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REVIEW

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ARTICLE

Primulina titan sp. nov. (Gesneriaceae) from a Limestone Area in Northern Guangxi, China

Zibing Xin[1,2] Longfei Fu[1,2] Zhangjie Huang[1,2] Wei-Chuen Chou[2] Yi Huang[2] Fang Wen[1,2*]

1. Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuangzu Autonomous Region and Chinese Academy of Sciences, CN-541006 Guilin, China
2. Gesneriad Conservation Center of China (GCCC), Guilin Botanical Garden, Chinese Academy of Sciences, CN-541006 Guilin, China

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ABSTRACT

A new species of Gesneriaceae, *Primulina titan,* is described and photographed from northern Guangxi, China. It resembles *P. hunanensis*, but can be distinguished by combined morphological characters of leaf, bract, corolla, stamen and pistil. We found only one population with approx. 800 mature individuals at the type locality. This species is provisionally assessed as vulnerable [VU D1] using IUCN criteria.

1. Introduction

The circumscription of *Primulina* has been revised and expanded based on molecular evidence [1-2]. Currently the genus *Primulina s.l.* represents the species richest genus in Chinese Gesneriaceae. By the end of May 2020, the genus *Primulina s.l.*, comprises more than 220 species (infraspecific taxa included) [3-5]. More than 50 new species of *Primulina* have been reported since last 5 years[6-10]. As this trend seemed to persist to date, it might suggest that there would be many new species to be discovered in the near future, as it is unlikely that the entirety of China can be surveyed any time soon [4].

During a botanical survey on limestone karst areas in Guangxi in early April 2018, an unknown plant of *Primulina* was discovered by the members of GCCC. The population was not in flowering at that time, only the white buds that about to bloom. Thus we visited the original locality again in early May 2020, when this unknown taxon is in flowering. After detailed comparison of this unknown plant with all reported *Primulina* species from Guangxi and adjacent areas [11-14], it neither fits

**Corresponding Author:*
Fang Wen,
Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuangzu Autonomous Region and Chinese Academy of Sciences, CN-541006 Guilin, China; Gesneriad Conservation Center of China (GCCC), Guilin Botanical Garden, Chinese Academy of Sciences, CN-541006 Guilin, China;
Email: wenfang760608@139.com

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the existing protologues nor conforms with the type specimens of these species. Nevertheless its inflorescence are most similar to those of *P. hunanensis* K.M. Liu & X.Z. Cai [15], it can be distinguished from the latter by combined of several morphological characters of leaf, bract, corolla, stamen and pistil. We confirmed that it represents a new *Primulina* species, which is described and photographed here.

2. Taxonomic Treatment

***Primulina titan* Z.B. Xin, W.C. Chou & F. Wen,** *sp. nov.* (Figure 1)

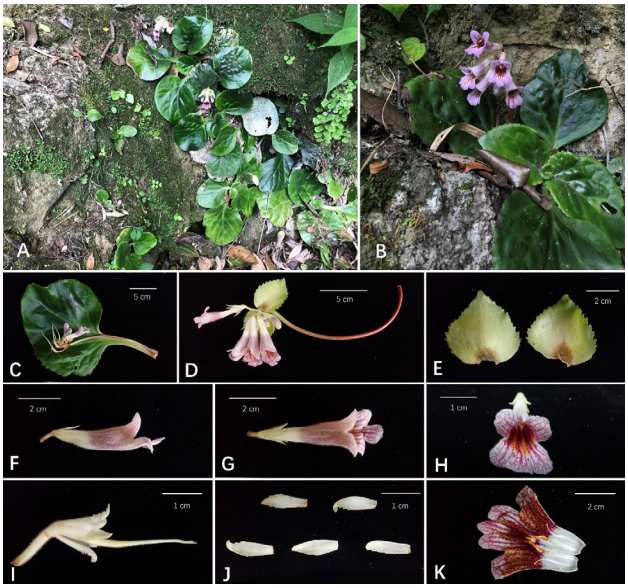

Figure 1. *Primulina titan.* A: Habitat. B: Habit with flowers. C: Leaf and the axillary cyme. D: Cyme. E: Bracts. F: Lateral view of the corolla. G: Top view of the corolla. H: Front view of the corolla. I: Pistil and Calyx. J: Calyx lobes. K: Opened corolla (Photographed by Z.B. Xin)

Diagnosis. *Primulina titan* differs from *P. hunanensis* by its leaf blade glabrous (*vs.* covered with straight, pointed, white trichomes), petiole 1.2-2.5 cm in diam. (*vs.* 0.4-1.0 cm); bract pale green inside, margin obviously numerous serrate, sparsely pubescent outside, glabrous inside (*vs.* pink or purple-red inside, margin entire or repand, densely trichomes and pubescent outside, sparsely trichomes and pubescent inside); corolla white outside, with red-purple and brown stripes and spots inside (*vs.* purple, with white stripes at corolla mouth); filament yellow, 1.5-1.7 cm long (*vs.* white, 0.8-1.2 cm); anther 7-8 mm long (*vs.* 3.5-4.5 mm); pistil pale green, 4.2-5.0 cm long (*vs.* pale pink, 2.6-3.8 cm).

Type. China. Guangxi Zhuangzu Autonomous Region: Guilin City, Yongfu County, Sanhuang, growing on moist and shaded rock surface on the limestone cliff, 25°3′52″ N, 109°41′20″E, alt. 345m, 09 May 2020, flowering, *Z.B. Xin et al., XZB20200509-01* (Holotype: IBK; Isotype: IBK).

Description. Herbs perennial, acaulescent, lithophytic. Leaves 4-10, basal, opposite; petiole 5-15 × 1.2-2.5 cm, glabrous; leaf blades broadly ovate, 15-35 × 12-30 cm, apex obtuse, base cuneate or cordate, margin repand, adaxial and abaxial surfaces glabrous; lateral veins 3-4, inconspicuous adaxially; apparently raised abaxially. Cymes 2-4, axillary, 5-10-flowered; peduncles stout, brown, 20-25 cm long, 5-8 mm wide, densely whitish pubescent; bracts 2, opposite, pale green, ovate or broadly ovate, 4.0-5.0 × 3.5-4.5 cm, obtuse at apex, margin obviously numerous serrate, sparsely pubescent outside, glabrous inside; pedicels pale green, 1.1-1.3 cm long, whitish pubescent. Calyx 5-parted, near to the base; lobes pale green, narrowly lanceolate, 9-13 × 3.0-4.0 mm, densely pubescent abaxially, nearly glabrous adaxially, apex acute, margin obviously serrate from middle to apex. Corolla white outside, with red-purple and brown stripes and spots inside, 5-6 cm long, densely pubescent outside, sparsely pubescent or glandular-pubescent inside; corolla tube narrowly funnel-shaped, almost straight, 3.6-4.0 × 1.0-1.4 cm; limb distinctly 2-lipped, adaxial lips short, bilobed, lobes ovate or broadly ovate, ca. 7 mm long, 8 mm wide; abaxial lips trilobed, lobes near equal, ovate, ca. 1 cm long, 8 mm wide. Stamens 2, adnate to 1.5-1.7 cm from corolla tube base, filaments 1.5-1.7 cm long, yellow, linear, geniculate strongly near middle, glandular-pubescent or trichomes; anthers glabrous, dorsi-fixed, reniform to elliptic, 7-8 mm long, 2 mm wide, coherent in pairs, thecae confluent at middle; staminodes 3, the middle one adnate to 8-10 mm from corolla tube base, 2-3 mm long, the lateral two adnate to 1.2-1.4 cm from corolla tube base, 1.0-1.1 cm long. Disc ca. 2 mm high, annular, margin repand. Pistil pale green, 4.2-5.0 cm long, densely glandular-pubescent; ovary linear, 2.0-2.5 × ca. 3 mm, densely glandular-pubescent; style ca. 1.2 × 1.5 mm, densely glandular-pubescent; stigma bilobed, lobe ca. 5.0 × 1.2 mm wide, puberulent. Fruit 5.5-6.5 cm long, apex obtuse, capsule cylindrical, densely glandular-pubescent.

Phenology. Flowers from the middle of April to June, fruits from the middle of June to August.

Distribution and habitat. The plant is known only from its type locality, near Sanhuang, Yongfu County, Guilin City, Guangxi Zhuangzu Autonomous Region, China, growing on moist and shaded rock surface on the limestone cliff, which is located in subtropical broad-leaved evergreen forest.

Etymology. Its specific epithet, "titan" comes from its quite big leaves.

Vernacular name. Tài Tǎn Bào Chūn Jù Tái (Chinese

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pronunciation).

Conservation status. The type population consists of approx. 800 mature individuals, growing on shaded and moist rock surface on the cliff. This species is provisionally assessed as vulnerable [VU D1] using IUCN criteria[16].

Notes. *Primulina titan* is morphologically close to *P. hunanensis,* but can be identified by combined characters (Table 1; Figure 2).

Table 1. Comparison between *Primulina titan* and *P. hunanensis*

Characters	*P. titan*	*P. hunanensis*
Leaf	glabrous	straight, pointed, white trichomes
Petiole	1.2-2.5 cm in diam.	0.4-1.0 cm in diam.
Bract	pale green inside, margin obviously numerous serrate, sparsely pubescent outside, glabrous inside	pink or purple-red inside, margin entire or repand, densely trichomes and pubescent outside, sparsely trichomes and pubescent inside
Corolla	white outside, with red-purple and brown stripes and spots inside	purple, with conspicuous white stripes at the corolla mouth
Corolla tube	2.4-2.9 cm long,	2.8-3.5 cm long
Filament	yellow, 1.5-1.7 cm long	white, 0.8-1.2 cm long
Anther	7-8 mm long	3.5-4.5 mm long
Staminode	3	2, rarely 3
Pistil	pale green, 4.2-4.8 cm long	pale pink, 2.6-3.8 cm long
Disc	ca. 2 mm high	1.2-1.8 mm

Figure 2. Comparison between *Primulina titan* (1) and *P. hunanensis* (2). A: Adaxial surfaces of the leaf blade. B: Cymes. C: Lateral view of the corolla. D: Front view of the corolla. E: Pistil. F: Stamens. (Photographed by Z.B. Xin)

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References

[1] Wang Y.Z., Mao R.B., Liu Y., Li J.M., Dong Y., Li Z.Y. & Smith J.F. Phylogenetic reconstruction of *Chirita* and allies (Gesneriaceae) with taxonomic treatments[J]. Journal of Systematics and Evolution, 2011, 49: 50-64. https://doi.org/10.1111/j.1759-6831.2010.00113.x

[2] Weber A., Middleton D.J., Forrest A., Kiew R., Lim C.L., Rafidah A.R., Sontag S., Triboun P., Wei Y.G., Yao T.L. & Möller M. Molecular systematics and remodeling of *Chirita* and associated genera (Gesneriaceae)[J]. Taxon, 2011, 60: 767-790. https://doi.org/10.1002/tax.603012

[3] IPNI. The International Plant Names Index. 2020. Available from: http://www.ipni.org

[4] Möller M. 2019: Species discovery in time: An example from Gesneriaceae in China[J]. Guangxi Sciences, 2019, 26(1): 1-16. https://doi.org/10.13656/j.cnki.gxkx.20190307.002

[5] Wen F., Wei Y.G., Fu L.F., Xin Z.B., Li S., Huang Z.J. & Meng D.C. The Checklist of Gesneriaceae in China. 2020. Available from: http://gccc.gxib.cn/about-46.aspx.

[6] Ning Z.L., Pan B., Wen F., Kang M. & Zhuang X.Y. *Primulina yingdeensis,* a new species from Guangdong, China, and P. rosulata, a new combination (Gesneriaceae), based on morphological and molecular evidence[J]. Willdenowia, 2016, 46: 399-409. http://dx.doi.org/10.3372/wi.46.46308

[7] Wu H.T., Zhou S.B., Ma W., Wang O.W. & Wen F. *Primulina dichroantha* (Gesneriaceae), a New Species from a Karst Cave from Guangxi, China[J]. Annales Botanici Fennici, 2017, 54(1-3): 95-98. https://doi.org/10.5735/085.054.0314

[8] Hong, X., Li, Z., Liu, J., Zhou, S., Qin, W. & Wen, F. Two new species of *Primulina* (Gesneriaceae) from limestone karsts of China[J]. PeerJ, 2018, 6: e4946.

 DOI: https://doi.org/10.30564/jrb.v2i3.1994

https://doi.org/10.7717/peerj.4946

[9] Li, S., Xin, Z.B., Chou, W.C., Huang, Y., Pan, B., Maciejewski, S. & Wen, F. Five New Species of the genus *Primulina* (Gesneriaceae) from Limestone Areas of Guangxi Zhuangzu Autonomous Region, China[J]. PhytoKeys, 2019, 127: 77-91. https://doi.org/10.3897/phytokeys.127.35445

[10] Xin Z.B., Huang Z.J., Fu L.F., Li S., Wang B.M. & Wen F. *Primulina spiradiclioides* (Gesneriaceae), a new species from limestone areas in Guangxi, China[J]. Annales Botanici Fennici, 2020, 57: 245-248.

[11] Wang W.T., Pan K.Y., Li Z.Y., Weitzman A.L. & Skog L.E. Gesneriaceae. In: Wu, Z.Y. & Raven, P.H. (Eds.) Flora of China[M]. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 1998: 244-401.

[12] Li Z.Y., Wang Y.Z. Plants of Gesneriaceae in China[M]. Henan Sci. Technol. Publ. House, Zhengzhou, 2005: 171-261.

[13] Wei Y.G., Wen F., Möller M., Monro A., Zhang Q., Gao Q., Mou H.F., Zhong S.H., Cui C. Gesneriaceae of South China[M]. Guangxi Science and Technology Publishing House, Nanning, 2010: 274-526.

[14] Vu P.X. Gesneriaceae. In: Tran, T.H. (Ed.) Flora of Vietnam[M]. Technology & Science Publishing House, Hanoi, 2018: 77-125.

[15] Cai, X.Z., Tian, J., Xiao S.Y., Peng L., Liu K.M. *Primulina hunanensis* sp. nov. (Gesneriaceae) from a limestone area in southern Hunan, China[J]. Nordic Journal of Botany, 2015, 33: 576-581. https://doi.org/10.1111/njb.00757

[16] Guidelines for Using the IUCN Red List Categories and Criteria[S]. version 14. Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission, 2019. http://cmsdocs.s3.amazonaws.com/RedListGuidelines.pdf

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REVIEW

Toxic Effect of Different Neem Formulations against Pests and Mammals

Nadia Zikry Dimetry*

Department of Pests and Plant Protection, National Research Centre, El-Bohouth St., Dokki, Cairo, 12622, Egypt

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ABSTRACT

Neem (*Azadirachta indica* A. Juss), prominently known as conventional medication is a local plant in India. Neem is regarded as a promising tree species which can be utilized in variety ways to benefit agricultural communities throughout the world. Neem based insect sprays were productive for the control of different insect species, yet their low lingering impact and absence of normalized definitions are issues for field application. Additionally, neem is fairly have harmful impact in nature. The use of nanotechnology as a mean for nanopesticides is in the beginning time of improvement. All things considered, the nanosphere definition demonstrated upgraded systemicity of the dynamic fixings and made its infiltration better in the plant, because of their little size. Nanoencapsulated pesticides can give controlled discharge energy, while proficiently upgrading piousness, dependability, and solvency. Nanoencapsulation can improve the vermin control proficiency over expanded spans by forestalling debasement of dynamic fixings under ecological conditions. This survey is hence composed to fundamentally evaluate the toxicological impacts i.e to examine the manifestations, systems and identifications of poisoning vertebrates particularly people. The prepared neem nano-plants contrasted with the bulk one have will be assessed on albino mice through two main approaches, i.e. determination of acute oral LD50 and study the toxic effect of sublethal dose (LD10) on some biochemical parameters. The effect of the prepared nano-formulations compared to the bulk one on various biomarkers, i.e. hematological, hepatotoxicity and nephrotoxicity in albino mice after an oral administration of sub-lethal dose during sub-acute treatment were taken in consideration. Hence, this review should thus offer an important guide for building up potential advantages are underlined, while little is known on security or the antagonistic impacts of nano-advances in the agro-foodsystem.

1. Introduction

Chemical synthesized pesticides are extensively used for controlling pests such as insects, mites, fungi, nematodes, rodents, weeds and others. The most basic models in crop production is to diminish decrease in crops and for delivering enough and solid food because of the expanding human populaces overall (7.7 billion) [1]. It has been recorded that around 3 million ton of insect sprays are utilized for crop insurance annually [2]. These inordinate utilization of harmful risky poisonous pesticides which may present expected ecolog-

**Corresponding Author:*
Nadia Zikry Dimetry,
Department of Pests and Plant Protection, National Research Centre, El-Bohouth St., Dokki, Cairo, 12622, Egypt;
Email: nadia_dimetry@yahoo.com

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ical and natural dangers to human health, non-target living beings and ecosystem[1]. The most widely recognized issues in crop protection are pesticide residues and pest resistance. Likewise, the pesticides deposits influence open health[3]. Insect sprays are utilized to ensure vegetables, plantations, fancy plants and put away item materials against numerous pests during storage and in the field.. In spite of the fact that the fundamental motivation behind these manufactured pest sprays was known, it can make numerous unfavorable impacts individuals,animals, feathered creatures and environment [3]. Also, widespread use of pesticides has led to the development of resistance to pesticides such as "organophosphorous compound, carbamates, pyrethroids, abamectin and indixacarb [4-10]". In this way, the persistent assessments of the plant insect sprays "for security poisonousness are extraordinarily required utilizing diverse creature models since the reactions by these creatures to synthetic operators changes widely [11]". At this moment, new example is going in the state of mind for using the basic plant extracts for instance "neem extracts or EOs [12,13] as regular pesticides to control insects with nanoformulations in green pest management [3]". These green and nanopesticides are safe, low or no mammalian destructiveness and have various goals of noxious action in insect pests, which lead to high selectivity and low restriction development [14,15]. Extracts from the neem tree "*Azadirachta indica,* A. Juss" green growth, organisms, microorganisms and fundamental oils of other plant roots are dynamic against several insect species including vertebrates and useful parasitoids and predators. They are utilized as options in contrast to the customary compound pesticides which lead to ecological unevenness as per their unsafe consequences for normal development, or the event of vermin opposition and the hurtful impact of pesticides deposits in soil or plant.

Low or no mammalian harmfulness and have numerous locales of poisonous activity in pests, which lead to high selectivity and low resistance development [14,15]. Extracts from the neem tree "*Azadirachta indica,* A. Juss" green growth, organisms, microscopic organisms and fundamental oils of other plant origin are dynamic against several pest species including vertebrates and helpful insects. They are utilized as options in contrast to the conventional synthetic pesticides which lead to the occurrence of pest resistance, ecological unevenness as per their hurtful consequences for natural enemies, or the event of harmful opposition and the unsafe impact of pesticides deposits in soil or plant. "As needs be, it is imperative to discover safe choices like plant extracts or basic oils which have demonstrated victories in pest control, as they have insecticidal action, influence the irritation fruitfulness, give antifeedant or obstacle impact just as influence the biochemical procedures inside the insect body". According to another perspective, one of the detriments of the customary organic items is their restricted soundness when applied in the field.

Extracts from the neem tree "*Azadirachta indica*, A. Juss" green growth, organisms, microorganisms and fundamental oils of other plant sources are dynamic against many insect species including vertebrates and valuable biological control species. They are utilized as options in contrast to the customary synthetic pesticides which lead to ecological unevenness as indicated by their unsafe consequences for common foes, or the event of anti-feedant or deterrent effect and the destructive impact of pesticides deposits in soil or plant. Likewise, it is critical to discover safe choices like plant extracts or natural oils which have indicated success in pest control, as they have insecticidal efficacy, influence the insect fertility, give antifeedant or obstruction impact just as influence the biochemical procedures inside the insect body. According to another perspective, one of the detriments of the customary herbal items is their restricted soundness when applied in the field.

