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Intergeneric Grafting of Ornamental Incense Cedar: First Results

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Abstract— *Horticultural grafting is important in propagating conifers, mainly because many species are hard to root, especially when using cuttings from mature trees. Incense cedar (Calocedrus decurrens (Torr.) Florins) was recently introduced in Albania as an ornamental tree in public and private urban green spaces and is now much in demand. Intergeneric grafts are rarely used in conifers, and there is little information regarding incense cedar grafting onto rootstocks from different genera. This work aimed to evaluate the effects of intergeneric grafting on the quality of ornamental incense cedar, which is little known in Albania. Scions were prepared by taking 8-10 cm apical shoot from young C. decurrens 'Aureovariegata' plants. Rootstocks were prepared from one-year-old Mediterranean cypress (Cupressus sempervirens L.) seedlings. First results show that the height of grafted plants tended to increase slowly from 30 to 90 days after grafting (DAG), with a minimum increase of 4,9 %; this is because the plant grafted takes several days to join scion and rootstock. The Relative Growth Rate (RGR) of the scion was generally higher than that of the rootstock, which even showed negative values 60 DAG; this trend grew after 90 DAG. At the end of our experimental work, we obtained 410 plants, representing an 82% graft success rate available for planting in different Albanian soils*.

Keywords— Calocedrus decurrens (Torr.) Florins; conifer; RGR; vegetative propagation.

I. INTRODUCTION

Vegetative propagation is an important tool in both ornamental and forest tree improvement [1]. Grafting, an ancient agricultural and vegetative propagation technique, generally involves connecting two plant segments, a portion of shoot ('scion') and a root piece ('rootstock') [2]. It is most commonly employed in woody perennial crops to indirectly manipulate scion phenotype. A wide range of classical grafting techniques can be found in Garner [3]. Among these, bark grafting is relatively easy and very successful; it can be performed in the spring, only when the bark slips or separates easily from the wood. Taxonomic proximity is a general requirement for successful graft-take and long-term survival of the composite plant [4]. Rootstocks are usually from the same species as the scion, although some interspecific graft combinations have been successful. Grafting is widely used to propagate conifers [5] because many species are hard to root, especially when using cuttings from mature trees [6]. In general, only few species can be grafted: i.e. dwarf conifers, which cannot be successfully cloned using cuttings [7]. For conifers, grafters have historically used the following combinations: *Picea abies* for all spruces, and *Pinus sylvestris* for all two-needled and some three-needled pines [8]. Vuksani et al. [9] showed that the grafting compatibility of Arizona cypress plants and Mediterranean cypress rootstock was 87% in Category A (perfect union).

The *Cupressaceae* family includes 21 genera and around 130 species of anemophilous trees and shrubs from the northern and southern hemispheres [10 and 11]. In the Mediterranean region, this family is widely represented both by native species, typically in woodlands, and by non-native species used for ornamental purposes and in reforestation programs. Albania is very rich in terms of flora: it is estimated that there are around 3,200 species of vascular plants, and the *Cupressaceae* family is quite widespread.

Incense cedar *(Calocedrus decurrens* (Torr.) Florins) is the only example of the small genus *Calocedrus* [12], (*syn. Libocedrus decurrens* Torr.). It belongs to the *Cupressaceae* family native to western North America, with the bulk of its range in the United States, from central western Oregon through most of California and the extreme west of Nevada. It grows at altitudes of 50-2,900 m.

Incense cedar was recently introduced in Albania as an ornamental tree in public and private urban green spaces and is now much in demand [13].

Its foliage is produced in flattened sprays with scale-like leaves 2–15 mm long; they are arranged in opposing decussate pairs, the successive pairs spaced at increasing distances; the facial pairs are flat, while the lateral pairs fold over their bases [14]. As an ornamental plant, '*Aureovariegata*' is a broadly columnar, evergreen, coniferous tree with exfoliating, red-brown to grey-green bark, slightly fragrant, flat sprays of linear, glossy, dark green and golden-yellow variegated leaves and, occasionally, erect, oval, red-brown female cones. Common propagation methods are seed and semi-hardwood cuttings.

Geographic variability in cone and seed production is great. There is considerable information available on incense cedar seedling production. Although a prolific seed bearer, it does not produce seeds every year; germination under controlled conditions may reach 98%, but usually averages from 20 to 40% [15].

Although incense cedar does not reproduce vegetatively in nature, it can be stimulated to do so in a nursery greenhouse: rooting, assessed in spring, is best (92% rooting) with 2500 p.p.m. NAA [16]. *Chamaecyparis lawsoniana* scion graft success is moderate with *C. decurrens*; there is little information regarding incense cedar grafting on rootstocks of other genera.

Research was, therefore, carried out to evaluate the effects of intergeneric grafting on the quality of ornamental incense cedar, which is little known in Albania.

II. MATERIALS AND METHODS

The experiment was carried out in an ornamental nursery, in a plastic greenhouse in the Laknas area (41° 22' 36" N, 19° 44' 14" E, Tirana, Albania).

Scions were prepared by taking 8-10 cm apical shoot from young *C. decurrens* '*Aureovariegata*' plants. They were individually transplanted in 30 cm diameter containers filled with a mix of Thumanes torfe (Albania) base and perlite (v:v=3:1)

Rootstocks were prepared from one-year-old Mediterranean cypress (*Cupressus sempervirens* L.) seedlings produced by seeds harvested from natural plants of the Kruja region (AL). Seedlings were grown in pots filled with a mixture of peat and perlite (v:v=3:1). The thickness of the rootstock was slightly greater than 1 cm; the length of the bark cut was 1.5 - 2.5 cm.

Grafting was carried out on 3 February 2015; in order to better connect rootstock and scion, the grafted point was tied down with raffia of the *Sagustaedinera* tropical palm bark. Five hundred grafted plants were grown under controlled conditions (T=22°C and UR 90%).

To assess grafting progress, outgrowth measurements were carried on grafted plants in the laboratory of the Horticulture and Landscape Architecture Department of the University of Tirana in three different periods: March 3 (30 days after grafting, DAG), April 3 (60 DAG) and May 3 (90 DAG) 2015.

The following bio-morphological parameters of the scion were measured: plant height (cm), leaf surface (cm^2), aboveground fresh and dry weight (g) (shoots were air-dried and then placed in an oven at T=70 °C). Ten plants were sampled. The aboveground fresh and dry weights (g) of the rootstock were measured.

The Growth index Relative Growth Rate (RGR) was calculated using dry weight data, according to Hunt (2002), as follows: $RGR = d_W/W * 1/dt$; in mg g^{-1} day^{-1}, where W = the sample's dry weight and dt= d2 - d1 is the interval of time. RGR was calculated for scion (RGR Scion), root system (RGR rootstock) and the whole plant (RGR-Plant).

Scion and rootstock RGR values were assessed throug ANOVA (Shapiro-Wilk Test). At the end of the experiment (90 DAG), graft compatibility was calculated as a percentage.

III. RESULTS

Figure 1 shows how the leaf surface area of grafted plant tends to increase slowly from 5.4 cm^2 (30 DAG) to 7.2 cm^2 (90 DAG).

Figure 2 shows that the scion height tends to increase slowly by 4.9 %, from 22.2 cm (30 DAG) to 23.3 (90 DAG); this slow growth may be ascribed to the time it takes (several days) for the grafted plant to connect scion and rootstock.

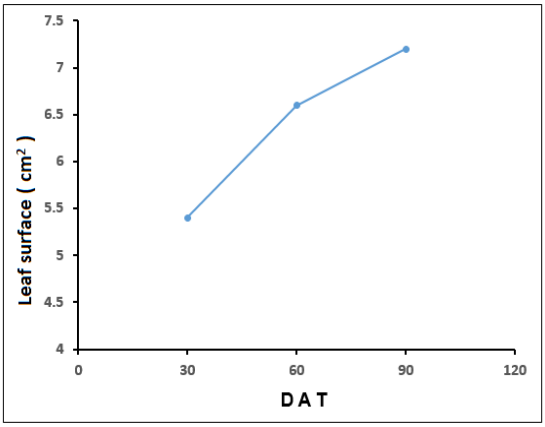

FIGURE 1: Leaf surface area of grafted plant at 30, 60 and 90 DAG.