Considering the real factors implied the massive number of plant extracts, it was found that neem extracts (got from the tree *Azadirachta indica*) is the most well known plant extracts among others. Its insecticidal activity relies upon its strategy for movement as interfering with larval turn of events and improvement, advancement obstacle, development impediment, or enlistment of malformation in the larval stage. "In the grown up adult insects, these concentrates block egg development and may in like cause sterilization in different insect pests and such impacts can likewise be prompted as hormone application [16,17]"

To conquer the inconveniences of the conventional organic extracts for example, instability chemical disintegration and consequently loss insecticide properties their pest spray properties, green nano-innovation is a fruitful way which is as of late used to improve the pesticide properties of plant insect sprays. Nano-innovation has become a significant examination field in all zones. The size, direction and physical properties of nano-particles have supposedly appeared to change the exhibition of any material [18]. Advancement of green procedures for the combination of nano-particles is developing into a significant part of nanotechnology [19]. Be that as it may, phytochemicals, for example, secondary metabolites and fundamental oils face issues of stability and cost adequacy. If there should be an occurrence of fundamental oils, their compound precariousness within the sight of air, light, dampness, and high temperatures that cause quick vanishing and corruption of

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some active components are significant concern. Joining of fundamental oils into a controlled-discharge nano-definition forestalls fast dissipation. Moreover, this sort of definition is required to be more successful than the bulk substances [21,22]. Then again, it has been discovered that pesticide nano formulations indicated less harmfulness towards non target organisms contrasted with bulk or business formulations and subsequently a higher explicitness was observed [23] corruption; prevents rapid evaporation and degradation; enhances stability improves solidness and keeps up the base viable measurements / application because of covering process [20].

To conquer the weaknesses of the customary natural extracts, for example instabiltiy, chemical decomposition and henceforth misfortune their insect spray properties, green nano-innovation is a fruitful way which is as of late used to improve the pesticide properties of herbal pest sprays. Nano-innovation has become a significant examination field in all zones. The size, direction and physical properties of nano-particles have purportedly appeared to change the exhibition of any material [18]. Advancement of green procedures for the blend of nano-particles is developing into a significant part of nanotechnology [19]. Be that as it may, phytochemicals, for example, auxiliary metabolites and basic oils face issues of strength and cost viability. If there should arise an occurrence of fundamental oils, their concoction shakiness within the sight of air, light, dampness, and high temperatures that cause quick vanishing and debasement of some dynamic parts are significant concern. Fuse of basic oils into a controlled-discharge nano-definition forestalls fast vanishing and corruption; improves strength and keeps up the base compelling measurement application because of covering process [20].

Additionally, such an enumerating is depended upon to be more reasonable than the mass substances [21,22]. On the other hand, it has been found that pesticide nano definitions exhibited less poisonous towards non target animals contrasted with mass or commercial formulations and in this manner a higher explicitness was watched. The target of the current survey is to focus on the harmfulness of Neem extracts and Neem nano-items as few is recorded on safety and the antagonistic impacts of the utilization of nano-advances in the agro-food sector [24].

2. Plant Extracts as Biological Control Agents

The characteristic issues achieved by maltreatment of pesticides have been the matter of stress for both scientists and people in recent time. It has been assessed that 3 million tons of pesticides[39] are used on crops each year and the general damage realized by pesticides comes to $100 billion every year. The reasons behind this are the high poisonousness and non-biodegradable properties of pesticides and the development in soil, water resources and harvests that impact open health [3].

Thusly, it must be considered to search for new significantly explicit and biodegradable pesticides to handle the issue of long haul harmfulness to vertebrate creatures, of course, one must analyze the environmental friendly pesticides and make techniques that can be used to decrease pesticide use while keeping up crop yields.

Natural products are a super option in contrast to synthetic pesticides as intends to diminish negative effects on human wellbeing and the earth. Advancing toward green science forms and the proceeding with requirement for growing new harvest security instruments with novel methods of activity makes disclosure pesticides as an alluring and productive interest that is telling consideration.. botanical insecticides are progressively pulling in research consideration as they offer novel methods of activity that may give viable control of pests that have just evolved protection from traditional insect sprays [25]. Neem "*Azadirachta indica*" is one of the most significant limonoid creating plants from Meliaceae family [26] has for some time been perceived as a wellspring of condition agreeable biopesticide. A few constitutions of its leaves and seeds show marked insect control potential and because of their relative selectivity, neem items can be suggested for some Integrated Pest Management (IPM) programs [27]. In any case, there are a couple of occurrences of things got from plant extracts, for instance, neem oil, China berry seed extracts among others that apply diverse method of activities to execute the focus insect pest. From those referenced techniques for activities of such plant extracts, the antifeedant [28,29], (insect development regulators) [30,16] and sterilizers [31]. Most work has focused on azadirachtin and other related compounds luxuriously from neem seed extracts which go about as both solid antifeedants and insect growth regulators. Azadirachtin and its contents has antifeedant effect due to either hydrogenation of - 22 twofold bonds or deacetylation caused any change by impeding of hydroxyl group affected the taking care of inhibitory activity, while acetylation of azadirachtin caused a decrease in the most extraordinary activity [29].

Further the sound framework synthetic structure around hemi acetyl region is critical for antifeedent development. Azadirachtin impacts the insect's reproductive organs, body improvement and other endocrine systems [31] and doesn't impact other biocontrol agent. Neem has impacted more than 300 insect pests [31]. Further neem items are bio-degradable, slight destructive or no noxious to non target living forms, while they are non-hurtful toward people and well evolved creatures [31].

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Neem roots have a capacity to recover sicknesses what's more give a couple of pest spray properties against insect pests [32]. In like way, the perniciousness characteristics of azadirachtin and the component of its insecticidal activity were besides considered [33]. Extracts of various parts of the tree, especially of the seeds, have been appeared to posses feeding prevention properties (antifeedant), repellency, toxicity and growth disruptive properties to different species and phases of insects of different orders "So that, one of the activities of neem is considered as insect growth regulator". A comprehensive review of the entomological properties of neem has been published [16,34,35] Be that as it Neem oil extracted from the seeds of neem tree has been known to contain various bioactive blends significant mixtures are tri-terpenoids of the class of limonoid. Noteworthy mixture as uncovered in published works seem to be (azadirachtin A), salanin, nimbin, 3-tigloylazadirachtol (azadirachtin B), and 1-tigloyl-3-acetyl-11-hydroxymeliacarpin (azadirachtin D) [36].

Neem subordinates don't kill insect pests straight forwardly, however behavioural and physiological properties and starvation antifeeding of insects on treated plants [17,37,38]. "The most significant active principal of neem compound is azadirachtin which unequivocally meddles with larval development and improvement. The morphological impacts are growth retardation, development hindrance, molting inhibition, or induction of abnormality."

Insect growth regulatory activity of neem debilitates the cuticle defense system of the larvae causing simple entrance of pathogenic organisms into insect framework. Azadirachtin, a naturally dynamic compound has been advanced as another new pest spray that is viewed as more eco-accommodating than synthetic insecticides. The pesticide adequacy, environmental security and open agreeableness of neem and its items has prompted its selection into different mosquito control programs [22]. Rao et al.,[40] demonstrated that the LC_{50} values for neonate and the second instar hatchlings of *Helicoverpa armigera* were 0.002 and 0.004 %, respectively when ate from Neem Azal-treated cotton leaves constantly. The LC_{50} values were 0.005, 0.02, and 0.03% for the first, second, and third instar hatchlings of *H. armigera,* respectively, when the acquaintance was compelled with 48 hr. Besides, they itemized that the concentration of 200 ppm of Neem Azal on a very basic level diminished larval and pupal weight in assessment with control.

Dimetry, et al.,[41] found that the LC_{50} values for second and fourth instar larvae of *Agrotis ipsilon* were 4.38 and 16.68 ppm individually when given semi-synthetic diet contain various convergences of azadirachtin when the introduction time frame was 96 hours. They included that LC_{90} carried on a similar action request as they were 8.57 and 34.74 ppm for the second and fourth instar individually.

Amin et al.,[42] brought up that neem oil at a sublethal portion (0.75ppm) azadirachtin when added to artificial diet and fed to second instar larvae of *Agrotis ipsilon* expanded the larval length to 22.71 days contrasted and 18.82 days for the control larvae and the level of larval mortalities expanded to 10 % contrasted and 5 % for the control. They additionally included that pupal span was drawn out concerning control treatment, Again, a noteworthy decrease (384.62 mg) was happened in pupal weight in correlation with the control 410.0 mg.

"Neem items have low poisonousness to birds, fish and vertebrates and are more less likely to induce resistance due to their multiple mode of action and their different method of activity on insects".

Generally, it could be concluded that the use of tested compounds with biological insecticides (azadirachtin; pyridalyl and quercetin) could be followed instead of conventional hazards insecticides and these may reduce the environmental pollution and hazard management program using tested compounds looking forward in integrated pest management.

3. Toxicological Evaluation of Plant Extracts against Mammals

Although a ton of work on pharmacological action of neem extracts has been done, very little toxicological assessment has been under taken. Toxicology worried about the investigation of the unfriendly impacts of synthetics on living creatures. The ecological issues came about because of the utilization of synthetic pesticides have been the explanation of worry for every single person and researchers in the ongoing decades. It has been recorded that around 3 million tons of pesticides are utilized on various crops every year. In this way, it must be taken in thought to scan for more safe and biodegradable pesticides to solve the problem of long term toxicity to mammals. Then again, ecological well disposed pesticides and create strategies which can be utilized to lessen pesticide application to get crop yield liberated from pesticide buildups. Herbal pesticides are sheltered option in contrast to synthetic pest sprays as a best mean to diminish negative effects on human well being, creatures, soil, water, *air and condition.* They are more good with the ecological segments than manufactured pesticides[43]. Neem items have low harmfulness to birds, and vertebrates and are more adverse to instigate opposition because of their numerous method of activity on insect pests. Moreover, insect growth regula-

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tor activity weakens the cuticle defense system of larvae causing simple infiltration of pathogenic life forms into insect pest framework. Toxicological examination of *A. indica* leaf extracts at 0.6 - 2.0 g/kg body weight didn't represent any deadly impacts on hematology, protein levels and histopathological boundaries of exploratory creatures though the leaf extracts at 200 g/kg body weight decreased the body weight of the animal and were joined by weakens, anorexia and histopathological defects[44]. The ethanolic root extract has likewise been accounted for to display a portion subordinate hepatotoxicity while the aquous extracts was not harmful to the liver [44].

Destructiveness profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats was explained by Ashafa et al. [11], differentiated and their different beginning body weight of the attempted animal, their last body weight extended ($P<0.05$) all through the introduction time period. The extract moreover extended the incomparable heap of the liver, kidney, lungs and heart of the animals [11]. (Table 1).

The 50 and 100 mg/kg body weight of the extract didn't fundamentally adjust the body weight of the animal, interestingly, the most noteworthy portion (300 mg/kg body weight) expanded the heaviness of the pancreas. So also, all the dosages of the extract expanded the liver-, kidney-, lung-and heart-body weight proportions. The spleen body weight proportion was not significantly different from the control at 50 and 100 mg/kg body weight. Moreover, the 200 mg/kg body weight of the extract diminished the spleen-body weight proportion while the 300 mg/kg body weight expanded it (Table 1). By and large, the adjustments in the biochemical parameters of toxicity effectively affect the ordinary working of the organs of the animals. In this manner, the ethanolic extract of *A. indica* stem bark at the dosages of 50, 100, 200 and 300 mg/kg body weight may not be totally safe as an oral cure and ought to be taken with alert if completely necessary [11].

The alterations in biochemical parameters by the ethanolic extract of *A. indica* stem bark are indications of adverse effects on the various organs of the animals. These will have consequential effects on the normal functioning of these organs. The ethanolic extract of *A. indica* stem barks may not be safe as an oral remedy most especially at 100, 200 and 300 mg/kg body weight. The dose of 50 mg/kg body weight appeared to be relatively safe [11].

Table 1. Efficacy of the stem bark of *A. indica* ethanolic extract on some organs of male rats (n= 10)

Parameters	Extract (mg/kg body weight)				
	check	50	100	200	300
Initial body weight (g)	185.00±8.93^a	188.00±5.26^a	184.00±7.11^a	187.00±4.44^a	184.00±7.74^a
Final body weight (g)	205.00±10.00^b	218.00±9.15^b	214.00±7.54^b	207.00±8.09^b	210.00±9.00^b
Weight of liver (g)	5.56±0.58^a	7.05±0.93^b	8.86±0.55^c	7.27±0.28^d	8.58±0.76^c
Weight of kidney (g)	0.80±0.05^a	1.03±0.07^b	1.09±0.09^b	1.07±0.20^b	0.98±0.07^b
Weight of lungs (g)	1.16±0.05^a	1.59±0.11^b	1.95±0.81^c	2.03±0.28^c	1.49±0.66^b
Weight of spleen (g)	0.56±0.03^a	0.57±0.02^a	0.54±0.06^a	0.27±0.07^b	0.71±0.07^c
Weight of heart (g)	0.51±0.01^a	0.77±0.04^b	0.72±0.04^b	0.74±0.09^b	0.73±0.03^b
Liver-body weight (%)	2.71±0.02^a	3.23±0.05^b	4.14±0.03^c	3.51±0.01^d	4.08±0.05^c
Kidney-body weight (%)	0.39±0.01^a	0.47±0.08^b	0.51±0.02^b	0.52±0.01^b	0.47±0.03^b
Lung-body weight (%)	0.57±0.03^a	0.73±0.02^b	0.91±0.01^c	0.98±0.02^c	0.71±0.07^b
Spleen-body weight (%)	0.27±0.02^a	0.26±0.04^a	0.25±0.05^a	0.13±0.01^b	0.34±0.01^c
Heart-body weight (%)	0.25±0.01^a	0.35±0.01^b	0.34±0.03^b	0.36±0.02^b	0.35±0.01^b

Note:
Mean±SD values carrying different superscripts from the check for each parameter are significantly different ($P<0.05$).
After Anofi Omotayo Tom Ashafa, Latifat Olubukola Orekoya, and Musa Toyin Yakubu (2012).*

In another examination done by Yun-xia Deng et al.[45], they found that in the acute toxicity test, the LD_{50} estimations of neem oil were seen as 31.95g/kg. "The 28 days subacute treatment with neem oil failed to change body weight gain, food and water consumption. Histopathological tests indicated that the objective organs of neem oil were testicle, liver and kidney. Serum biochemistry investigation demonstrated no significant contrasts in any of the boundaries inspected under the portion of 1600mg/kg/day".

They included that in no-watched antagonistic impact level (NOAEL) of presentation and target organs of neem oil for building up security standards for human exposure, the subchronic toxicity extract with neem oil in mice was assessed. The mice (10 for each sex for each portion) was orally managed with neem oil with the dosages of 0 (to fill in as a control), 177, 533 and 1600 mg/kg/day for 90 days. After the treatment time frame, perception of reversibility or perseverance of any poisonous impacts, mice were constantly taken care of without treatment

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for the accompanying 30 days. During the two trials, the serum biochemistry, organ weight and histopathology were inspected. The outcomes demonstrated that the serum biochemistry and organ coefficient in exploratory gatherings had no significant difference compared with control group. At the 90th day, the histopathological assessments demonstrated that the 1600 mg/kg/day portion of neem oil had differing degrees of harm on every organ with the exception of heart, uterus and ovarian. Following 30-day recovery, the level of sores to the tissues was decreased or even reestablished. The safe dose of neem oil was 177 mg/kg/day for mice and the objective organs of neem oil were resolved to be testicle, liver and kidneys [89].

Toxicity to other Mammals and Birds

New neem leaves given to Goat and Pigs for 7 days in 200mg/kg portion caused passing of creatures at 5 th day and posthumous revealed clog in brain [47]. SDS PAGE examination of heart proteins of the Bioneem treated chick embryo didn't show any noteworthy contrast in protein profile when contrasted with that of the control.

In study done to broiler chicks by Nety et al. [88] who announced that the hydro-alcoholic extract of *Azadirachta indica* (Neem Leaves) as an alternative in contrast to anti-biotic growth promoter- Bacitracin methylene diasalicylate (BMD) was assessed in broiler chicks. The extract was prepared by 50 % methanol and 50 % water AIE, 0.4 g/L) and was placed in drinking water and given to the chicks of particular treatment for 42 days. 90 broiler chicks (day-old) were arbitrarily allocated to 3 groups and each group with 3 replicates with 10 chicks of each.

They found decreased body weight, body weight gain and decreased feed conversion in (AIE) *Azadirachta indica* extract supplemented birds suggest that *A. indica* leaf extract contains toxic substance. The results of this study are in accordance with the findings of previous workers in acute toxicity study on neem leaf aqueous extract in chicken. The elevated activity of serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) in the serum of AIE supplemented birds for 6 weeks indicated severe liver damage and increase level of uric acid and creatinine concentration indicated hepato-nephro toxicity, significant lower level of packed cell volume and lymphocyte count was observed in AIE supplemented group. Nety et al. [88] concluded that supplementation of hydro-alcoholic extracts of *Azadirachta indica* leaves (AIE) in broiler birds caused death of 20 % of birds during the 4th and 5th weeks and toxicity is mainly associated with the hepatotoxicity, nephrotoxicity, suggesting that AIE should be used with caution in ethano-veterinary practice. Rafeeq Alam Khan& Maryam Aslam [48] expressed that acute oral poisonousness (LD_{50}) was seen in albino mice utilizing standard conventions where as sub-chronic, hematological and histopathological contemplates were surveyed on 24 albino rabbits after giving herbal formulations for 60 days in two dosages (20 and 60 mg/kg) against control gatherings.

Likewise, It is accounted for that leaves of neem brought about harmful impacts on sheep[49], goats and guinea pigs [50]. A portion higher than prompting passing in guinea pigs. In any case, 200 mg/kg in a similar course was seen as non-poisonous to rabbits [51]. Ethanol neem containing 3000 ppm azadirachtin (±10%) is recorded by the Environmental Protection Agency (EPA), USA. The information submitted on intense oral harmfulness in rodents showed no negative impact up to a portion of 5ml/kg (National Academy Press, Washington D.C., 1992) [71].

In another assessment, methanolic leaf and bark extracts showed an oral LD50 (Lethal segment, half) of about 13g/kg in acute harmfulness assessment on mice's. Creature gave general signs of wiped out prosperity and disquiet, gastro-intestinal spam, lack of concern, refusal of water.

4. Nanotechnology in Pest Control

Pesticides denotes a wide range of agro-chemicals, those are extensively used in agriculture for protection of crops from diseases, pests and weeds. Pesticides are exploited both extensively and intensively to minimize these losses and around 3 billion tons of pesticides are employed for the same[2]. The biggest hurdle that lead to the failure of these pesticides was the water-insolubility of these formulations. Purchaser familiarity with the health hazard from the residual harmfulness and the issue of most efficient protection from pesticides has contend the researchers during the most recent two decades everywhere throughout the world to look for progressively safe methods [52]. One of the most effective options is utilizing nanotechnology [20]. Nanoemulsions are emulsions whose minuscule size is uniform and incredibly little with the size ranges that of bulk materials and can be figured without the utilization of organic solvents [54]. In excess of 1300 business nanomaterials with wide spread of potential applications are as of now available [55-58]. Kamaraj, et al. [59] orchestrated a neem gum nano-plan (NGNF) as antifeedant, larvicidal and pupicidal exercises against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) at 100 ppm. The NGNF showed significant (100%) antifeedant, larvicidal and pupicidal practices against *H. armigera* and *S. litura.* The LC50 estimations of 10.20, 12.49 and LC90 estimations of 32.68, 36.68 ppm on H. armigera and S.

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litura, exclusively were resolved at 100 ppm.