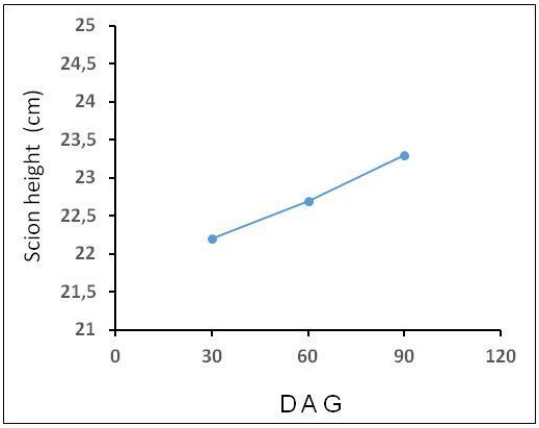

FIGURE 2. Scion height at 30, 60 and 90 DAG.

As for the Relative Growth Rate, the RGR of scion (RGR-sc) is higher than that of rootstock (RGR-rst), which even yield negative values at 60 DAG (Figure 3).

This trend grows after 90 DAG (Figure 4).

The difference is thought to be due to genetic variation in components deriving from different species.

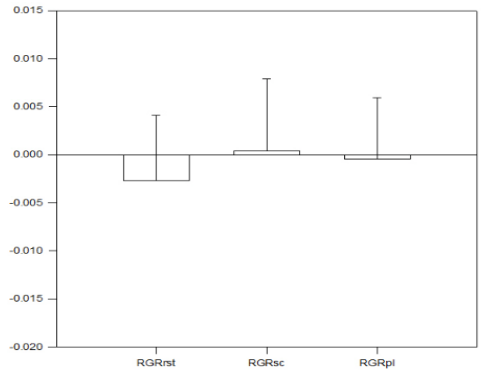

FIGURE 3. Height Relative growth rates of plant (RGR-Pl), relative growth rates of scion (RGRsc) and relative growth rates of rootstock (RGRrst) at 90 DAG.

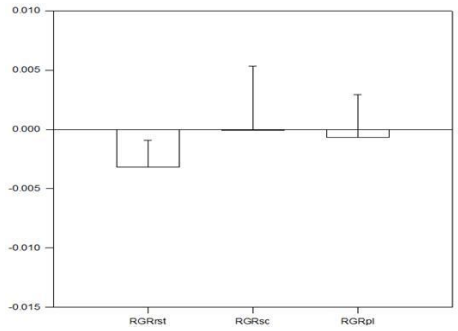

FIGURE 4. Relative growth rates of plant (RGR-Plant), relative growth rates of scion (RGRst) and relative growth rates of rootstock (RGRrst) 90 DAG.

This trend was statistically estimated by means of RGR analysis of variance (Shapiro-Wilk Test) $P < 0.050$.

TABLE 1
THIS RELATIVE GROWTH RATE OF ROOTSTOCKS (RGRRST), RELATIVE GROWTH RATE OF PLANTS (RGRPLANT) AND RELATIVE GROWTH RATE OF SCIONS (RGRSC) 60 AND 90 DAG (MEAN VALUES ± STD.DEV)

	Measurement 60 DAG	Measurement 90 DAG
RGR rst	-0.00267±0.00681	-0.00319±0.00226
RGR sc	0.000421±0.00746	-0.0000473±0.00539
RGR plant	-0.000410±0.00637	-0.000689±0.00360
Probability (P)	0.589	0.192

The differences in the mean RGRrst, RGRsc and RGRplant values among the processed groups are not great enough to exclude random sampling variability, i.e. differences (P1 = 0.589, P2 = 0.589) are not statistically significant.

Another interesting statistical finding is that the Standard Deviation (StdDev) value is large, indicating that growth rates vary widely among plants: when all growing conditions are the same, this may be ascribed to variations in the quality of manual grafting. At the end of our experimental work, we obtained 410 plants (representing an 82% grafting success rate) ready for planting in Albanian different soils. Estimated RGR rootstock values tend to decrease over time, even reaching negative values, a trend that grows stronger more than 90 DAG. It's thought that changes in RGRrst are influenced by changes in the physiological grafted component; in our case, this is even more pronounced because we joined two different genetic species. This is of particular concern for plant viability in the future, although the grafting success rate seems satisfactory.

IV. DISCUSSION

Some studies have reported similar or better success rates for scions grafted onto different rootstocks [17 and 18]. In some cases also taxonomically distant species have been grafted successfully [19].

Intergeneric grafts are rarely used in conifers. Great variations in anatomy, physiology and morphology among some genera often prevent successful grafting [19].

Nevertheless grafting of Nootka cypress [*Chamaecyparis nootkatensis* (D. Don) Spach] cultivars onto Chinese arbor-vitae (*Thuja orientalis* L.) stocks has yielded up to 94% success rates [20].

White cedar (*Thuja occidentalis* L.) rootstocks are used commercially for grafting Lawson cypress scions [21].

As for intergeneric grafting, two *Cedrus* species on *P. pinea* L. (Stone pine) rootstocks were incompatible, although several grafts survived for two years [22].

There is evidence that rootstocks can alter the scion growth rate in conifers: several studies [23-25] report slower scion growth when scions are grafted onto other species.

V. CONCLUSIONS

Grafting success requires specialists with long experience in the field. For best results, rootstock and scion should have a similar consistency to not compromise the quality of the plants produced.

In order to produce quality seedlings suitable for growth in Albania's soil with abundant skeleton and to meet growing market demands, it is important to develop suitable methods, such as grafting, for producing plants quickly.

Estimated RGR-rootstock values tend to decrease over time, even reaching negative values, a trend that grows stronger more than 90 DAG.

This is of particular concern for plant viability in the future, although grafting success seems satisfactory.

We will therefore continue our experimental work by monitoring the intergenerically grafted plants to ensure their success.

AUTHORS CONTRIBUTION

Conceptualization, G.V.; methodology, B.D. and G.C.; software, H.K.; validation, G.V., G.C. and B.D.; data curation, GC.; writing—original draft preparation, G.V.; writing—review and editing, BD and GC; funding acquisition, B.D. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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First report of *Meloidogyne* species infecting *Turnera subulata* J. E. Smith from Chhattisgarh, India

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Abstract— *Gall symptoms were observed on the roots of Turnera subulata a flowering shrub of medicinal importance. Rhizosphere soil and infected root samples of T. subulata were collected from Jora, Krishaknagar, IGKV campus Raipur, Chhattisgarh. Second-stage juveniles (J2) of root knot nematode were isolated from diseased roots showing excessive gall formations on primary as well as secondary roots. Morphological and morphometrical findings after examination of the species conformed with descriptions of Genus Meloidogyne. This is the first report of Meloidogyne species infecting T. subulata in India as per the available literature scan.*

Keywords— Turnera subulata, Gall symptoms, Root Knot nematode.

I. INTRODUCTION

Turnera subulata belonging to the Turneraceae family is one of the most extensively distributed perennial flowering weed in the tropical and subtropical regions of Asia and Africa. *T. subulata* is infact a polymorphic polyploid complex of perennial weeds commonly called 'Butter cup, sulphur alder and white alder'. Plants are compact, thick herb with dark green foliage and light yellow or white flower having a dark base (Saravanan *et al*., 5). It is most widely adopted as an ornamental garden plant and important medicinal herb used in alternative traditional folk medicine for treatment of several types of chronic diseases. It has been reported as a fast naturalizing weed in Indian subcontinent (Kumar *et al*.,3)

Nematodes account for an estimated 14% of all worldwide plant losses and among them root-knot nematodes are the most common and destructive nematode pathogens. Most root-knot nematodes have a very wide host range (Mitkowski & Abawi,4). In India, the distribution of nematodes in different states has been documented in nematode distribution atlas by All India Coordinated Research Project (Nematodes) and published by Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, New Delhi, India during 2010.

Stunting and leaf wilting symptoms were observed in few plants of *Turnera subulata*, (Figure 1a & b) grown as hedges in the Indira Gandhi Krishi Vishwavidyalaya Campus, Raipur. The plants when uprooted revealed the presence of galls on primary as well as on secondary roots (Figure 1c & d). Rhizospheric soil and infected root samples of *T. subulata* were collected from University campus. Around 20 percent of total plants were found to be associated with multiple galling on roots

1 (a)

1 (b)

1 (c)

1 (d)