At a time when the conventional pesticides pose an immense threat to the quality of environment and the health of the organisms, more emphasize should be given on development of nano-formulation of pesticides, which proves to be more potent and selective over the conventional formulations. Though these formulations have not reported to cause any acute toxicity in the non-target organisms till date, more thrust should be given on this aspect to come up with ways to nullify the short comings before its ingression in agriculture[90]. Dimetry, et al.[60] found that the utilization of nanomaterials will bring about the advancement of productive and expected methodologies towards the management of insect pests. They arranged the planned neem oil nanoemulsion just as loaded neem was described by Transmission electron microscopy. Likewise, the harmfulness of neem oil free as bulk, nano and loaded neem have been evaluated against the second and fourth larval instar of *Spodoptera littoralis* under lab conditions. The toxicity of the tried oil dependent on LC_{50} of loaded neem was lower 2.22 ppm contrasted and neem oil nano emulsion and bulk neem (5.09 and 6.71 ppm separately for the second larval instar. A similar pattern was found concerning the fourth larval instar.

Dimetry et al. [41] assessed the harmfulness of neem and peppermint oil nano plans against *Agrotis ipsilon* hatchlings. They revealed that the LC_{50} estimation of loaded neem or pepper mint were lower (0.62 and 36.47 ppm) contrasted and neem or pepper mint oil nano-emulsion and bulk neem for the second larval instar. They included that the various definitions of neem are more strong than if there should be an occurrence of peppermint oil, as LC_{50} and LC 90 qualities were essentially lower.

Amin et al. [42] considered the capability of nano-details of neem and peppermint oils on the bionomics and enzymatic potancy of *Agrotis ipsilon* hatchlings. They exhibited critical stretching of the larval span, rate mortalities were extended similarly as larval malformations. Aftereffects of enzymatic potency indicated significant impacts of the three formulations of neem and pepper mint oil. Essential oils increase in the activities of cuticle phenoloxidase and chitinase were viewed, in any case, noteworthy restraints were recorded for amylase, invertase, trehalase, protease and alkaline phosphatase. Potential uses of nanotechnology in agribusiness are: transport of nano-pesticides epitomized in nano-materials for controlled release; alteration of biopesticides with nano-materials; slow arrival of nano material helped fertilizers, biofertilizers and micronutrients for capable use; and field employments of agrochemicals, nanomaterials helped movement of nano material for crop improvement. Nano-sensors for plant pathogen and pesticide disclosure, and NPs for soil protection or remediation are various zones in agribusiness that can benefit by nano-technology [20].

The utilization of miniaturized scale and nano-emulsions as carriers of pesticides diminishes the utilization of natural dissolvable and expands the dispersity, wettability, and penetration properties of the droplets and may prompt improvement of the organic viability of pesticides [61]. Papanikolaou, et al. [62] expressed that expanding insecticidal action of nano-detailed pyrethrins in combination with the nonappearance of antagonistic consequences for non-target aphid predators make them compatible plant protection items in natural cultivating and IPM procedures in different crops. Additionally, they added that their outcomes confirmation to the utilization of nano-innovation in improvement of pest spray definition for the advancement of solid and decreased natural hazard plant insurance items.

4.1 Toxicity of Nano-particles

Little information is available in relation to the toxic effects of the prepared nano-particles on the mammalian organisms. From the available information and some facts related to the toxicity of the nano-particles is that a large number of scientific evidence such as increasing the heart, pulmonary and neurological diseases caused by the nano-particles, were pushed many international scientific organization to recognize the harmful effects of nano-particles even the emergence of Nano-toxicology. Furthermore, the long term exposure to the nano-structures agents may lead to new/unforeseen harmful effects. Also, it is not known how the ingested nano-particles will behave in the body. In addition, Absorption, distribution, metabolism and Excretion profiles of nano-materials are different from bulk equivalents. So that, the long term health consequences of ingested bio-persistent nano-particles are not known. Based on such facts, FAO/WHO Expert Meeting on the Application of Nano-technologies decided to carry out a risk assessment of each nano-particles based products[63]. Dimetry and Hussein [64] called attention to that there is an extraordinary concern with respect to the nano-materials which can possibly apply unsafe consequences for nature and human wellbeing and when we have a nano-pesticide, it turns out to be twofold edged weapon". Bayoumi,[65] mentioned the important points that have to be in consideration when testing the nano-formulations which could be summarized as the following: Exposure route and exposure period (acute and chronic) of the tested organisms, Existence of impurities and agglomeration

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of nano-materials.

Also, Mossa et al. [66] called attention to that no indications of harmfulness or mortality in male rodents acquainted with nanoemulsion of camphor or the EO. Biochemical cutoff points likewise show insignificant changes in all liver biomarkers in serum of male rats. The liver is the basic organ in the body, expect a colossal action in xenobiotic detoxification. "It is the basic goal to harmful xenobiotic and their metabolites. Accordingly, changes in liver cutoff biomarkers are conventionally utilized as biomarkers for liver noxiousness and damage [67, 68]. It has been represented that the development in the potency of liver synthetic compounds and change in grouping of protein, albumn and globulin can be direct result of cell injury [68, 69]" hepatotoxicity and change in proteins

Deng, Yun-xia et al. [45] found that, acute and 28-day sub acute harmfulness tests were done. They found that subacute treatment with neem oil did not succeed to change body weight increase, food and water utilization. intense poisonousness depicts the horrible effect of a substance that result either from a solitary presentation [70] or from various exposures in short space of time (for the most part under 24 hours). While, sub acute harmfulness, can be delineated as the negative effects should occur inside 14 days of the association of substance. Intense oral poisonousness of ethanol neem extracts containing 3000 ppm azadirachtin (± 10%) is enrolled with the Environmental Protection Agency (EPA), USA. The data submitted on acute oral harmfulness in rodents showed no negative effect up to a doze of 5ml/kg[71]. In another assessment, methanolic leaf and bark extracts showed an oral LD50 of about 13g/kg in intense poisonousness concentrates on mice [45].

Deng, Yun-xia et al. [45] found that, acute and 28-day sub acute poisonousness tests were completed. In the acute poisonousness test, the LD50 estimations of neem oil were seen as 31.95g/kg. The subacute treatment with neem oil did not affect to change body weight gain, food and water utilization. Serum natural chemistry investigation indicated no huge contrasts in any of the boundaries analyzed under the dose of 1600mg/kg/day. Histopathological tests indicated that the objective organs of neem oil were testicle, liver and kidneys up to the portion of 1600mg/kg/day.

"Raizada et al. [74] communicated that a single oral dose of azadirachtin (5000 mg/kg) to male and female rat didn't convey any sign of harmfulness nor demise in the treated animal. The LD50 regard along these lines is more than 5000 mg/kg both in male and female rodents"

Dorababu et al. [75] exhibited that acute similarly as sub acute harmfulness mulls over demonstrated no mortality with 2.5 g/kg dose of *Azadirachta indica* extract in mice.

4.1.1 Acute Toxicity

"Acute harmfulness depicted the adversarial effect of a substance that result either from a solitary presentation [70] or from a several exposures in short space of time (commonly under 24 hours)". While, sub acute harmfulness, can be portrayed as the troublesome effects should occur inside 14 days of the organization of substance. "Acute oral poisonousness of ethanol neem extracts containing 3000 ppm azadirachtin (±10%) is enrolled with the Environmental Protection Agency (EPA), USA [71]. The data submitted on acute oral poisonousness in rats showed no negative effect up to a portion of 5ml/kg[71]".

In another examination, methanolic leaf and bark extracts demonstrated an oral LD50 (Lethal portion, half) of about 13g/kg in acute poisonousness evaluations on mice[47]. Animal gave general indications of sick wellbeing and uneasiness, gastro-intestinal fit, unresponsiveness, refusal of water and feed and hypothermia. Mice passed on under terminal spasms. No gross tiny injury was found on autopsy[91]. The information submitted on acute oral harmfulness in rats showed no negative impact up to a portion of 5ml/kg [71]. Target organs of poisonous impacts were the central nervous system and lungs[92]. Be that as it may, methanol solvent and insoluble parts, from an equeous leaf extracts were not harmful inside 24hr at an oral portion of 200mg/kg in mice [93]. In this way, all the above examination is done on the rodents, rabbits and guinea pigs and they indicated their response [47].

Upon acute presentation, nimbidin, disconnected from neem seeds,dose conditionally decreased intense paw oedema in rodents. The medium compelling dose (ED50 esteem) was 79.4 mg/kg body weight in rats [72]. Once more, the LD 50 values for Neem Azal (Neem based pesticidal item) were higher than 2g/kg body weight in mice [73].

The consequences of the accessible oral poisonousness tests demonstrated that, intense harmfulness at high dosages may occur [76,77] depending upon the particle size, covering and synthetic chemical composition of the nano particles. "Raizada et al.,[74] conveyed that a solitary oral dose of azadirachtin (5000 mg/kg) to male and female rats didn't make any indication of hurtfulness nor passing in the creatures. The LD50 value in this manner is in excess of 5000 mg/kg both in male and female rats". Dorababu et al. [75] showed that acute also as sub acute noxiousness examines demonstrated no mortality with 2.5 g/kg portion of *Azadirachta indica* extracts on, hematological profile and distinctive liver and kidney work tests in rats when rewarded for 28 days with 1 g/kg portion of *Azadirachta*

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indica extract. Acute, subacute and subchronic destructiveness following oral introduction have been explored in rats for a few nano particles.

Deng, Yun-xia et al. [45] found that, acute and 28-day sub acute poisonousness tests were investigated. In the acute noxiousness test, the LD50 estimations of neem oil were exhibited as 31.95g/kg. "The subacute treatment with neem oil did not change body weight increment, food and water use.

Youssef [78] pointed out that the toxic effect of bulk, nano-emulsion and loaded nano-emulsion of neem extract on the Swiss albino mice clearly determined acute oral $LD_{50,}$ values were 113.33, 134.83 and 140.90 mg/kg body weight for loaded nanoemulsion, nano-emulsion and bulk neem extract respectively. This result revealed that the nano formulations were more toxic than the bulk one. In addition, the sub-acute administration of sub-lethal dose (LD_{10}) during 14 days showed significant alterations between induction and reduction in the selected biomarkers, *i.e*. hematological toxicity (hemoglobin increased to reach +107.53% after 3 days from continues treatment of bulk oil, RBCs was +343.89% after 3 days in loaded nano-emulsion treatment, WBCs was +150.00 after 5 days in bulk treatment), hepatotoxicity (GOT) +57.27% after 3 day in bulk treatment, GPT -51.96% after 14 days in loaded nano-emulsion, glutathione S-transferase +241.38 after 5 days in bulk oil, reduced glutathione +86.85% after 5 days in nano-emulsion and bilirubin + 355.88% after 20 days in bulk oil), nephrotoxicity (creatinine +330.56 after 20 days in loaded nano-emulsion), total ATPases +8.56% after 3 days in nano-emulsion and total protein in liver samples -74.60% after 7 days in loaded nano-emulsion and in brain samples was -74.16% after 3 days in loaded nano-emulsion

An another report was completed to explore the toxic effect of the prepared neem nano formulation on albino mice as mammalian model. Human adventitious ingestion of 20ml neem oil declared the harmful encephalopathy [72]. Fresh neem leaves offered to Goat and Pigs for 7 days in 200mg/kg portion caused demise of creatures at 5 th day and after death revealed obstruct in cerebrum [73]. SDS PAGE examination of heart proteins of the Bioneem treated chick embryo didn't exhibit any valuable effect in protein profile when contrasted with that of control. Diminished body weight, body weight gain and diminished feed transformation in the *Azadirachta indica* extract (AIE) enhanced supplemented birds propose that *A. indica* leaf extracts contains poisonous substance. The after effects of this investigation are as per the discoveries of past laborers in acute toxicity on neem leaf water extracts in chicken 5,9. The raised movement of ALT and AST in the serum of AIE supplemented birds for about a month and a half demonstrated extreme liver harm and increment level of uric Most of neem based products might be toxic. The assorted neem formulations regulated once or chronically conflictingly sway animal health and once in a while now and again even reason demise with medium deadly concentration LC50 values varying in go from 1.6 to 16 ml/kg body weight.

Treatment of mice had no influence on liver, spleen, thymus or body weight records and an update of macrophage relocation prevention and foot cushion thickness [79]. The non-hepatotoxic nature of Neem Leaf Preparation was illustrated. The level of serum urea remained not changed and run of the mill designing of the cortical and medullary bits of kidney were also observed after neem leaf arrangement treatment corrosive and creatinine related with nephrotoxicity [79].

4.1.2 Chronic Toxicity

"A large portion of neem based products may be harmful. The diverse neem formulations regulated once or chronically alternately sway creature wellbeing and now and then once in a while even reason demise with medium lethal concentration (LC_{50}) values changing in go from 1.6 to 16 ml/kg body weight".

Treatment of mice had no influence on liver, spleen, thymus or body weight records and an overhaul of macrophage movement deterrent and foot cushion thickness[79]. The non-hepatotoxic nature of Neem Leaf Preparation was illustrated. The degree of serum urea remained not changed and regular building of the plant planning of the cortical and medullary bits of kidney were moreover seen after neem leaf preparation treatment.

Panda and Kar [80] nounced that dose dependent impacts were seen in mice treated with neem fluid leaf extract. Neem application diminished tri-iodothyronine (T3) what's more, extended serum thyroxine (T4) centers and hepatic lipid peroxidation and decreased glucose-6-phosphatase development while improving the activities of super oxide dismutase and catalase. "Khosla et al. [81] found that Sub-chronic organization of neem leaf extracts caused a decrease in glucose levels in regular and diabetic rabbits. The concentrate was more convincing than seed oil." "Abdel Megeed et al. [82] indicated that when rats were treated with azadirachtin, expanded serum SGOT and SGPT activities and bilirubin content were seen". Histopathological thinks about exhibited over the top changes in the liver to the degree blockage, hydropic degeneration, defilement and lymphocytic infiltration. The eventual outcomes of the activities of liver and serum AST, ALT, ALP, S-bilirubin, S-albumn, S-cholesterol and S-protein of an-

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alyzed and control rats displayed no large changes in the clinico-engineered cutoff points of creatures treated with various convergences of azadirachtin [74].

Dehghan et al. [83] observed a decline in ATPase activity in caput and cauda epididymis of sperm of mice when treated with neem seed alcoholic extract. The vast majority of the neem-based products are poisonous. For Praneem and Nimbokil-60 effects on reproduction and fertility are accounted for. Every single other agent, regulated once or chronically, contrarily influence animal health and sometimes even reason demise with medium deadly focus (LC_{50}) values changing in the range from 1.6 to 16ml/kg of the neem-based items are noxious. For Praneem and Nimbokil-60 impacts on reproduction and fertility are represented. Each and every other agent, controlled once or chronically, oppositely influence animal health and once in a while even explanation downfall with medium destructive center (LC50) values changing in the range from 1.6 to 16ml/kg.

Haque et al. [84] "announced that essential obstruction of progress of Ehrlich's carcinoma was watched following prophylactic treatment on Swiss albino mice with Neem Leaf Preparation (NLP-1 unit) when consistently for about a month. "Harmful impacts of this specific part (1 unit), close by 0.5 unit and 2 units of NLP portions, were assessed on various murine physiological frameworks. 100% of mice could drive forward through 4 infusions of 0.5 and 1 unit Neem leaf course of action (NLP) dosages". "Body weight, particular organ-body weight extents and physical conduct of treated mice remained absolutely unaltered during treatment with different NLP doses. These Neem Leaf Preparation doses were seen to animate hematological systems as confirm by the expansion in total account of RBC, WBC and platelets and hemoglobin percentage". As histological changes also as climb in serum alkalinee phosphatase, SGOT, SGPT were not found in mice treated with three distinct dosages of NLP, the non-hepatotoxic nature of NLP was represented. The degree of serum urea stayed unaltered and normal plan of the cortical and medullary bits of the kidney were in like way safeguarded after NLP treatment. Increased immunizer creation against B16 melanoma antigen was perceived in mice inoculated with 0.5 unit and 1 unit of NLP. Number of splenic T lymphocytes (CD4+ and CD8+) and NK cells were in like way observed to be stretched out in mice infused with 0.5 unit and 1 unit of NLP. Regardless, NLP portion of 2 units couldn't show such safe stimulatory changes.

NLP safe incitement was associated well with the improvement restriction of murine carcinoma. In end, tumor advancement impediment was watched precisely when mice were injected with immuno stimulatory dosages of NLP (0.5 unit and 1 unit).).

"Portion subordinate effects were found in mice treated with neem aquous leaf extract. Neem application diminished tri-iodothyronine (T3) and expanded serum) glucose-6-phosphatase activity while overhauling the activities of super oxide dismutase and catalase".

4.1.3 Toxicity of Sub-lethal dose (LD_{10}) of Neem Bulk and Nano-formulations on Some Biochemical Markers

The sub-lethal. dose (LC10) impact of neem bulk and nano formulations were done to Swiss Albino mice orally during a week and the exposure procedure was stopped during fourteen days as a recovery period the presentation dependent on the information got from Hayes and Youssef [85,78]. To investigate the harmful impacts of the various formulations utilized in examination with the bulk form, some biochemical markers were estimated during the subacute treatment (heamatological, hepatotoxicity and renal biomarkers. Additionally, acute and sub choronic toxicity were evaluated by Rafeeq Alam Khan et al.[48] who researched Acute oral harmfulness (LD50) in albino mice using standard shows though sub-interminable, hematological and histopathological looks at were reviewed on 24 albino rabbits after giving herbal formulations for 60 days in two dosages (20 and 60 mg/kg) against control bunch. The results of this investigation showed that the medication is sheltered up to 5000 mg/kg body weight following acute oral poisonousness test and no mortality was seen during sub chronic harmfulness contemplates. Consequences of sub-chronic poisonousness didn't show any significant changes in biochemical, dosage (LC10)) effect of neem bulk and nano extracts were done to Swiss Albino mice orally during a week and the introduction strategy was quit during fourteen days as a recuperation period the introduction subject to the data got from Hayes and Youssef [85,78], ematological and histopathological boundaries. In any case,some markers, for instance, urea, creatinine, hemoglobin, and RBC check were changed, yet these progressions don't compare with the histopathological results and may be identified with intra singular varieties.

NLP interceded resistant incitement was related well with the advancement restriction of murine carcinoma. All things considered, tumor advancement confinement was observed exactly when mice were infused with immuno stimulatory dose of NLP (0.5 unit and 1 unit).

Dose subordinate effects were found in mice treated with neem watery leaf extract. Neem treatments diminished tri-iodothyronine (T3) and extended serum

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) glucose-6-phosphatase activity while upgrading the exercises of super oxide dismutase and catalase

4.2 Long Term Toxicity

"Information from harmfulness evaluations with various courses of presentation show that several systemic effects for different organ structures may occur after long term introduction to Nano-Particles, including the invulnerable system, provocative effects and cardiovascular framework. Effects on the safe framework may fuse oxidative pressure or enactment of master fiery cytokines in the lungs, liver, heart and cerebrum. Effects on the cardiovascular system may fuse pro- thrombotic impacts and negative outcomes on the cardiovascular capacity (intense myocardial localized necrosis and unfavorable consequences for the pulse."

In addition, genotoxicity, and conceivable carcinogenesis and teratogenicity may occur, for the going with endpoints, further investigates are expected to insist. In an overview on the health evaluation of Neemazal TM-T/S (Neem-based item), this item didn't have any impact on reproduction and didn't cause skin or eye disturbance. No cancer-causing nature was watched and 100ppm didn't have any impact after 90 days administration in rats [73].

4.3 Conclusions and Future Prospective

Worldwide market patterns towards crops protectants were progressively focused on items gotten from natural sources. As these biopesticide framework, plant extracts contain at least one or more chemical compounds, the safety evaluation to human being become more necessary, so as to guarantee security to man. A considerable lot of the botanicals have not been completely explored for their mammalian toxicity.

Once more, a deficiency of the toxicological data of various arranged nano-particles, studies on the toxicity of nano-particles demonstrated that there is a requirement for future exploration about the synthesis of new materials and assessment of their harmfulness. There is a solid thought that biological activity of nano particles will rely upon physiochemical studies and don't considered in toxicity screening contemplates. These limits that may be important in understanding the unsafe impact of the attempted materials consolidate particle size and size appropriation, shape, crystal structure, chemical arrangement, surface zone, surface chemistry and agglomeration state [86]. "The expanded surface reactivity predict that NSPs display more biological activity prominent natural movement per given mass contrasted with bigger particles, they ought to be taken into living beings". This increased biological activity can be either positive as for example cancer prevention agent action or negative for example harmfulness (subsequently its utilization in huge sums may demonstrate risky [87] or mixture of both "Data from toxicity evaluation demonstrate that several systemic consequences for various organ system may happen after long term exposure to nano-particles (NPs), including immune system, inflammatory effects and cardio-vascular system". Likewise, genotoxicity and possible carcinogenesis and teratogenicity may happen. These will affect the normal functioning of these organs. Likewise further investigates are needed to affirm the safeness of these neem or nano - formulations for warm blooded creatures with unique reference to human beings and their animals.

References

[1] Aktar, M.W., D. Sengupta, A. Chowdhury. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplin. Toxicol., 2009, 2: 1-12.

[2] Pan-UK. Current Pesticide Spectrum, Global Use and Major Concerns. http://www.pan-uk.org/briefing/SIDA_Fil/Chap1.html (january 18, 2003)

[3] Koul,O., S.Walia, G.S. Dhaliwal. Essential oils as green pesticides. Potential and constrains. Biopestic. Int., 2008, 4(1): 63-84.

[4] Siqueira H.A.A., Guedes R.N.C., Picanco M.C. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Agricultural and Forest Entomology, 2000, 2 (2): 147-153. DOI: https://doi.org/10.1046/j.1461-9563.2000.00062.x

[5] Siqueira H.A.A., Guedes R.N.C.;, Fragoso D.B., Magalhaes L.C. Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). International Journal of Pest Management, 2001, 47(4): 247-251. DOI: 10.1080/09670870110044634

[6] Reyes M., Rocha K., Alarcón L., Siegwart M., Sauphanor B. Metabolic mechanisms involved in the resistance of field populations of *Tuta absoluta* (Meyrick)(Lepidoptera:Gelechiidae) to spinosad. Pesticide Biochemistry and Physiology, 2012, 102(1): 45-50. https://doi.org/10.1016/j.pestbp.2011.10.008

[7] Campos M.R., Silva T.B., Silva W.M., Silva J.E., Siqueira H.A.A. Spinosyn resistance in the tomato borer *Tuta absoluta* (Meyrick)(Lepidoptera: Gelechiidae). Journal of Pest. Science, 2015, 88: 405-412. DOI: 10.1007/s10340-014-0618-y

[8] Haddi K., Berger M., Bielza P., Rapisarda C., Wil-

liamson M.S., Moores G., Bass C. Mutation in the ace-1 gene of the tomato leaf miner (*Tuta absoluta*) associated with organophosphates resistance. Journal of Applied Entomology, 2017, 141(8): 612-619. DOI: 10.1111/jen.12386

[9] Zibaee I., Mahmood K., Esmaeily M., Bandani A.R., Kristensen M. Organophosphate and pyrethroid resistances in the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) from Iran. Journal of Applied Entomology, 2018, 142(1-2): 181-191.

[10] Guedes R.N.C., Roditakis E., Campos M.R., Haddi K., Bielza P., Siqueira H.A.A., Tsagkarakou A., Vontas J., Nauen R. Insecticide resistance in the tomato pinworm. *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. Journal of Pest Science, 2019, 92(4): 1329-1342. DOI: 10.1007/s10340-019-01086-9

[11] Anofi Omotayo Tom Ashafa, Latifat Olubukola Orekoya, Musa Toyin Yakubu. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. Asian Pac J Trop Biomed. 2012, 2(10): 811-817. DOI: 10.1016/S2221-1691(12)60234-2

[12] Duarte, J.L., J.R.R. Amado, A.E.M.F.M. Oliveira, R.A.S. Cruz, A.M. Ferreira et al., "Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. Rev. Bras. Farmacogn., 2015, 189-192.

[13] Ebadollahi, A. Iranian plant essential oils as sources of natural insecticide agents. Int. J. Biol. Chem., 2011, 53: 266-290.

[14] Ehsanfar, S., S.A. Modarres-Sanavy. Crop protection by seed coating. Commun. Agric. Applied Biol. Sci., 2004, 70: 225-229.

[15] Elaissi, A., Z. Rouis., N. Abid Ben Salem, S. Mabrouk, Youssef ben Salem et al. Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complement. Altern. Med., 2012, 12. DOI: 10.1186/1472-6882-12-81

[16] Dimetry, N.Z. Prospects of botanical pesticides for the future in integrated pest management program (IPM) with special reference to neem uses in Egypt. Archive of Phytopathology and Plant Protection, 2012, 45: 1138-1161.

[17] Dimetry, N.Z., A.Y. El-Laithy, A.M.E. Abd El-salam, A.E. El-Saiedy. Management of the major piercing sucking pests infesting cucumber under plastic house conditions. Archives of Phytopathology and Plant Protection, 2013, 46: 158-171.

[18] Mubayi, A., S. Chatterji, P.M. Rai, G. Watal. Evidence based green synthesis of nanoparticles. Advanced Materials Methods., 2012, 3(6): 519-525.

[19] Raveendran, P., J. Fu, S.L. Wallen. A simple and ''green'' method for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles. Green Chem., 2006, 8: 34 -38.

[20] Ghormade, V., V. D. Mukund, M.P. Kishore. Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotech. Adv.,2011, 29: 792 -803.

[21] Anjali, C.H., S.S. Khan, K.M. Goshen, S. Magdassi, A. Mukherjee, V. Chandrasekaran. Formulation of water-dispersible nanopermethrin for larvicidal applications. Ecotoxicology and Environmental Safety, 2010, 73: 1932-1936.

[22] Anjali, C.H., Y. Sharma, A. Mukherjee, N. Chandrasekaran. Neem oil (*Azadirachta indica*) nanoemulsion as potent larvicidal agent against *Culex quinquefasciatus*. Pest Manag Sci, 2012 68(2): 158-163.

[23] Frederiksen, H.K., H.G. Kristensen, M. Pedersen. Solid lipid microparticle formulations of the pyrethroid gamma-cyhalothrin-incompatibility of the lipid and the pyrethroid and biological properties of the formulations. J. Control. Release., 2003, 86: 243-252.

[24] Bouwmeester, H., S. Dekkers, M.Y. Noordam, W.I. Hagens, A.S. Bulder, D. Heer, S. E.C. Ten Voorde, S.W.P. Wijnhoven, A. Marvin; H.J.P. Adriënne, J.A.M. Sips. Review of health safety aspects of nanotechnologies in food production. Regulatory Toxicology and Pharmacology, 2009, 53: 52-62.

[25] Amoabeng, B.W., G.M. Gurr, C.W. Gitau, H.I. Nicol, L. Munyakazi, P.C. Stevenson. Tri-Trophic Insecticidal Effects of African Plants against Cabbage Pests. Plos one, 2013, 8(10): 1371-1382.

[26] Connolly, J. Chemistry of the limonoids of the Meliaceae and Cneoraceae. In Chemistry and Chemical Taxonomy of the Rutales, (.eds P. G. Waterman and M. F. Grunden), 1983: 175-213.

[27] Schmutterer, H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol., 1990, 35: 271-297.

[28] Kraus W., Cramer R., Bokel M., Sawitzki, G. New insect antifeedant from *Azadirachta indica* and *Melia azedarach*. In Schmutterer H., Ascher KRS, Remboldt H. Proc. 1st Neem Conf. Rottach- Egern,1981, 53 - 62.

[29] Roy, A., S. Saraf. Limonoids: overview of significant bioactive. Nature, 2006, 363: 685 -693.

[30] Satasook, C., M.B. Isman, P. Wiriyachitra. Activity of rocaglamide, an insecticidal natural product, against the variegated cutworm, Peridroma saucia (Lepidoptera: Noctuidae). Pestic. Sci.,1992, 36: 53-58.

DOI: https://doi.org/10.30564/jrb.v2i3.2032

[31] Mordue, A. J., Blackwell, A. Azadirachtin: an update. J. Insect Physiol.,1993, 39: 903-924.

[32] Ragasa, C.Y., Z.D. Nacpil, G.M. Natividad, M. Tada, J.C. Coll, J.A. Rideout, Tetranortriterpenoids from Azadirachta Indica. Journal of Phytochemistry, 1997, 46: 555-558.

[33] Champagne, D. E., M.B. Isman, G.N. Towers. Insecticidal activity of phytochemicals and extracts of the Meliaceae. Insecticides Plant Orig.,1989, 387: 95-109. (London: Academic Press)

[34] Jacobson, M. Botanical Insecticides Past, Present and Future, In: Philogene BJR, Morand P, (Eds.), Insecticidal of Plant Origin. Am Chem Soc Symp Ser., Washington, DC, 1986, 387.

[35] Saxena, R.C. Insecticides from Neem. In: Insecticides of plant origin. Arnason, J. T., Philogene, B.J.R., Morand, P. (Eds). American Chemical Society, Washington., 1989: 110-135.

[36] Kumar, J., B.S. Parmar. Physicochemical and chemical variation in neem oils and some bioactivity leads against *Spodoptera litura* F. J. Agric. Food Chem.,1996, 44: 2137-2143.

[37] Isman, M.B. Botanical insecticides, deterrent and repellents in modern agriculture and an increasingly regulated world. Ann Rev Entomic, 2006, 51: 45-66.

[38] Schmutterer, H. The neem tree (Azadirachta indica) and other Meliceous plants. In Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other porposes. 1st edition. Mumbai: Neem Foundation, 2002.

[39] Peshin R., Dhawan A.K. Integrated pest management. Innovation-Development Process. Cornell University, Ithaca, United States, 2009.

[40] Rao, B.R., P. Rajasekhar, M. Venkataiah, N.V. Rao, Bio-efficacy of "Neem Azal" (azadirachtin 10,000 ppm) against cotton bollworm, *Helicoverpa armigera* (Hübner). Journal of Entomological Research, 1995, 19(4): 329-333.

[41] Dimetry, Nadia Z., Amin A.H., Bayoumi A.E., Abdel-Raheem M.A., Youssef, Dalia. Comparative toxicity of neem and peppermint oils nano-formulations against *Agrotis ipsilon* (Hufn.) larvae (Lepidoptera: Noctuidae). Journal of Botanical Research, 2019, 1(1): 13-19.
DOI: doi.org/10.30564/jrb.Nlil.590

[42] Amin, Abdel Rahman, H., A.E., Bayoumi, Dimetry, Nadia Z., Youssef, Dalia, A. Efficiency of Nano formulations of Neem and Peppermint Oils on the Bionomics and enzymatic Activities of *Agrotis ipsilon* Larvae (Lepidoptera: Noctuidae). International Journal of Natural Resource Ecology and Management, 2019, 4(5): 102-112.
DOI: 10.11 648/j.ijnrem.20190405.11

[43] Isman, M.B., C.M. Machial. Pesticides based on plant essential oils: from traditional practice to commercialization. In M. Rai and M.C. Carpinella (eds.), Naturally Occurring Bioactive Compounds, Elsevier, BV, 2006: 29-44.

[44] Nwachukwu, N., Igwenyi, I. Influence of extraction methods on the hepatotoxicity of *A. indica* root. J Res Biosci. 2006, 2: 10-23. [Google Scholar]

[45] Deng, Yun-xia, Cao, Mei, Shi, Dong-xia, Yin, Zhong-qiong, Jia, Ren-yong, Xu, Jiao, Wang, Chuan, Lv, Cheng, Liang, Xiao-xia, He, Chang-liang, Yang, Zhi-rong, Zhao, Jian. Toxicological evaluation of neem (Azadirachta indica) oil acute and sub-acute toxicity. Environ. Toxicol. Pharmacol., 2013, 35(2): 240-246.

[46] Ajay Mishra, Nikhil Dave. Neem oil poisoning: Case report of an adult with toxic encephalopathy. Indian J. Crit Care Med., 2013: 17(5): 321-322.
DOI: 10.4103/0972-5229.120330

[47] Raj, A. Toxicological effect of *Azadirachta indica*. As. J. Multidisciplinary Studies, 2014, 2(9): 29-33.

[48] Rafeeq Alam Khan, Maryam Aslam, Shadab Ahmed. Evaluation of Toxicological Profile of a Polyherbal Formulation. Scientific Research, 2016, 7(1).
DOI: 10.4236/pp.2016.71008

[49] Ali, B.H., Salih, A.M.M. Suspected Azadirachta toxicity in sheep (Letter). Veterinary Record, 1982, 111(494).

[50] Ali, B.H., The toxicity of *Azadirachta indica* leaves in goats and guinea pigs". Veterinary human Toxicology, 1987, 29: 16-19.

[51] Thompson, E.B., Anderson, C.C. Cardiovascular effects of Neem extract. Journal Pharmaceutical Sciences, 1978, 67: 1476-1478.

[52] Debnath, N., S.Das, D. Seth, R. Chandra, S. C. Bhattacharya, A. Goswami. Entomotoxic effect of silica nanoparticles against *Sitophilus oryza* (L.). J. Pest. Sci., 2011, 84: 99-105.

[53] Leiderer P., DekorsyT. Interactions of nanoparticles and surfaces tag der mAundlichen Pr Aufung. 25. April 2008, URL:
http://www.ub.uniknostanz.de/kops/volltexte/5387/;URN:http://nbn-resolving.de/urn:nbn:de:352-opus-53877

[54] Taylor R., Walton DRM. The chemistry of fullerenes. Nature, 1993, 363: 685-693

[55] Podsiadlo, P., Kaushik A.K., Arruda EM, Waas, A.M., Shim BS, Xu J., Nandivada H., Pumplin BG; Lahann J., Ramamoorthy, A., Kotov, N.A. Ultrastrong and stiff layered polymer nanocomposities. Science, 2007, 318(5847): 80 - 83.

[56] Henkes,Y.A.E., Chris Bauer, J., Schaak, R.E. Nano-

crystal conversion chemistry: a unified and materials-general strategy for the template- based synthesis of nano crystalline solids. Journal of Solid State Chemistry, 2008, 181(7): 1509-1523.

[57] Kong, L., Tang, J., Wang, Y., Wang, L., Cong, F. Fluorescent nano Blocks of lanthanide complexes on nanosilicon dioxide and carbon nano tube donors with ligand- antenna integration (ALI) structure. Materials Science and Engineering C, 2009, 29(1): 85-91.

[58] Mehta, R.J., Zhang Y., Karthik C., Singh B., Siegel RW., Borca-Tasciuc T., Ramanath, G. A new class of doped nanobulk high-fiogure-of-merit thermorelectrics by scalable bottom -up" assembly Nat Mater. 2012, 10, 11(3): 233 -240.
DOI: 10.1038/nmat3213

[59] Kamaraj, C., P. R. Gandhi, G. Elango, S. Karthi, M. Chunge, G. Rajakumar. Novel and environmental friendly approach; Impact of Neem (*Azadirachta indica*) gum nano formulation (NGNF) on *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.). International Journal of Biological Macromolecules, 2018, 107: 59-69.

[60] Dimetry, Nadia Z., A.H. Amin, A. E. Bayoumi, E. M. Hoballah, Dalia A. Youssef. Neem Nano Formulations as a Green Revolution in the Future For Controlling The Cotton Leaworm (*Spodoptera oflittoralis*) (BOISD.). Int. Conf. on Agriculture, Forestry and Life Sciences, 6 - 8, 2018: 257-272.

[61] Kalaitzaki, A., N.E. Papanikolaou, F. Karamaouna, V. Dourtoglou, A. Xenakis, V. Papadimitriou. Biocompatible colloidal dispersions as potential formulations of natural pyrethrins: a structural and efficacy study. Langmuir, 2015, 31(21): 5722-5730.

[62] Papanikolaou, A.D., I. Kuhn, M. Frenzel, O. Sech-weiger. Semi-natural habitats mitigate the effects of temperature rise on wild bees. J. Appl. Ecol., 2017, 54: 527-536.

[63] Buzea, C., I.I. Pacheco, K. Robbie. Nanomaterials and nanoparticles: sources and toxicity. Biointerphase, 2 (4): 17 - 71.

[64] Dimetry, Nadia Z., Hussein, Hany M. Role of nanotechnology in agriculture with special reference to pest control. Int. J. Pharm Tech Research CODEN (USA): IJPRIF, 2016, 9(10): 121-144.
ISSN (Online): 2455-9563

[65] Bayuomi, A.E. Nanoparticles: their risks and Hazards. King Saud University for Scientific Publication and Printing, Riadh, Kingdom of Saudi Arabia: 491. (In Arabic)

[66] Mossa, Abdel-Tawab, Halim, Nilly Ahmed Hassan Abdelfattah, Samia Mostafa Mohamed Mohafrash. Nanoemulsion of Camphor (*Eucalyptus globulus*) Essential Oil, Formulation, Characterization and Insecticidal Activity against Wheat Weevil, *Sitophilus granaries*. Asian Journal of Crop Science, 2017, 9: 50-62.
DOI: 10.3923/ajcs.2017.50.62

[67] Yamamoto, I., J.E. Casida. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor. Springer, Tokyo, 1999: 300.
ISBN: 978-4-431-70213-9

[68] Yang, F.L., X.G. Li, F. Zhu, C.L. Lei. Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Agric. Food Chem., 2009, 57: 10156-10162. CrossRef | Direct Link.

[69] Yang, Y.C., H.C. Choi, W.S. Choi, J.M. Clark, Y.J. Ahn. Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). J. Agric. Food Chem., 2004, 52: 2507-2511.

[70] Anonymous. Acute toxicity safety emporium. Archived from the original Material Safety Data Sheet (MSDS), 2006.

[71] Anonymous. Board on science and technology for international development, National Research Council Report of ad hok panel on neem, a tree for solving global problem. National Academy Press, Washington D.C. 1992, 60-113.

[72] Pillai, N.R., G. Santhakumari. Toxicity studies on nimbidin, a potential antiulcer drug. Planta Medica, 1984, 50: 146-148.

[73] Niemann, L., Hilbig. Neem seeds extracts as an example for health evaluation of naturally occurring substances to be applied in plant protection. Gesund Pflanzen, 2000, 52(5): 135 -141.

[74] Raizada, R.B., M.K. Srivastava, R.A. Kaushal, R.P. Singh. Azadirachtin, a neem biopesticide: Subchronic toxicity assesment in rats. Food and chemical toxicology, 2001, 39: 477-483.

[75] Dorababu, M., M.C. Joshi, G. Bhawani, M. M. Kumar;, A. Chaturvedi, R.K. Goel. Effect of aqueous extract of neem (*azadirachta indica*) leaves on offensive and diffensive gastric mucosal factors in rats. Indian J. Physiol. Pharmacol., 2006, 50(3): 241-249

[76] Wang, B., W.Y. Feng, M. Wang, T.C. Wang, Y.Q. Gu, M.T. Zhu. Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice". J Nanopart Res, 2008, 10: 263- 276.

[77] Kong, T., S. Zhang, J. Zhang, Z. Hao1, F. Yang, C. Zhang, Z. Yang, M. Zhang, J. Wang. Acute and cumulative effects of unmodified 50-nm nano-ZnO on mice. Springer nature, 2018, 180: 124-134.

DOI: https://doi.org/10.30564/jrb.v2i3.2032

[78] Yousef, Dalia A.; Bayoumi, A.E.; Dimetry, N.Z.; Amin,A.H. and Hoballah,E.M, "Evaluating Effect of Pepper Mint Oil (*Mentha pipreta*) and its Nano-Formulations on some Enzym atic Activities and Bionomics of Cotton Leaf Worm *Spodoptera littoralis* (Boisd.)" Arab Univ., J. Agric Sci., Ain Shams Univ., Cairo, Special Issue, 2018, 26 (2 C), 1977- 1991.

[79] Ray, A., B.D. Banerjee, P. Sen. Modulation of humoral and cell-mediated immune responses by *Azadirachta indica* (neem) in mice. Indian J. Exp. Biol., 1996, 34: 698-701.

[80] Panda, S., A. Kar. How safe is neem extract with respect to thyroid function in male mice. Pharmacol. Res., 2000, 41(4): 419- 422.