FIGURE 1: Excessive galling/Multiple gall formation on *Tunera subulata*

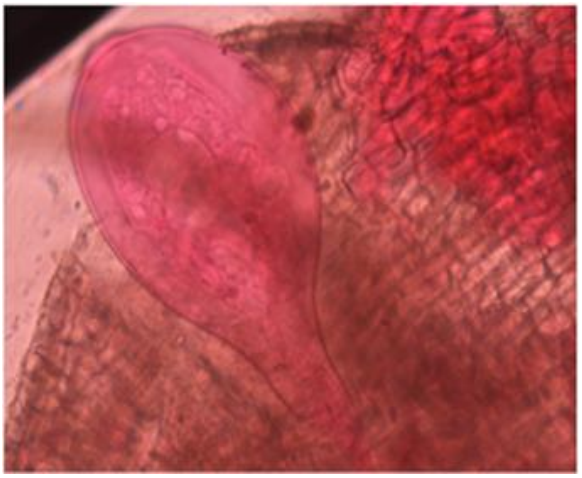

FIGURE 2: Mature root-knot females *Meloidogyne* species endoparasitically infecting the root tissue was observed.

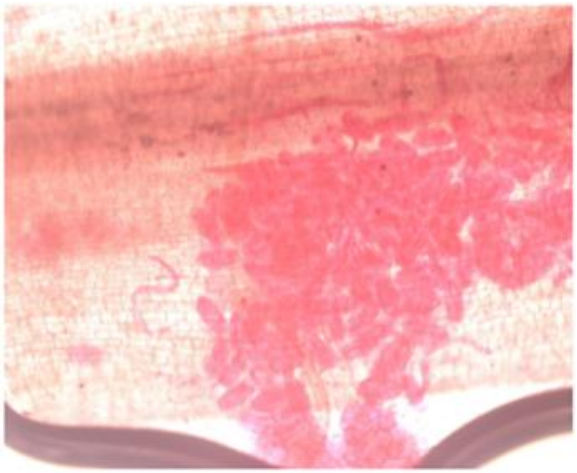

FIGURE 3: Second-stage juveniles (j2) of *Meloidogyne* species, the infective stage and vermiform in shape.

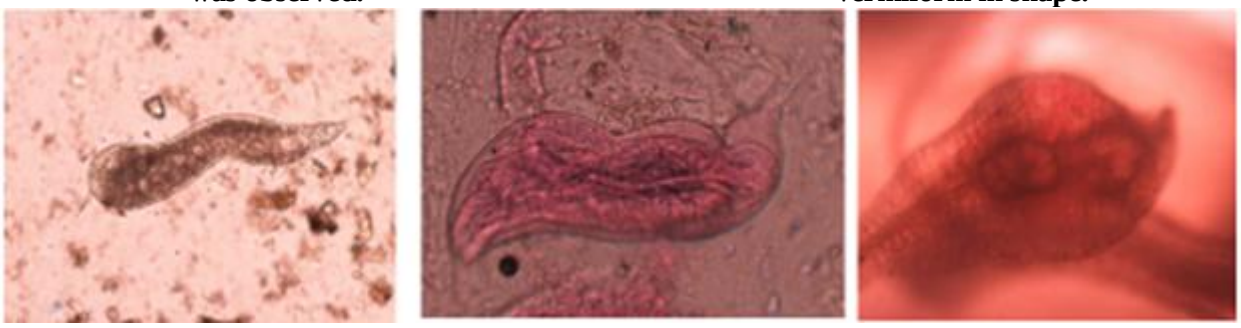

FIGURE 4: J4 stage of *Meloidogyne* species depicting the progression from juvenile to globose adult females

II. RESULTS

The infected roots (vegetative propagules) was boiled in acid fuschin lactophenol solution for few minutes and destained in clear lactophenol. Different stages of Meloidogyne retained the red stain deeper than the plant tissue (Coyne *et al.*,1). Mature root-knot females endoparasitically infecting the root tissue was observed (Fig 2). The females of root-knot nematode had a globose body, with a short "neck," containing the stylet. Second-stage juveniles (J2), the infective stage and vermiform in shapes along with males were also observed (Figure 3).

The J4 stage, depicting the progression from juvenile to globose adult females or to vermiform adult males was also clearly visible (Figure 4).

III. CONCLUSION

The morphological and morphometrical findings after examination of different stages of *Meloidogyne* species in *Turnera subulata* conformed with descriptions and measurements provided by Whitehead (6) and Hunt & Handoo (2).

To our knowledge and literature scan, this is the first report of *Meloidogyne* species infecting *Turnera subulata* in India.

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