[81] Khosla, P., Bhanwra, J. Singh, S. Seth and R.K. Srivastava. A study of hypoglycamic effects of *Azadirachta indica* (Neem) in normal and alloxan diabetic rabbits. Indian J. Physiol. Pharmacol., 2000, 44: 69-74.

[82] Abdel Megeed, M.I., U.M. Radwan, A.Z. Hindy, A. El Zarook. Liver functions under stress of certain common pesticides residue used on fruits and vegetables orally administrated. Annals of Agricultural Science Cairo, 2001, 46: 383-404.

[83] Dehghan, M. H., T. Martin, R. Dehghanan. Antifertility effect of Iranian neem seed alcoholic extract on epididymal sperm of mice. Iranian Journal of Reproductive Medicine, 2005, 3(2): 83-89.

[84] Haque, E, I. Mandal, S. Pal, R. Baral. Prophylactic Dose of Neem (*Azadirachta indica*) Leaf Preparation Restricting Murine Tumor Growth is Nontoxic, Hematostimulatory and Immunostimulatory. Immunopharmacology and immunotoxocology, 2006, 28(1): 33-50.

[85] Hayes, W.J.. General principles: Dosage and other factors influencing toxicity. In: Toxicity of pesticides (W.J.Hayes, ed.), Wavely press, USA, 1975.

[86] Lewinski, N., V. Colvin, R. Drezek. Cytotoxicity of nanoparticles. Small, 2008, 4(1): 26-49.

[87] Nat Vander, J.M., Sluis Vander, W.G., Desilva, K.T.D., Labadie, R.P. Ethnopharmacolo-gnostical survey of neem. A. Juss. (Meliaceae). J. Ethno pharmacology, 1991, 35: 1-24.

[88] Nety S., K.M. Koley, Neelu Gupta, V. Kumar, M. Chowdhary. Toxicological effect of hydroalcoholic extract of *Azadirachta indica* (Neem) in broiler birds. Indian J. Vet.Pathol., 2017, 41(2): 112-115. DOI: 10.5958/0973-970X.2017.0025.6

[89] Wang, C., M. Cao, D-X Shi, Z-Qyin, R- Jia et al. A 90 day Sub chronic Toxicity Study of Neem oil, *Azadirachta indica* Oil in Mice. Hum. Exp. Toxicol, 2013, 32(9): 904-913. DOI: 10.1177/0960327113475677

[90] Rohit Ramesh, Dabhi MR, Vinod, B. Mor. Nano pesticides as emerging agri-chemical formulations for income maximization. International Journal of Chemical Studies, 2018, 6(5): 2607-2610.

[91] Okpanyi, S.N., Ezeukwv, G.C. Anti-inflammatory, anti-pyretic activities of Neem. Planta Medica, 1981, 41: 34-39.

[92] Gandhi, M., Lal; R., Sankaranarayanan, A., Banerjee, C.K., Sharma, P.L. Acute toxicity Study of the oil from Neem Seed (oil). J.Ethnopharmacology, 1988, 23: 39-51.

[93] Singh, P.P., Junnarkar, A.Y., Reddi, G.S., Singh, K.V. Neem: neuropsycho pharmacological and anti-microbial studies. Fitoterapia, 1987, 58: 235-238.

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ARTICLE

A Primary Study for Checking the Occurrence of Plant Parasitic Nematodes with the Crop Banana at Agricultural Areas of Palakkad Taluk, India

Ashfak Ahammed O.[1*] Usman A.[2] Rasmi A.R.

1. PG & Research Department of Botany, Government Victoria College (Affiliated to University of Calicut), Palakkad, Kerala, India
2. Department of Botany, KAHM Unity Women's College (Affiliated to University of Calicut), Manjeri, Kerala, India

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ABSTRACT

The reviews on production of banana during the recent years were not a satisfying one with respect to fourth position of Kerala in area of cultivation in India. Among so many factors for this declination, plant parasitic nematodes also found as a major negative factor. Thus the present study tried to prove this predict and conducted a survey in the unexplored rhizosphere region of an important crop banana (Nendran) in Palakkad taluk of Kerala, India during the post monsoon season of 2017. A total of twenty seven samples each were collected from banana rhizosphere soil and roots and processed for this study. The analysis revealed that the most abundant nematode population was Radopholus spp. and most frequently occurred genus was Meloidogyne spp. in the studied banana fields. The major diversity showing area were Elappully panchayath for rhizosphere soil samples and Kannadi panchayath for root samples. Different plant parasitic nematodes such as Aphelenchus spp., Criconemoides spp., Dory laimoides spp., Helicotylenchus spp., Hoplolaimus spp., Meloidogyne sp p., Pratylenchus spp., Radopholus spp., Rotylenchulus spp., Tylenchoryn chus spp. and Tylenchus spp. were observed in both soil and root samples examined.

1. Introduction

The productivity rate of banana expressed in agricultural statistics reports of 2016 and 2017 was not a satisfying one with respect to the fourth position in area of cultivation in the Indian state Kerala [3,4]. Plant parasitic nematodes are reported to cause a yield loss in banana [21]. The reviews on the plant disease surveys of Kerala showed that there was no much attention given to the crop loss due to plant parasitic nematodes. Consequently a systematic study on species abundance, distribution and pathogenicity of nematodes are lacking. Any disease management studies should also focus on occurrence and diversity of nematodes on a crop area. Knowledge on diversity and occurrence of nematode, as well as the major environmental and agronomical cues for understanding their distribution in specific areas is of vital importance for designing its control measures

**Corresponding Author:*
Ashfak Ahammed O.,
PG & Research Department of Botany, Government Victoria College (Affiliated to University of Calicut), Palakkad, Kerala, India;
Email: ashfubot@gmail.com

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[2]. To make more practical management, such baseline studies provide information to make suitable strategies. Along with identification of plant nematodes of a region its diversity analysis is important to assess the pathogenic potential of that region and became an important criterion for identification of hot spots of nematode attack [20]. Nematodes affect crops through feeding plant roots and it also leads to infestation of secondary pathogens such as fungi and bacteria [13].

The major parasitic nematode species of banana such as *Radopholus similis, Helicotylenchus* spp., *Pratylenchus coffeae* and *Meloidogyne* spp. are to be controlled not only because of the damage they cause but also due to their pronounced variability and interactions with different banana cultivars [1,10]. In the present study diversity and occurrence of plant parasitic nematodes were assessed by a survey. The sampled region of the present study is a valuable benchmark area where passes the Palghat gap in Nilgiri Biosphere Reserve in Western Ghats. Diversity studies on fauna suggested influence of this gap for diversification of various taxa [19].

2. Materials and Methods

2.1 Survey and Sample Collection

The study area Palakkad taluk of India lies at geographic co-ordinates between 10°53′37″ N; 76°36′52″ E in Northern end, 10°50′39″ N; 76°49′37″ E in Eastern end, 10°43′43″ N; 76°40′22″ E in Southern end and 10°44′27″ N; 76°33′09″ E in Western end. The identification and characterization of nematode fauna was done by following methods. Samples were collected from rhizosphere region of banana (*Musa* AAB "Nendran") by an intensive survey from different banana fields in Palakkad taluk during August to December of 2017. The collection sites designated as per local self government bodies namely "panchayath"/Municipality. They were included Akathethara panchayath, Elappully panchayath, Kannadi panchayath, Kodumba panchayath, Malampuzha panchayath, Marutha road panchayath, Mundur panchayath, Pudupariyaram panchayath and Pudussery panchayath. A total number of twenty seven samples with three samples each from a panchayath/Municipality were collected from both rhizosphere soil and root samples. At each place a random of rhizosphere soil and root samples were collected from banana plants at 25-30 cm away from the bole of the plant and to a depth of 25-30 cm. Samples were collected in polythene bags, properly labeled and stored at room temperature until it was processed for nematode extraction in Laboratory.

2.2 Extraction of Nematodes from Soil Samples

The collected soil samples were processed for nematode assay by Cobb's decanting and sieving followed by the modified Baermann [6] funnel technique [16]. All collected samples were taken in uniform quantity of 250 g. Then it was transferred to a plastic container and mixed well with tap water. After settlement of large soil particles it was poured into meshes having different mesh size arranged one above the other. The nematodes trapped in the lower most mesh (BSS 400) were gently decanted into a plastic beaker by adding clear water. Then it was poured onto a tissue paper over layered on wire gauge mesh which was placed in a plastic petridish with clear water. This set up was maintained for 12 hours to collect nematodes.

2.3 Extraction of Nematodes from Root Samples

The infected root bits were taken from semi hard portion of the main roots. Roots were washed thoroughly to remove adhered soil particles and then cut into 4 cm sized pieces. These pieces having 10g (fresh weight) were taken from each sample and macerated gently using kitchen mixer grinder (Panasonic, Japan). Then it was poured onto a tissue paper over layered on wire gauge mesh which was placed in a plastic petridish with clear water. This set up was maintained for 12 hour to come down nematodes towards clear water stores in plastic petri dish.

2.4 Identification and Analysis of Samples for Nematodes

Nematodes collected from soil samples were killed and fixed by using 4% hot formaldehyde solution. Nematode population is estimated by using a stereomicroscope (Magnus MSZ-TR) and images were taken by using camera attached Compound microscope (Olympus CX2l*i*). The nematodes present in the suspension were identified up to generic level based on morphology using nematode identification key of Tarjan *et al.* [19] . Occurrences of population of each nematode in each sample were recorded. To check the nematode diversity, nematode density and nematode population abundance measures such as Absolute Density (AD), Absolute Frequency (AF) and Prominence value (PV) were calculated by using the formula proposed by Norton [12] in which:

$$\text{Absolute frequency} = \frac{\text{Number of samples containing nematodes}}{\text{Number of samples collected}} \times 100$$

$$\text{Absolute density} = \frac{\text{Number of nematodes in all samples}}{\text{Number of sample collected}} \times 100$$

$$\text{Prominence value} = \text{Absolute density} \times \sqrt{\text{Absolute frequency}}$$

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$$\text{Occurrence (\%) of a genus at a study area} = \frac{\text{Total number of a genus}}{\text{Total number of nematodes at a study area}} \times 100$$

3. Results

3.1 Analysis of Soil and Root Samples

From twenty seven soil and root samples collected from banana fields of Palakkad taluk twelve plant parasitic nematodes were obtained. One genus was seeming to be new in morphological features. Even though plant parasitic nematodes were found in all banana fields, they were found below Economic Threshold Level (ETL) only. ie., the maximum nematodes observed from a collection cite was 216 only from 250g soil at Elappully panchayath. The different types of plant parasitic nematodes observed at this study area were *Aphelenchus* spp., *Criconemoides* spp., *Dorylaimoides* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus* spp., *Rotylenchulus* spp., *Tylenchorynchus* spp. and *Tylenchus* spp..

3.2 Diversity Analysis between Panchayaths

The nematode diversity studies revealed that Elappully panchayath and Kannadi panchayath had maximum diversity for studied soil samples and Kannadi panchayath showed maximum diversity for studied root samples. The maximum number of genus observed per panchayath was seven for soil samples and six for root samples. The root analysis for plant parasitic nematodes showed that Kodumba panchayath and Pudupariyaram panchayath were represented by two genera only. *Meloidogyne* spp. occurred in both panchayaths and other genus was *Tylenchus* spp. for Kodumba panchayath and *Hoplolaimus* spp. at Pudupariyaram panchayath. None of the genera was found in cent percentage in both soil and root samples collected from the study area. All twelve nematodes spp. were not observed in a single panchayath under study.

On analysing the soil samples, major contribution of 21.16% nematodes were found in Elappully panchayath followed by Kannadi panchayath, Mundur panchayath, Marutha road panchayath, Malampuzha panchayath, Pudupariyaram panchayath, Pudusery panchayath and Kodumba panchayath with a shares of 16.55%, 14.0%, 13.32%, 13.12%, 9.01%, 7.54% and 3.43% respectively and Akathethara panchayath with the least share of 1.86%. Kannadi panchayath (32.31%) showed a remarkable higher variables for percentage of occurrence of nematodes on analysis of root samples and least was observed at Marutha road panchayath (1.46%) and others are observed in the order of Malampuzha panchayath (19.94%), Elappully panchayath (16.74%), Pudussery panchayath (10.19%), Mundur panchayath (9.33%), Akathethara panchayath (4.66%), Pudupariyaram panchayath (3.2%) and Kodumba panchayath (2.18%). For understanding the distribution, patterns of nematodes population and population abundance of each nematode in each panchayath were clearly given in the Tables 1, 2 & 3 for both soil and root samples.

Table 1. Nematode distribution in rhizosphere soil and root of banana "Nendran" (AAB) samples at Attappady hill area

Panchayaths	*media*	*Aph*	*Cri*	*Dor*	*Hel*	*Hop*	*Mel*	*Pra*	*Rad*	*Rot*	*Tyl*	*Tyr*	*Unk*
Akathethara	Soil	-	-	++	-	+++	+	-	-	-	++	-	-
	Root	-	-	-	-	+++	+	+++	-	-	-	-	-
Elapully	Soil	-	-	+	-	+++	+++	+++	+++	+++	+++	-	-
	Root	-	++	-	-	+++	++	+++	+++	-	-	-	-
Kannadi	Soil	-	-	+	+++	+++	++	-	+++	+++	+++	-	-
	Root	-	-	++	+++	++	++	-	+++	+++	-	-	-
Kodumba	Soil	-	-	-	+++	-	+	-	+++	-	-	-	-
	Root	-	-	-	-	-	++	-	-	-	+++	-	-
Malampuzha	Soil	-	-	++	+++	-	+++	-	-	+++	-	+++	-
	Root	-	-	++	+++	-	+++	-	-	-	-	+++	-
Marutha road	Soil	+++	-	+	++	-	+++	-	+++	+++	-	-	-
	Root	-	-	+	-	-	+	-	-	-	+++	-	-
Mundur	Soil	-	-	++	+++	-	+++	-	+++	+++	-	-	-
	Root	-	-	++	-	+++	+++	-	+++	++ -	-	-	-
Pudupariyaram	Soil	-	-	+	-	-	+++	-	+++	-	-	-	-
	Root	-	-	-	-	+++	++	-	-	-	-	-	-
Pudussery	Soil	-	++	-	+++	+++	+++	-	+++	-	-	-	+
	Root	+	-	-	+++	-	+++	-	+++	-	-	-	-

Notes:
Aph - Aphelencus spp.; Cri - Criconemoid Spp.; Dor - Dorylaimoides spp.; Hel - Helicotylenchus spp.; Hop -Hoplolaimus spp.; Mel - Meloidogyne spp.; Pra - Pratylenchus spp.; Rad -Radopholus spp.; Rot - Rotylenchulus spp.; Tyl - Tylenchus spp.; Tyr - Tylenchorynchus spp.; Unk -Unknown spp.

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Table 2. Percentage of occurrence and population density of each plant parasitic nematode species in rhizosphere soil samples from banana *var*. "Nendran" (AAB) at different panchayaths in Palakkad taluk, India

Panchayaths	Total per panchayath	% of occurrence		AF	AD	PV
Akathethara	19	1.86	*Dor*	66.67	166.67	1360.86
			Hop	100	333.33	3333.33
			Mel	33.33	66.67	384.88
			Tyl	66.67	66.67	544.35
Elapully	216	21.16	*Dor*	33.33	66.67	384.88
			Hop	100	533.33	5333.33
			Mel	100	3133.33	31333.3
			Pra	100	966.67	9666.67
			Rad	100	1766.67	17666.7
			Rot	100	600	6000
			Tyl	100	133.33	1333.33
Kannadi	169	16.55	*Dor*	33.33	33.333	192.44
			Hel	100	833.33	8333.33
			Hop	100	333.33	3333.33
			Mel	66.67	66.67	544.35
			Rad	100	766.67	7666.67
			Rot	100	2800	28000
			Tyl	100	800	8000
Kodumba	35	3.43	*Hel*	100	366.67	3666.67
			Mel	33.33	66.67	384.88
			Rad	100	733.33	7333.33
Malampuzha	136	13.32	*Dor*	66.67	166.67	1360.86
			Hel	100	2133.33	21333.3
			Mel	100	166.67	1666.67
			Rot	100	1500	15000
			Tyr	100	566.67	5666.67
Marutha road	134	13.12	*Aph*	100	566.67	5666.67
			Dor	33.33	66.67	384.88
			Hel	66.67	233.33	1905.21
			Mel	100	1200	12000
			Rad	100	1300	13000
			Rot	100	1100	11000
Mundur	143	14	*Dor*	66.67	66.67	544.35
			Hel	100	500	5000
			Mel	100	833.33	8333.33
			Rad	100	3033.3	30333.3
			Rot	100	333.33	3333.33
Pudupariyaram	92	9.01	*Dor*	33.33	133.33	769.76
			Mel	100	733.33	7333.33
			Rad	100	2200	22000
Pudussery	77	7.54	*Cri*	66.67	66.67	544.35
			Hel	100	666.67	6666.67
			Hop	100	433.33	4333.33
			Mel	100	500	5000
			Rad	100	833.33	8333.33
			Unk	33.33	66.67	384.88

Notes:

Aph - Aphelencus spp.; Cri - Criconemoid Spp.; Dor - Dorylaimoides spp.; Hel - Helicotylenchus spp.; Hop -Hoplolaimus spp.; Mel - Meloidogyne spp.; Pra - Pratylenchus spp.; Rad -Radopholus spp.; Rot - Rotylenchulus spp.; Tyl - Tylenchus spp.; Tyr - Tylenchorynchus spp.; Unk -Unknown spp.

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Table 3. Percentage of occurrence and population density of each plant parasitic nematode species in root samples from banana var. "Nendran" (AAB) at different panchayaths in Palakkad taluk, India

Panchayaths	Total per panchayath	% of occurrence	Nematode genera	AF	AD	PV
Akathethara	32	4.66	*Hop*	100	233.33	2333.33
			Mel	33.33	100	577.32
			Pra	100	733.33	7333.33
Elapully	115	16.74	*Dor*	66.67	133.33	1088.69
			Hop	100	133.33	1333.33
			Mel	66.67	133.33	1088.69
			Pra	100	966.67	9666.67
			Rad	100	2466.67	24666.67
Kannadi	222	32.31	*Dor*	66.67	133.33	1088.69
			Hel	100	1033.33	10333.33
			Hop	66.67	100	816.52
			Mel	66.67	166.67	1360.86
			Rad	100	466.67	4666.67
			Rot	100	5500	55000
Kodumba	15	2.18	*Mel*	66.67	66.67	544.34
			Tyl	100	433.33	4333.33
Malampuzha	137	19.94	*Dor*	66.67	100	816.52
			Hel	100	2500	25000
			Mel	100	1433.33	14333.33
			Tyr	100	533.33	5333.33
Marutha road	10	1.46	*Dor*	33.33	33.33	192.44
			Mel	33.33	66.67	384.88
			Tyl	100	233.33	2333.33
Mundur	64	9.32	*Dor*	66.67	266.67	2177.38
			Hop	100	233.33	2333.33
			Mel	100	633.33	6333.33
			Rad	100	833.33	8333.33
			Rot	66.67	166.67	1360.86
Pudupariyaram	22	3.2	*Hop*	100	600	6000
			Mel	66.67	133.33	1088.69
Pudussery	70	10.19	*Aph*	33.33	66.67	384.88
			Hel	100	300	3000
			Mel	100	866.67	8666.67
			Rad	100	1100	11000

Notes:
Aph - Aphelencus spp.; Dor - Dorylaimoides spp.; Hel - Helicotylenchus spp.; Hop -Hoplolaimus spp.;
Mel - Meloidogyne spp.; Pra - Pratylenchus spp.; Rad -Radopholus spp.; Rot - Rotylenchulus spp.; Tyl - Tylenchus spp.; Tyr - Tylenchorynchus spp.

3.3 Distribution Analysis on Nematode Genera Observed

The analysed data on percentage of occurrence of different nematode genera at Palakkad taluk revealed that the mostly observed genus in soil samples was *Radopholus* spp. with a share of 31.24% and least observed genus *Criconemoides* spp. and unknown nematode were at 0.2%. But in root samples the most observed genus was *Rotylenchulus* spp. with a share of 24.75% and least observed one was *Aphelenchus* spp. at 0.29%. AD (%) of *Radopholus* spp. was notably higher than other genera in rhizosphere soil samples. But the measure of frequency of observation [Absolute Frequency (AF) distribution] was highest for the genus Analysis in root samples also showed

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higher AF distribution (%) for the genera *Meloidogyne* spp. and AD (%) for the genus *Rotylenchulus* spp. The most frequently observed genus *Meloidogyne* spp. had an absolute frequency of 81.48% in rhizosphere soil samples and 66.67% in root samples.

For analysing the population abundance of an organism a summative figure of AD and AF known as prominence value was checked. Even though the value of AF (%) and AD (%) for *Radopholus* spp. were lower than other genera, while considering the prominence value as a measure of population abundance highest value (10419.69) was showed by *Radopholus* spp. in both rhizosphere soil samples and root samples. Among the soil samples least prominence value (14.26) was observed by new variant spp. Dealing with the root samples lowest value (20.16) was seen for *Aphelenchus* spp.. The highest prominence value, showing genus *Radopholus* spp. was not observed in two panchayaths such as Akathethara panchayath and Malampuzha panchayath of this studied area. The survey for plant parasitic nematodes in banana var. Nendran (AAB) showed that five genera were prevalent in Palakkad taluk, Kerala on rhizosphere soil and root. The plant parasitic nematode such as *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Rotylenchulus* spp. and *Radopholus* spp. were those genera with respect to absolute frequency and absolute density. The percentage of occurrence, frequency of distribution and population abundance of different types of nematodes observed in soil and root samples were given in the Tables 4& 5.

Table 4. Percentage of occurrence, frequency of distribution and population abundance of different nematodes in rhizosphere soil of banana var. Nendran (AAB) in Palakkad taluk, India

Nematode genus	Occurrence (%)	Absolute frequency	Absolute density	Prominence value
Aphelenchus spp.	1.67	11.11	62.96	209.88
Criconemoides spp.	00.2	7.41	7.41	20.16
Dorylaimoides spp.	12.06	37.04	77.78	473.34
Helicotylenchus spp.	13.91	62.96	525.93	4173.18
Hoplolaimus spp.	4.8	33.33	181.48	1047.78
Meloidogyne spp.	19.88	81.48	751.85	6786.75
Pratylenchus spp.	2.84	11.11	107.41	358.02
Radopholus spp.	31.24	77.78	1181.48	10419.69
Rotylenchulus spp.	18.61	55.56	703.7	5245.1
Tylenchorynchus spp.	1.67	11.11	62.96	209.88
Tylenchus spp.	2.94	29.63	111.11	604.81
Unknown spp.	0.2	3.7	7.41	14.26

Table 5. Percentage of occurrence, frequency of distribution and population abundance of different nematodes in rhizosphere soil of banana var. Nendran (AAB) in Palakkad taluk, India

Nematode genus	Occurrence (%)	Absolute frequency	Absolute density	Prominence value
Aphelenchus spp.	0.29	3.7	7.41	20.16
Dorylaimoides spp.	2.91	33.33	74.07	427.67
Helicotylenchus spp.	16.74	33.33	425.93	2495.08
Hoplolaimus spp.	5.68	51.85	144.44	1040.12
Meloidogyne spp.	15.72	66.67	400	3265.99
Pratylenchus spp.	7.42	22.22	188.89	890.43
Radopholus spp.	21.25	44.44	540.74	3604.94
Rotylenchulus spp.	24.75	18.51	629.63	2709.49
Tylenchorynchus spp.	2.91	11.11	59.26	197.53
Tylenchus spp.	2.33	22.22	74.07	349.19

The nematodes seem to be new with morphologically distinguishable feature were observed in rhizosphere soil collected from Pudussery panchayath with a share of 0.2%. It had stylet to show plant parasitic mode of nutrition. *Tylenchorynchus* spp. was observed only in Malampuzha panchayath for both rhizosphere soil and root samples. *Criconemoides* spp. found only in soil samples collected from Pudussery panchayath. The nematode genera *Aphelenchus* spp. occurred only at Marutha road panchayath for soil sample analysis and at Pudussery panchayath for root samples. The *Pratylenchus* spp. was observed only in both rhizosphere soil and root samples of Elappully panchayath and root samples collected from Akathethara panchayath. *Dorylaimoides* spp. and *Tylenchus* spp. were observed only in Marutha road panchayath for root samples. On considering the species diversity and more number of nematodes per panchayath the Kannadi panchayath had more diversity showing region in Palakkad taluk for soil samples.

4. Discussion

After a nationwide survey in Palakkad district, a widespread occurrence of *Radopholus similis, Meloidogyne incognita, Helicotylenchus multicinctus, Heterodera oryzicola* and *Pratylenchus coffeae* in banana was reported [8]. In this study, these results were also in accordance with the results except none of the surveyed banana growing areas of Palakkad taluk showed the presence of *Heterodera oryzicola.* Plant parasitic nematodes of banana from vellayani, Kerala documented [15]. It was found that seven phytonematodes were associated with the banana crop, of which *R. similis, H. multicinctus* and *P. coffeae* were recorded as abundant

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nematodes. This result was in confirmation with the findings of present study. *Pratylenchus, Meloidogyne, Helicotylenchus, Tylenchorhynchus, Hoplolaimus, Rotylenchulus, Hirschmanniella, Criconemoides* were observed in West Bengal in banana [7]. In all soil samples analysed, *M. incognita* was found to occur at the highest frequency in banana fields of Malaysia [14]. But the present study contradicted that result. While many of results for nematodes associated with banana showed the widespread presence of *R. similis* and the present study report also was satisfying with that mainstream view [5,17]. The number of *Radopholus* spp. found high in both soil and root samples. But it was very much higher in soil samples than root samples. In India, the first occurrence of *Radopholus* spp. ie, *R. similis* was reported on banana from Palakkad District [11]. Subsequently this nematode was reported from banana in South India [9]. Both *Pratylenchus* spp. and *Radopholus* spp. were co-exist with *Meloidogyne* spp. in both types of samples in the present study. Almost all nematodes showed in Palakkad taluk were reported from the Tanjavur district of Tamilnadu, India also [18].

5. Conclusion

With respect to the soil and root samples, Kannadi panchayath found as more diverse region in Palakkad taluk. The genera which has been seriously affecting on banana plants ie, *Radopholus* spp. was observed as the most abundant one and most frequency of distribution was showed by *Meloidogyne* spp. in this studied area. The plant parasitic nematode such as *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Rotylenchulus* spp. and *Radopholus* spp. showed prominent occurrence in the present study. These results demonstrate the importance of these five nematode genera in banana production as an inverse relationship occurred between the nematodes and growth of banana. The presence five identified population of nematodes in higher density were seems to be hazardous for the better growth of banana. The lost caused by this pathogen should be addressed. So the care should be taken in an economical and eco-friendly manner.

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Importance of the Study

The productivity rate of an important crop banana expressed in agricultural statistics reports in recent years was not a satisfying one with respect to the fourth position in area of cultivation in the Indian state Kerala. To analyse the reasons behind it, one of the important pathogen such as plant parasitic nematodes were taken under consideration. The severity of its attack can be revealed only by an intensive survey. The reviews on the plant disease surveys for Kerala showed that there was no much attention given to the effects of plant parasitic nematodes. There are only less than two works were reported in this agriculturally important state. The study are included in Palakkad district of the state Kerala which ranks first in an important food crop Banana production and cultivation. Consequently species abundance, distribution and pathogenicity in Kerala were unidentified. Knowledge on diversity distribution in specific areas is of vital importance for designing its control measures to make more practical management. This study also important to assess the pathogenic potential of the study area and became an important criterion for identification of hot spots of nematode attack along with identification of plant nematodes of a region. Another importance comes under he point that the life cycle of this pathogen have an effect by the study area. The sampled region of the present study is valuable benchmark area where passes the Palghat gap in Nilgiri Biosphere Reserve in Western Ghats. Diversity studies on fauna suggested influence of this gap for diversification of various taxa.

Conflict Of Interest

This research work was carried out without any research grants or horarium. I do not have any potential conflict of interest (financial or other).

Informed Consent

I hereby confirmed that written consent was obtained from all participants prior to the study.

Ethical Approval

It is not applicable for this study. I hereby assure that no any animal used or like research work carried out in this study to take ethical approval from the Ethical committee.

Trial Registration

Eg. Name of Trial Registry: Trial Registration Number

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Contributorship

The research work jointly conceived and designed by all three authors (Ashfak Ahammed O., Usman A. and Rasmi A.R). The work carried out and manuscript preparation was done by the first author (Ashfak Ahammed O.). The data analysis work was done by the second author (Usman A.) and preliminary and final proof reading of the manuscript was done by the third author (Rasmi A.R). In each step of the research, strict supervision was handled by both second and third authors.

Declaration

I hereby state that the manuscript manuscript entitled A Primary study at Agricultural areas of Palakkad taluk of India for checking the occurrence of a negative growth factor Plant parasitic Nematodes with the crop Banana *var.* "Nendran" has not been submitted to any other journal for consideration and has not been published or presented previously (partly or in full). I also states that no data has been fabricated including images to support conclusions. Proper acknowledgements of works are given.

References

[1] Almeida N.O., Teixeira R.A., Carneiro F.A., Oliveira C.M., Ribeiro V.A., Júnior M.L., Rocha M.R. Occurrence and correlations of nematodes, Fusarium oxysporum and edaphic factors on banana plantations. Journal of Phytopathology, 2018, 166: 265-272. https:// doi.org/10.1111/jph.12683

[2] Archidona-Yuste A., Cantalapiedra-Navarrete C., Liébanas G., Rapoport H.F., Castillo P., Palomares-Rius J.E. Diversity of root knot nematodes of the genus Meloidogyne Göeldi, 1892 (Nematoda: Meloidogynidae) associated with olive plants and environmental cues regarding their distribution in southern Spain. PLoS ONE, 2018, 13(6): e0198236. https://doi.org/10.1371/journal.pone.0198236

[3] Anonymous. Agricultural statistics 2016-17. Department of Economics and Statistics, Government of Kerala, 2016, 18.

[4] Anonymous. Agricultural statistics 2017-18. Department of Economics and Statistics, Government of Kerala, 2017, 18.

[5] Araya M., De Waele D., Vargas R. Occurrence and population densities of nematode parasites of banana (Musa AAA) roots in Costa Rica. Nematropica, 2002, 32: 21-33.

[6] Baermann G. Eine einfache Methode zur Auffindung von Ankylostomum (nematoden) Larven in Erdproben. Geneesk. Tijdschr. Ned-Indië, 1917, 57: 131-137.

[7] Khan M.R., Hasan M.A. Nematode Diversity in Banana Rhizosphere from West Bengal, India. Journal of Plant Protection Research, 2010, 50(3): 263-268.

[8] Khan M.R., Jain R.K., Sing, Pramanik A. Economically Important Plant Parasitic Nematodes Distribution - ATLAS. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, New Delhi, 2010, 89.

[9] Koshy P.K., Sundararaju P., Sosamma V.K. Occurrence and distribution of Radopholus similis (Cobb) Thorne in South India. Indian Journal of Nematology, 1978, 8: 49-58.

[10] Luquine L., Barbosa D.H.S., Ferreira C., Rocha L., Haddad F., Amorim E. First report of the root knot nematode Meloidogyne enterolobii on bananas in Brazil. Plant Disease, 2018. https://doi.org/10.1094/PDIS-04-18-0602-PDN.

[11] Nair M.R.G.K., Das M.N., Menon M.R. On the occurrence of the burrowing nematode Radopholus similis (Cobb, 1893) Thorne, 1949 on banana in Kerala. Indian Journal of Entomology, 1966, 28: 553-554.

[12] Norton D.C. Ecology of plant parasitic nematodes. Awiley intersciences publication, John Wiley and Sons. New York, U.S.A., 1978, 268.

[13] Powell N.T. Interaction of plant parasitic nematodes with other disease causing agents. In: B.M. Zuckerman, W.F. Mai, R.A. Rohde, (Eds.), Plant parasitic nematodes, Academic Press Inc., London, 1971, 2:119-136.

[14] Rahman S.A.S.A., Mohd zain S.N., Mat B.M.Z., Sidam A.K., Othman R.Y., Mohamed Z. Population Distribution of Plant-parasitic Nematodes of Bananas in Peninsular Malaysia. Sains Malaysiana, 2014, 43(2): 175-183.

[15] Roy K., Roy S., Sarkar S., Rathod A., Pramanik A. Diversity of migratory nematode endoparasites of banana. Journal of Crop and Weed, 2014, 10(2): 375-391.

[16] Southey J.F. Laboratory methods for work with plant and soil nematodes. Ministry of agriculture, Fisheries and Food, HMSO, London, 1986, 202.

[17] Speijer P. R., De Waele D. Screening of Musa germplasm for resistance and tolerance to nematodes. Montpellier: International Network for the Improvement of Banana and Plantain (INIBAP) Technical Guidelines, 1997, 1-1-47.

[18] Srinivasan R., Kulothungan S., Sundararaju P., Govindasamy C. Biodiversity of Plant parasitic nematodes associated with Banana in Thanjavur district of Tamilnadu, International Journal of Plant, Animal and Environmental Sciences, 2011, 1(2): 63-69.

 DOI: https://doi.org/10.30564/jrb.v2i3.2085

[19] Tarjan A.C., Esser R.P., Chang S.L. Interactive Diagnostic Key to Plant Parasitic, Free living and Predaceous Nematodes. Journal of the Water Pollution Control Federation, 1977, 49: 2318-2337.

[20] Vijayakumar S.P., Menezes R.C., Jayarajan A., Shanker K. Glaciations, gradients and geography: multiple drivers of diversification of bush frogs in the Eastern Ghats Escarpment. Proceedings of the Royal Society B: Biological Sciences, 2016, 283: 20161011. http://dx.doi.org/10.1098/rspb.2016.1011

[21] Zalpuri L, Tara J.S., Singh V.K. Plant and Soil nematodes: Diversity and Interactions. Global Journal of Biology, Agriculture and health sciences, 2013, 2(2): 123-124.

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ARTICLE

Biodiverse, Productive, and Socially Just Silvopastures: a Solution for the Brazilian Drylands

Felipe Machado Pinheiro[1*] Patrick Hunt[2]

1. University of Florida (UF) School of Natural Resources and Environment and Tropical Conservation & Development Program, PhD candidate Intedisciplinary Ecology, Gainesville, FL, 32611, US
2. UF Latin American Stuides, Tropical Conservation & Development Program, MA student, Gainesville, FL, 32611, US

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ABSTRACT

Drylands constitute more than 40% of global land and are particularly vulnerable to the impacts of climate change. In many of these drylands, livestock activities are a major form of land-use. In Brazil, the two major dryland biomes, Cerrado and Caatinga, play a key role in the country's livestock activities. While important economically, these activities also contribute to the emission of high amounts of greenhouse gases. One suggested strategy for mitigating the impacts of climate change is the adoption of silvopastoral systems (SPS) which combine trees, pasture, and animals simultaneously on the same unit of land. Farmers in the drylands of Brazil have a long history of practicing SPS. The practice of silvopasture is relevant to both climate change and the economy, but not necessarily to the issues of biodiversity loss and economic inequality. The lack of interdisciplinarity in rural agricultural development projects in the past, such as those related to the "Green Revolution", resulted in the aggravation of economic inequalities and biodiversity loss. The present work, focusing on the Brazilian Drylands, reviews these issues to justify the need for interdisciplinary projects considering multiple variables like soil quality, tree density, biodiversity richness, and farmers' perception.

1. Introduction

The present work intends to justify the need of developing and supporting biodiverse and socially-just silvopastoral systems. Focusing on the Brazilian drylands, scientific information was gathered in order to discuss the role of agriculture in climate change, biodiversity loss and poverty; as well on how an interdisciplinary/agroecological approach is capable of resulting in multiple benefits for a truly sustainable rural development.

2. Climate Change and Agriculture: The Brazilian Role

Climate change is one of the greatest challenges of our time and its adverse impacts undermine the ability of all countries to achieve sustainable development [1]. Climate

**Corresponding Author:*
Felipe Machado Pinheiro,
University of Florida (UF) School of Natural Resources and Environment and Tropical Conservation & Development Program, PhD candidate Intedisciplinary Ecology, Gainesville, FL, 32611, US;
Email: felipinheirorj@gmail.com

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change also exacerbates other challenges that humanity faces such as natural resource depletion, environmental degradation, desertification, drought, freshwater scarcity, and loss of biodiversity. Facing these challenges is relevant considering the expected increase of the world population to 9.1 billion by 2050, pushing up the demand for food production by about 70% [2].

Since the industrial revolution, the main gas associated with climate change is atmospheric carbon dioxide (CO_2), the concentration of which increased by 46% from 1750 to 2019, from 280 ppm to 410 ppm [3]. Agriculture is the economic sector that is most vulnerable to climate change, as well as a major cause of it. Agricultural activities directly account for about 14% of the global greenhouse gases (GHG) emissions, and as the main driver of deforestation and land-use change, are indirectly responsible for another 17% of global emissions [4]. The Intergovernmental Panel for Climate Change (IPCC) states that the economic sector of agriculture, forestry and other land-use (Figure 1) directly emits per year 24% of the total GHG emissions, or 11.76 $GtCO_2$ equivalent (eq) (IPCC 2014).

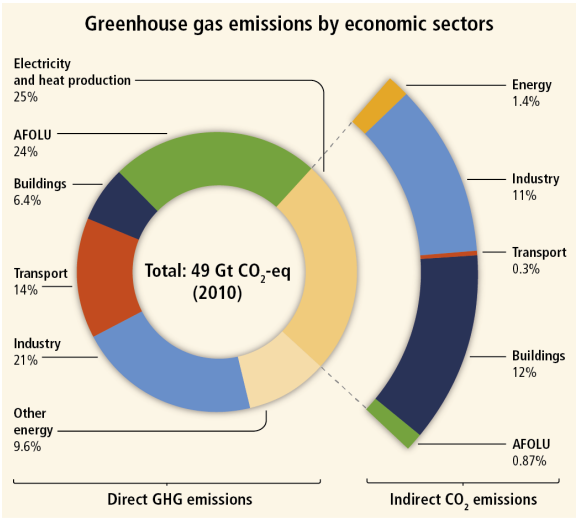

Figure 1. Agriculture, Forestry and Other Land-use (AFOLU) and other economic sectors contributions to total worldwide greenhouse gas emissions in 2010

Source: IPCC (2014).

A key sub-sector of agriculture that plays a significant role in GHG emissions worldwide is livestock supply chains. Beef and dairy cattle contribute 41% and 20%, respectively, of the sector's GHG emissions. For 2005, the sub-sector was estimated to have emitted 7.1 of $GtCO_2$ equivalent, representing 14.5% of all human-induced emissions in the year [5]. In Brazil, agriculture accounts for about 37% of the jobs in the country, and 25% of its gross domestic product (GDP) [6]. Brazil has the second largest herd of livestock on the planet, more than 238 million head of cattle, and is the world's leading beef exporter [7]. The agriculture sector, especially the livestock sub-sector, plays a significant role in GHG emissions.

In Brazil, the total GHG emissions (direct and indirect) from the agriculture sector in 2016 were 1,696 $MtCO_2$ eq, about 70% of the country's emissions. An increase of 165% from 1970 to 2016. Currently, the country accounts for 8.4% of the global agriculture sector GHG emissions, the 3rd greatest emitter [8]. This large contribution of agriculture activities to Brazilian GHG emission, also made the country a top-10 world GHG emitter [8], even though a majority of Brazil's energy comes from renewable sources.

The direct GHG emissions of the agricultural sector in Brazil in 2016 were 499 $MtCO_2$ eq, 1.7 % higher than that of the previous year. Beef and dairy were directly responsible with 69%, and 10%, or 342 $MtCO_2$ eq and 50 $MtCO_2$ eq, respectively. The agriculture sector's indirect GHG emissions were 1,197 $MtCO_2$ eq, and from this total, 1,167 $MtCO_2$ eq were related to land-use change associated with agricultural expansion into native vegetation for crop or cattle ranching [9].

The high emissions in the agriculture sector and livestock sub-sector in Brazil can, however, be potentially mitigated. A large area in the country has ruminant systems operating at low productivity, which have the potential to use management practices that can reduce their emissions while also providing economic benefits [5,9]. To support such practices, the Brazilian government passed a Decree in 2010, with the objectives of reducing the GHG emission of the country by 2020. The National Plan for Low Carbon Emission in Agriculture (ABC Plan), a part of the Decree, lays out some targets and plans, however not much was accomplished after its publication in 2010 [9].

In the ABC plan, the main targets of the mitigation of livestock activities were to recover 15 million ha of degraded pasture, by proper vegetation management and fertilization, potentially sequestering annually 8.3-10.4 $MtCO_2$ eq; and convert open pastures to silvopastoral and other agroforestry systems in 4 million hectares (Mha), potentially sequestering annually 1.8- 2.2 $MtCO_2$ eq [6]. The sequestration rates per ha in the ABC program are in line with the literature on carbon sequestration from the restoration of degraded grassland sites [10].

3. Drylands Vulnerability

Several definitions of drylands exist, the Millennium Ecosystem Assessment [11] describes drylands considering the aridity index classification, including hyper-arid, arid,

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semiarid, and dry subhumid categories. These regions are home to 2 billion people and occupy more than 60 million km^2, 41% of the earth's land area. The UN Environment Programme -World Conservation Monitoring Centre [12] included additional areas as drylands for their relevance on biodiversity conservation, e.g. Cerrado biome and other dry subhumid forests (Figure 2).

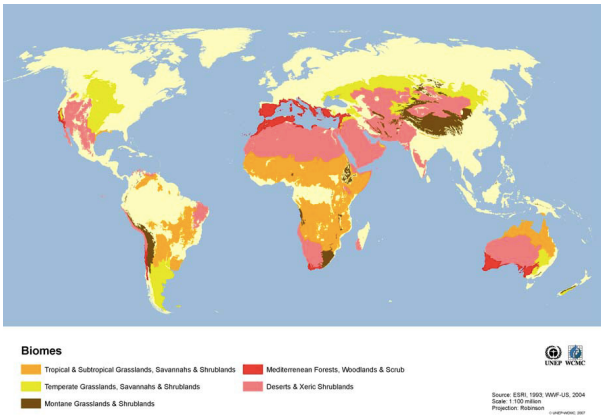

Figure 2. Worldwide drylands delimitation

Source: UNEP-WCMC (2007).

The dryland area affected by desertification is in the range of 6-12 million km^2, reducing their capacity to have enough primary productivity to sustain human livelihoods. The associated socio-environmental crises caused by desertification in these regions are often aggravated by the fact that the local populations are often excluded from policy processes, including lack of political dialogue, and denied appropriate investments from sustainable development projects [11]. In addition to the desertification processes in these regions, drylands are already being impacted by climate change [13] and there is a high probability that the extent of drought-affected areas will increase in coming years. The expected decrease in water resources might affect multiple sectors beyond agriculture, including water supply, energy production and health [4].

As livestock activities help to sustain many communities in drylands, this fact conveys the urgency of the adoption of livestock management practices that are capable of climate change mitigation and adaptation while also increasing productivity. The adoption of these practices becomes even more urgent when considering the expected demand for meat and milk in 2050 compared to 2010 are projected to increase by 73% and 58%, respectively [14]. Considering the drylands fragile nature and inherently low productive capacity, designing and adopting productive resilient land-use systems is a particularly challenge of land management in these regions.

4. Brazilian Drylands and Livestock Activities

The drylands regions in Brazil have a key role in the livestock activities of the country, as well as contributing significantly to GHG emissions. Brazil, the fifth largest country in the world, has approximately 35%, or circa 2.8 million km^2 of drylands [12,15]. These areas are represented mainly by the Cerrado (Brazilian savanna) and the Caatinga (Brazilian semiarid) biomes (Figure 3). These regions are also placed among the most endangered eco-regions on Earth due to high rates of conversion and few protected areas [16].

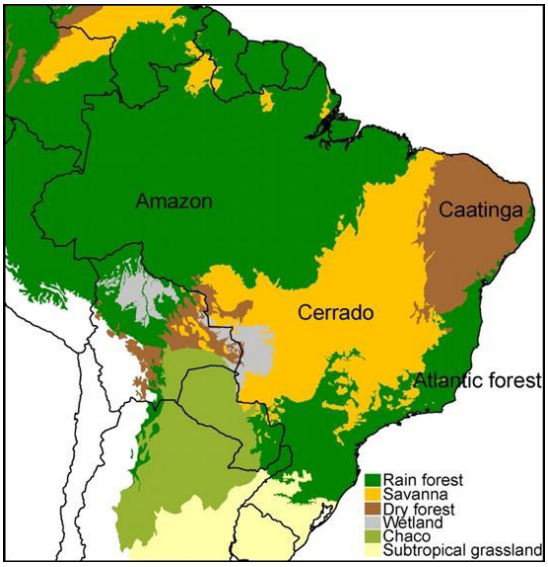

Figure 3. Brazilian biomes

Source: Simon et al. (2009).

4.1 Caatinga Biome

The semiarid Caatinga region is an unique biome of Brazil, located in the Northeast of the country (3° to 17° S, and 35° to 45° W) and occupies 845,000 km^2, about 10% of the country [15]. The annual average rainfall is 750-800 mm, with a rainy season usually lasting 3-5 months . Every three to four decades severe drought periods occur, lasting 3-5 years, with rainfall remaining around 260 mm for several years. High annual average temperatures are another striking feature of the Caatinga, with values between 25 to 29° C [15].

The Caatinga's proximity to three wetter arboreal biomes, the Cerrado savannah, and the tropical forests, Amazon and the Atlantic Forest biomes, contributes to the biome's rich diversity of plants and very unique flora of drought resilient tree species. The Caatinga has 4,320 species of angiosperms, 744 of which were

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described as endemic [17]. In addition, 620 belong to the Fabaceae family (Leguminous), which contains many nitrogen fixing species, which have special relevance for agricultural systems.

Currently, 45% of the Caatinga has been deforested, from 1985 to 2017 the Caatinga lost a 5 Mha of forest area. Although several experiences exist in the biome integrating animal activities with forest conservation, the predominance of livestock activities using, e.g., slash-and-burn with a fallow period shorter than 50 years, overgrazing, and intense firewood gathering, are the main reasons for deforestation, and desertification processes in many regions in the biome [18,19].

Historically and currently, livestock-related activities were/are the primary occupation of the Caatinga inhabitants, in a drought year, the agricultural production in a major state of the biome declines 84%, while livestock activity drops 20% [20]; which reinforces the value of livestock farming for the local communities. The recognition of the negative impact of some management practices and the recommendations on tree growing in the Caatinga's pastures for improving livestock productivity date back to the 1860s [21]. Today, a substantial body of knowledge on SPS, is available in the form of numerous reports and books describing these practices [20,22-24].

4.2 Cerrado Biome

The Cerrado biome is the largest woodland-savanna in South America and second largest vegetation formation after the Amazon [25]. Stretching over most of east-central Brazil, Cerrado is slightly bigger than Mexico, covering approximately 205 Mha, about 24% of Brazil's land area [26]. The climate in the region is characterized by two well-defined seasons: dry winters and rainy summers, the precipitation ranges from 800 to 1,800 mm [27].

Cerrado is one of the 34 worldwide *Biodiversity Hotspots* [28] due the its high level of species richness and endemism, and the rapid loss of habitat due the conversion of native vegetation to pasture and cropland. The Cerrado has the highest plant diversity among tropical savannas with ca. 11,384 species of flowering plants (Angiosperms), where 29,7% or 4,151 are endemic to the biome. In addition, 1,158 species in the biome are Fabaceae [17], suggesting great potential for exploiting native nitrogen fixing trees.

The Cerrado region is the main cattle production area of Brazil, with an estimated herd of 75 million animals, or 44% of the Brazil's herd [29]. About 28% of the biome, 60 Mha, is occupied by pastures[30]. Traditionally, beef cattle production is a major source of income for many farmers in the Cerrado region [31,32]. It is estimated that 39% of the Cerrado pastures have some level of degradation, representing about 18 million ha [33].

The land-use change of the Cerrado biome has been significant in past decades, with great areas of forest changing to farming. The biome lost 20.8 Mha of natural forest formation from 1985 to 2017 [34]. This high land-use change of native arboreal vegetations to farmland, is considered a threat to the Cerrado biodiversity. According to the Brazilian Greenhouse Gases Inventory [35], the carbon emissions due to deforestation in the Cerrado increased from 0.05 petagrams (Pg) C yr^{-1} (1988 to 1994) to 0.06 Pg C yr^{-1} (2002 to 2008).

In addition to vulnerability to land degradation, the Cerrado economy and farmer livelihoods might be greatly impacted by climate change [27]. The estimated changes of temperature in the biome for the year 2100 is 4-6 °C in the most severe prediction, and 2-4 °C considering the least severe prediction (IPCC SRES A2 and B2 emissions scenarios)[36]. For precipitation, the most severe scenario predicts a decrease of 20-50% of current levels in the central and southern parts of the Cerrado, and reductions of about 70% in the northeastern part, which is near the Caatinga region; the projections with less severe changes indicate a reduction of 30% in the central and southern parts of the Cerrado, and a reduction of 50% in the northeastern area. These predictions show how great the reduction of water availability could be and call for urgent actions to adopt agricultural alternatives with proven mitigation and adaptation potential to climate change.

5. The Silvopasture Alternative

Solutions are needed and some are already being applied in dryland regions. In Brazil, estimations show how it might be feasible for the country to achieve sustainability and productivity. An increase in the grasslands productivity from ~32% to 50% would satisfy the demand for meat and milk until ~2040, without the need for native forest conversion/deforestation to cultivated grasslands [37]. Adding to the feasibility to achieve better productivity, the growth of trees could also help to achieve mitigation. Tree planting in agricultural lands is indicated as a relatively efficient and cost-effective method compared with other mitigation strategies and provides a range of co-benefits important for improved farm family livelihoods and climate change adaptation[38].

Agroforestry systems (AFS) involve the intentional integration of trees and shrubs into crop and animal farming systems to create environmental, economic, and social benefits and has been practiced around the world for centuries [39]. Trees and shrubs can enrich biodiversity in the landscape, increase ecosystem stability as well

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as diminish the effects of extreme weather events, such as heavy rains, droughts and windstorms. Agroforestry systems can also contribute to preventing erosion, stabilizing soils, raising infiltration rates, and halting land degradation [38].

Considering the need for strategies to fight climate change, AFS has been recognized as having the greatest potential for carbon (C) sequestration of all the land-uses analyzed in the Land-Use, Land-Use Change and Forestry report of the IPCC [40]. Since the Kyoto Protocol, AFS has gained increased attention as a strategy to sequester C for its potential to do it by at least two ways: 1) increasing direct C sequestration through the addition of C into the tree and shrub components and stored as wood, and, 2) increasing C storage in the soil organic carbon (SOC), which has also the added advantage related to soil fertility improvement and moisture retention content [41].

Several factors contribute to the ability of AFS to sequester C more efficiently than monocultures, including the efficient C (and nutrient) cycling within the soil-plant system, increased return of biomass (C) to soil, decreased biomass decomposition and sequestration of soil C in deeper layers of soil [42-47]. In addition, AFS provides a more shaded environment which contributes to the increase of animals' comfort (and productivity) due to microclimate amelioration [48,49], which will be an important adaptation strategy in a climate change scenario of higher temperatures.

The available estimation of C stored annually in AFS in the aboveground biomass varies from 0.29 to 15.21 Mg C ha year^{-1}; the below ground C stock varies from 1.25 to more than 300 Mg ha^{-1} up to 1 m depth [50]. As an example of the potential mitigation of AFS for livestock activities, in Southern Brazil a study estimated that the tree component (*Pinus ellioti*) could mitigate the GHG emitted by at least 3.58 cows ha^{-1} [51]. In addition, Resende et al. (2019) describe a 100 ha of SPS being capable of mitigating a herd of 150 cows.

In drylands, SPS is one of main types of AFS, and it is characterized by the integration of trees (either for wood, oils, fruits, etc.) with animals being managed in the same area [53]. Silvopastoral systems are traditional land-use systems in many subhumid [54], semiarid and arid regions [55]. In the drylands, SPS plays an important role in the sustainability of many communities as a more resilient activity in comparison with crop production or tree-less pastures.

Several management practices are related to SPS, which include the use of fodder banks, an assemblage of tree and shrub species that are predominantly fodder species. These trees may also provide several products (e.g. fruits) and services (e.g. soil fertility improvement). The fodder trees can be planted as live fences, wind breaks, woodlots, soil conservation barriers, etc. Usually, the fodder is cut and carried to stall-feed the animals, but in some systems the animals are allowed to graze on the fodder bank in a controlled manner for defined periods of time [23].

As deficiency of nitrogen (N) is common in dryland pastures and can significantly affect the growth of grass and trees, a general solution is the use of chemical fertilizers in pasture lands, however the cost is high and the application would have to be repeated indefinitely. Another resilient alternative and low-cost solution is rhizobium inoculation with nitrogen fixing plants, mainly trees species, which would help fertilize soils over the years and, in addition, these plants could be used to feed livestock and produce timber, honey, etc. Dubeux et al. (2017) reviewed the importance of nitrogen fixing trees/tree legumes for the tropics and argued that tree legumes are an underexploited resource in warm-climate grasslands.

The mitigation potential of SPS for drylands was described in a global meta-analysis study [56], in which SPS, compared to pastures, showed 89% higher SOC stock at the topsoil (0-20 cm) and 27% higher at the 0-100 cm depth. In the semiarid region of Brazil, SPS has been shown as the most efficient land-management system in the Caatinga to minimize losses of carbon [24]. Also in the Caatinga, SPS management, when compared to many other practices commonly applied in the region, such as intensive cropping, slash and burn, firewood collection, and secondary forest in natural stands, was considered one of the systems with higher SOC stock [22]. In addition, studies in the Caatinga described higher SOC stock near trees (*Zyziphus joazeiro, Spondias tuberosa* and *Prosopis juliflora)* than away from trees [24,57].

Silvopastoral systems are also of great relevance for optimization of land-use systems, meeting productive and conservation goals. To meet the demand of 500 Brazilians for grains, meat, and energy, the Cerrado's conventional systems (i.e. monocultures) would required 420 ha, while the SPS with Eucalyptus trees would only require 70 ha [58]. In addition, compared to conventional systems, the SPS decreased the climate change potential by 55%, improved the quality of employment, and decreased the total production costs by 54% [58].

In Brazil, the most common tree species used in SPS are exotic Eucalyptus hybrids. This land-use system is practiced over about 2 million ha, an area that has increased due to governmental incentives [59]. On the other hand, many SPS experiences exist in the Caatinga

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region using native trees feeding animals [55], although many of these examples have not been properly studied. In the Caatinga, new designs are also being developed. For example, some innovative small holder farmers are growing native trees in a cactus (*i.e. Opuntia ficus-indica)* plantation, increasing the plantation's biodiversity and soil resilience; a system maintaining a high yield of > 250 Mg ha^{-1} $year^{-1}$ of green/fresh biomass after 17 years of use [60].

6. Agriculture and Biodiversity loss, Inequality and Poverty

As described in the previous sections, SPS are an important strategy for climate change mitigation, but their proper development and adoption may also help to solve additional relevant issues. Currently, the whole planet is at a high-risk of biodiversity loss [61], and the decline of biodiversity (including biodiversity for food and agriculture) has been a feature of conventional agricultural intensification, leaving agricultural systems impoverished, vulnerable, and dependent on continuous use of external inputs [11]. If the role of biodiversity is considered in SPS intensification, it could improve the sustainability of land-use systems as well supporting the biodiversity recovery.

The danger of agricultural intensification focusing on only one target, i.e. productivity, can be exemplified by the adoption of "Green Revolution" practices in Bolivian communities that replaced traditional SPS [54]. Silvopastoral systems that had developed and adapted over generations, underwent significant changes after external actors encouraged the use of chemical fertilizers and pesticides for expanding cash crops, resulting in soil erosion and decrease in the local well-being [54].

In addition, while production and productivity of the major food crops continue to increase due to agricultural intensification, the number of people who are food insecure and malnourished remains high at nearly 1 billion and reached a record high in 2009 [38]. If social aspects are given more consideration when discussing/developing SPS projects, it could support a reduction on the rural inequality, as the 2030 Agenda for Sustainable Development [1] says that "This will only be possible if wealth is shared and income inequality is addressed" [1]. For example, 59% of the variation in the Cerrado pastures' degradation are explained by poverty and low income. The more degraded a pasture is, the lower the social and economic indicators will be and the capacity of rural populations to invest on the the recovery of degraded pastures will also be lower [33].

The development of SPS that consider social aspects is of key importance to Brazil, a country ranking second in inequality and worst when measuring the share of national wealth held by the poorest 10% of the population, with most living in the countryside [62]. To discuss the rural inequality in the country it is important to notice that large rural establishments (>1,000 ha) receive 43% of funds (subsidized low-interest credit) and contribute 24% of production value, while small farms (<50 ha) with 23% of funds, produce 41% of agricultural value (Figure 4).

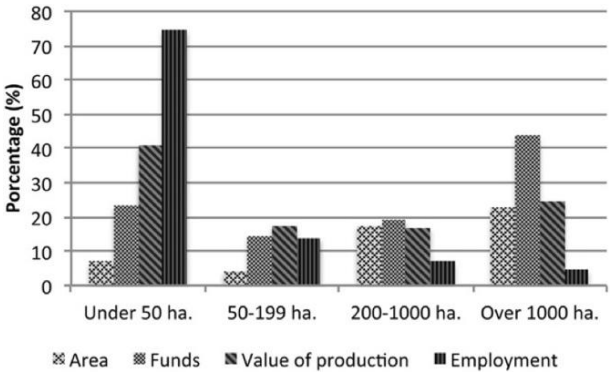

Figure 4. Brazilian agricultural indicators according to establishment size (ha), defined as a percentage of all declared and undeclared arable land [83]

Source: Paulino (2014).

Small-scale agriculture produces nearly double the amount generated by establishments with more than 1,000 ha, even though these large establishments control more than three times the area [63]. In addition, small farmers are responsible for ~70% of Brazil's food consumed by the Brazilian population [64], and have outstanding success with job creation, 74% of the countryside's economically active population[63]. In this way, better financial support and enabling land policies targeting on these successful small farmers, if considering the adoption of SPS, and other AFS, have the potential to both reduce the rural inequality and increase the use of resilient biodiverse rich systems.

Based on the fact that not all small farmers are using sustainable management practices, it is relevant to develop extension projects and funds for the adoption of SPS and other AFS. In the Caatinga region, the lack of interest to adopt sustainable practices was associated with the poor education level of farmers [65], developing accessible learning resources for these is also of great relevance. In addition, in the same Caatinga region 90% of the farmers are male, and as poverty alleviation and rural business development programs were successful when women were involved in the programs [66], gender dynamics should be considered for any future projects.

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7. Considering the Interdisciplinary Agroecological Approach

Agroecology is described as both a science and a set of practices [67], which includes AFS. As a science, agroecology includes ecology, natural, environmental, social and agricultural sciences (e.g. Figure 5). Based on the beneficial biological interactions and synergies among the components of the agroecosystem, agroecological systems can allow the regeneration of soil fertility, enhancement of soil organic matter and soil biological activity, and maintaining food productivity [67,68].

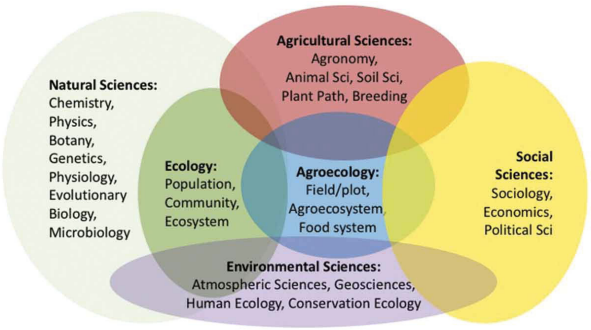

Figure 5. The interdisciplinary science of agroecology

Source: Picasso (2018).

The integration of traditional knowledge and modern technologies has been widely recognized for the development of sustainable land-use systems [69]. The importance of giving value to the farmers' knowledge, perceptions and management practices, is highlighted in agroecology, due to a highly knowledge-intensive system, based on techniques that are not delivered top-down, but developed on the basis of farmers' knowledge and experimentation [70]. In this way, it is described to be important for scientists to recognize farmers' experiences and emphasizes the capability of local communities to experiment, evaluate, and scale-up innovations through farmer-to farmer research and grassroots extension approaches [67,70,71]. Even moreso considering that the most relevant relation between climate change and peasant agriculture is that many small farmers are already mitigating and adapting to climate change by using biodiversity rich systems [72]. This includes farmers that are adopting a variety of sustainable practices, including SPS.

One example of the potential success of such approach in research projects is the recent documentation of an innovative SPS designed by a Caatinga small farmer, who planted cactus with Caatinga native fodder trees [60], both used to feed his animals. In relation to rural extension projects, Cuba probably has the greatest example of how participatory approaches can increase the adoption of sustainable practices by farmers, where, in 10 years of extension projects, the number of families adopting agroecology practices went from 200 to 110,000 families [71].

The Brazilian drylands due to their great plant biodiversity have great potential to explore and develop biodiverse rich systems for livestock activities, which can

Table 1. Common Fodder trees and shrubs of the Caatinga region and their main characteristics.

Common name Species	Height and Succession stage	Growth habits and Coppicing ability	Fodder availability, Animals' preference	Other possible uses
Aroeira *Myracrodruon urundeuva*	Up to 25m; End of secondary succession, near climax	Moderate growth; No coppicing	Leaves consumed either green or dried	Wood for construction; Medicinal; Bees (nectar and pollen)
Catingueira *Poincianella pyramidalis*	Up to 10m; Secondary succession (intermedium)	Slow growth; No coppicing	Leaves highly preferred when dry (dry season - up to 35% of the animal's diet)	Wood for construction; Bees (nectar and pollen)
Juazeiro *Ziziphus joazeiro*	Up to 10m; End of secondary succession, near climax	Slow growth; Inverted phenology, trees grow new leaves in the dry season; No coppicing	Leaves consumed when green in the dry season; Fruits consumed in wet season	Strategic fodder bank for drought periods; Medicinal; Fruit tree (human and animal consumption); Bees (nectar and pollen)
Jurema-preta *Mimosa tenuiflora*	Up to 8m; Beginning of the secondary succession	Fast growth; Leaves remain green during the dry season (even more if coppiced); Coppicing recommended	Leaves highly preferred when green (wet season); Fruits avidly consumed in the beginning of dry season	Strategic fodder bank for drought periods; Nitrogen fixing; Firewood; Medicinal; Bees (nectar and pollen)
Mororó *Bauhinia cheilantha*	Up to 8m; Pioneer and in secondary succession (intermedium)	Slow growth; Coppicing recommended	Consumed when green (wet season)	Firewood; Bees (nectar and pollen); Nitrogen fixing
Sabiá *Mimosa caesalpiniifolia*	Up to 9m; Secondary succession (intermedium)	Fast growth; Coppicing recommended	Highly preferred when green (wet season)	Firewood; Bees (nectar and pollen); Easy to spread, roots may grow out of branches; Nitrogen fixing

Source: Pinheiro and Nair (2018).

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put the region as a global SPS hot spot and showpiece of the future. The Caatinga biome already has a traditional use of the native vegetation for livestock activities, where many of the native tree species were described as fodder trees (Table 1) and belong to the nitrogen fixing plant group, the Fabaceae family. In addition, there exists a need to intensify the research efforts addressing domestication and utilization of native nitrogen fixing trees in warm-climate grasslands, especially considering the biodiversity rich Brazilian drylands where there are already many native species that could be targets for development. Some studies in Brazil already showed the value of using native nitrogen fixing plants in SPS, which can increase the N accumulation in the system and its pathway cycling, estimated to sustain the forage productivity for several years [49,73]

8. Conclusions

An interdisciplinary approach seems to be necessary for the Brazilian livestock sector and feasible thought the use of biodiverse SPS by the innumerous small/medium holder farmers in the country. In addition, the interdisciplinary approach aligns with several international country agreements.

Brazil as a signatory of the Paris Agreement [74], agreed to reduce its greenhouse gases emissions and as an efforts to limit the temperature increase to 1.5 °C above pre-industrial levels. In 2015, the country pledged to reduce 43% of its 2005 emissions level by 2030 [75]. Since SPS can sequester carbon above and below ground, it has been considered as a main strategy. On the other hand, is important to note that this commitment is currently threatned due to the recent actions (2019-2020) of the central goverment. The current Bolsonaro admistration is receiving the attention of the international academic community for attacking scientists who are reporting the increase in the deforestation rate, for cutting the budget related to science and education, scaling back enforcement of environmental laws, and pushing forward with proposals to shrink the size of protected areas [76-78]; actions that are completely against any intention to in fact reduce its emissions.

When the development of SPS considers vulnerable small farmers, it also aligns with several Sustainable Development Goals of the 2030 Agenda[1] and the associated targets (integrated and indivisible). From the 17 goals, it is mainly connected to these goals:

Goal 1: End poverty in all its forms everywhere. Especially connected to the Target 1.4: highlighting the importance of appropriate new technology for the poor and the vulnerable.

Goal 2. End hunger achieve food security and improved nutrition and promote sustainable agriculture. Especially Target 2.3: expecting to double the agricultural productivity and incomes of small-scale food producers by 2030 through, for example, access to inputs and knowledge.

Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all.

Goal 13. Take urgent action to combat climate change and its impacts.

Goal 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.

The value of the development of SPS using local and native plant species is also reinforced by its connection with international and Brazilian national targets for biological conservation. As a signatory of the United Nations Convention on Biological Diversity [79] and having developed its National Biodiversity Strategies and Action Plan [80] to be accomplished by 2020 (expiring), from the 20 national targets, those related to the use and development of resilient and biodiverse productive land-use systems (i.e. SPS) are:

National Target 1: Brazilian people are aware of the values of biodiversity and the steps they can take to conserve and use it sustainably.

National Target 2: Biodiversity values, geo-diversity values, and sociodiversity values have been integrated into national and local development and poverty reduction and inequality reduction strategies, and are being incorporated into national accounting, as appropriate, and into planning procedures and reporting systems.

National Target 7: The incorporation of sustainable management practices is disseminated and promoted in agriculture, livestock production, aquaculture, silviculture, extractive activities, and forest and fauna management, ensuring conservation of biodiversity.

National Target 15: Ecosystem resilience and the contribution of biodiversity to carbon stocks has been enhanced through conservation and restoration actions, including restoration of at least 15% of degraded ecosystems, prioritizing the most degraded biomes, hydrographic regions and ecoregions, thereby contributing to climate change mitigation and adaptation and to combatting desertification.

National Target 19: The science base and technologies necessary for enhancing knowledge on biodiversity, its values, functioning and trends, and the consequences of its loss, are improved and shared, and the sustainable use

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of biodiversity, as well as the generation of biodiversity-based technology and innovation are supported, duly transferred and applied.

The development of biodiverse and socially just SPS should be considered as a key strategy for the rural development of the Brazilian drylands. Future agricultural projects considering such interdisciplinary approach might contribute to a transition to systems that bring at the same time environmental, economic, and social benefits, resulting in climate change mitigation/adaptation, recovery of biodiversity and sustainable development of neglected rural populations.

References

[1] United Nations. Transforming Our World: The 2030 Agenda for Sustainable Development. 2015.

[2] FAO. How to Feed the World in 2050. 2017.

[3] Dlugokencky, E., Tans, P. NOAA/ESR, 2019 [Online]. Available: www.esrl.noaa.gov/gmd/ccgg/trends/ [Accessed: 25-Nov-2019]

[4] IPCC. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment. IPCC, Geneva, Switzerland, 2007.

[5] Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., Tempio, G. Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities. Food and Agriculture Organization of the United Nations (FAO), 2013.

[6] MAPA. Plano Setorial de Mitigação e de Adaptação Às Mudanças Climáticas Para a Consolidação de Uma Economia de Baixa Emissão de Carbono Na Agricultura: Plano ABC (Agricultura de Baixa Emissão de Carbono), Ministério da Agricultura, Pecuária e Abastecimento, Ministério do Desenvolvimento Agrário, coordenação da Casa Civil da Presidência da República, Brasília, DF, Brazil, 2012.

[7] USDA. Foreign Agricultural Service. Livestock and Poultry: World Markets and Trade. 2019.

[8] WRI. CAIT. WRI's Climate Data Explorer. 2019. [Online]. Available: http://cait.wri.org/ [Accessed: 20-May-2019].

[9] SEEG. Emissões Do Setor de Agropecuária: Período 1970 - 2016, 2018.

[10] Conant, R. T., Paustian, K. Potential Soil Carbon Sequestration in Overgrazed Grassland Ecosystems. Global Biogeochem. Cycles, 2002, 16(4): 90-1-90-9.

[11] MEA. Ecosystems and Human Well-Being. Island Press, Washington, DC, 2005.

[12] UNEP-WCMC. A Spatial Analysis Approach to the Global Delineation of Dryland Areas of Relevance to the CBD Programme of Work on Dry and Subhumid Lands. Cambridge, UK, 2007.

[13] Burney, J., Cesano, D., Russell, J., La Rovere, E. L., Corral, T., Coelho, N. S., Santos, L. Climate Change Adaptation Strategies for Smallholder Farmers in the Brazilian Sertão. Clim. Change, 2014, 126(1-2): 45-59.

[14] FAO. World Livestock 2011 - Livestock in Food Security, Food and Agriculture Organization of the United Nations, Rome, Italy, 2011.

[15] IBGE. Mapa de Biomas e de Vegetação Do Brasil, Rio de Janeiro, RJ, Brazil, 2004.

[16] Beuchle, R., Grecchi, R. C., Shimabukuro, Y. E., Seliger, R., Eva, H. D., Sano, E., Achard, F. Land Cover Changes in the Brazilian Cerrado and Caatinga Biomes from 1990 to 2010 Based on a Systematic Remote Sensing Sampling Approach. Appl. Geogr., 2015, 58: 116-127.

[17] Forzza, R. C., Baumgratz, J. F. A., Bicudo, C. E. M., Canhos, D. A. L., Carvalho Junior, A. A., Costa, A. F., Costa, D. P., Hopkins, M., Leitman, P. M., Lohmann, L. G. Catálogo de Plantas e Fungos Do Brasil. Andrea Jakobsson Estúdio and Jardim Botânico do Rio de Janeiro, Rio de Janeiro, RJ, 2010.

[18] MMA, IBAMA. Monitoramento Do Desmatamento Nos Biomas Brasileiros Por Satélite: Monitoramento Do Bioma Caatinga 2008-2009. Ministério do Meio Ambiente / Instituto Brasileiro do Meio Ambiente, 2011.

[19] Santana, M. O. Atlas Das Áreas Susceptíveis à Desertificação Do Brasil. MMA / SRH / UFPB, Brasília, DF, Brazil, 2007.

[20] Araújo Filho, J. A. Manejo Pastoril Sustentável Da Caatinga. Projeto Dom Helder Camara, Recife, PE, Brazil, 2013.

[21] Braga, R. História Da Comissão Científica de Exploração. Imprensa Universitária do Ceará, Fortaleza, CE, 1962.

[22] Aguiar, M. I. de, Maia, S. M. F., Xavier, F. A. da S., de Sá Mendonça, E., Filho, J. A. A., Oliveira, T. S. Sediment, Nutrient and Water Losses by Water Erosion under Agroforestry Systems in the Semi-Arid Region in Northeastern Brazil. Agrofor. Syst., 2010, 79(3): 277-289.

[23] Cândido, M. J. D., Araújo, G. G. L., Cavalcante, M. A. B. Pastagens No Ecossistema Semi-Árido Brasileiro: Atualização e Perspectivas Futuras. In: Reunião Anual Da Sociedade Brasileira De Zootecnia, Sociedade Brasileira de Zootecnia, Goiânia, GO, 2005, 85-94.

[24] Menezes, R. S. C., Salcedo, I. H. Influence of Tree Species on the Herbaceous Understory and Soil

 DOI: https://doi.org/10.30564/jrb.v2i3.2102

Chemical Characteristics in a Silvopastoral System in Semi-Arid Northeastern Brazil. Rev. Bras. Cienc. Solo, 1999, 23(4): 817-826.

[25] Pacheco, A. R., Chaves, R. de Q., Nicoli, C. M. L. Integration of Crops, Livestock, and Forestry: A System of Production for the Brazilian Cerrados. Eco-efficiency from Vis. to Real., 2013, 51-60.

[26] IBGE. Mapa de Biomas e de Vegetação Do Brasil. Rio de Janeiro, RJ, Brazil, 2004.

[27] Bustamante, M., Nardoto, G., Pinto, A., Resende, J., Takahashi, F., Vieira, L. Potential Impacts of Climate Change on Biogeochemical Functioning of Cerrado Ecosystems. Brazilian J. Biol., 2012, 72(3): 655-671.

[28] Mittermeier, R. A., Gil, P. R., Hoffmann, M., Pilgrim, J., Brooks, T., Mittermeier, C. G., Lamoreux, J.. Fonseca, G. A. B. Da. Hotspots Revisited. CEMEX, México City, 2004.

[29] IBGE. Censo Agropecuário 2017, Rio de Janeiro, RJ, Brazil, 2018.

[30] Parente, L., Ferreira, L., Faria, A., Nogueira, S., Araújo, F., Teixeira, L., Hagen, S. Monitoring the Brazilian Pasturelands: A New Mapping Approach Based on the Landsat 8 Spectral and Temporal Domains. Int. J. Appl. Earth Obs. Geoinf., 2017, 62: 135-143.

[31] Klink, C., Moreira, A. Past and Current Human Occupation, and Land Use. The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna., P. Oliveira, and R. Marquis, eds., Columbia University Press, New York, USA, 2002: 69-88.

[32] Diniz-Filho, J. A. F., Oliveira, G. de, Lobo, F., Ferreira, L. G., Bini, L. M., Rangel, T. F. L. V. B. Agriculture, Habitat Loss and Spatial Patterns of Human Occupation in a Biodiversity Hotspot. Sci. Agric., 2009, 66(6): 764-771.

[33] Pereira, O., Ferreira, L., Pinto, F., Baumgarten, L. Assessing Pasture Degradation in the Brazilian Cerrado Based on the Analysis of MODIS NDVI Time-Series. Remote Sens., 2018, 10(11): 1761.

[34] Project MapBiomas. Collection [Version3.1] of Brazilian Land Cover & Use Map Series. [Online]. Available: http://mapbiomas.org/ [Accessed: 03-Dec-2019].

[35] MMA. Segundo Inventário Brasileiro de Emissões e Remoções Antrópicas de Gases de Efeito Estufa - Emissões de CO2 Pelo Uso Da Terra, Mudança Do Uso Da Terra e Florestas. Brasília, DF, Brazil, 2010.

[36] Marengo, J. A., Jones, R., Alves, L. M., Valverde, M. C. Future Change of Temperature and Precipitation Extremes in South America as Derived from the PRECIS Regional Climate Modeling System. Int. J. Climatol., 2009, 29(15): 2241-2255.

[37] Latawiec, A. E., Strassburg, B. B. N., Valentim, J. F., Ramos, F., Alves-Pinto, H. N. Intensification of Cattle Ranching Production Systems: Socioeconomic and Environmental Synergies and Risks in Brazil. Animal, 2014, 8(8): 1255-1263.

[38] FAO, PAR. Biodiversity for Food and Agriculture: Contributing to Food Security and Sustainability in a Changing World. Rome, Italy, 2011.

[39] Nair, P. K. R., Garrity, D. Agroforestry -The Future of Global Land Use Advances in Agroforestry. 2012.

[40] Watson, R. T., Noble, I. R., Bolin, B., Ravindranath, N. H., Verardo, D. J., Dokken, D. J. IPCC Special Report on Land Use, Land-Use Change, and Forestry. IPCC, Geneva, Switzerland, 2000.

[41] Nair, P. K. R., Kumar, B. M., Nair, V. D. Agroforestry as a Strategy for Carbon Sequestration. J. Plant Nutr. Soil Sci., 2009, 172(1): 10-23.

[42] Apolinário, V. X. O., Dubeux, J. C. B., Lira, M. A., Ferreira, R. L. C., Mello, A. C. L., Coelho, D. L., Muir, J. P., Sampaio, E. V. S. B. Decomposition of Arboreal Legume Fractions in a Silvopastoral System. Crop Sci., 2016, 56(3): 1356-1363.

[43] Montagnini, F., Nair, P. K. R. Carbon Sequestration: An Underexploited Environmental Benefit of Agroforestry Systems. Agrofor. Syst., 2004, 61-62(1-3): 281-295.

[44] Nair, P. K. R. Carbon Sequestration Studies in Agroforestry Systems: A Reality-Check. Agrofor. Syst., 2012, 86(2): 243-253.

[45] Oelbermann, M., Voroney, R. P., Thevathasan, N. V., Gordon, A. M., Kass, D. C. L., Schlönvoigt, A. M. Soil Carbon Dynamics and Residue Stabilization in a Costa Rican and Southern Canadian Alley Cropping System. Agrofor. Syst., 2006, 68(1): 27-36.

[46] Saha, S. K., Nair, P. K. R., Nair, V. D., Kumar, B. M. Carbon Storage in Relation to Soil Size-Fractions under Tropical Tree-Based Land-Use Systems. Plant Soil, 2010, 328(1): 433-446.

[47] Tonucci, R. G., Nair, P. K. R., Nair, V. D., Garcia, R., Bernardino, F. S. Soil Carbon Storage in Silvopasture and Related Land-Use Systems in the Brazilian Cerrado. J. Environ. Qual., 2011, 40(3): 833.

[48] Paciullo, D. S. C., Pires, M. F. A., Aroeira, L. J. M., Morenz, M. J. F., Maurício, R. M., Gomide, C. A. M., Silveira, S. R. Sward Characteristics and Performance of Dairy Cows in Organic Grass-Legume Pastures Shaded by Tropical Trees. 2014, Animal, 8(8): 1264-1271.

[49] Xavier, D. F., da Silva Lédo, F. J., de Campos Paciullo, D. S., Urquiaga, S., Alves, B. J. R., Boddey, R. M. Nitrogen Cycling in a Brachiaria-Based Silvopastoral System in the Atlantic Forest Region of Minas

  DOI: https://doi.org/10.30564/jrb.v2i3.2102

Gerais, Brazil. Nutr. Cycl. Agroecosystems, 2014, 99(1): 45-62.

[50] Nair, P. K. R., Nair, V. D., Mohan Kumar, B., Showalter, J. M. Carbon Sequestration in Agroforestry Systems. Advances in Agronomy, Elsevier, 2010: 237-307.

[51] Oliveira, E. B. De, Ribaski, J., Augusto, É., Ferreira, J., Junior, P. Produção , Carbono e Rentabilidade Econômica de Pinus Elliottii e Eucalyptus Grandis Em Sistemas Silvipastoris No Sul Do Brasil. Pesqui. Florest. Bras. Colombro-PR, 2008, 57(1): 45-56.

[52] Resende, L. O., Müller, M. D., Kohmann, M. M., Pinto, L. F. G., Cullen Junior, L., Zen, S., Rego, L. F. G. Silvopastoral Management of Beef Cattle Production for Neutralizing the Environmental Impact of Enteric Methane Emission. Agrofor. Syst., 0123456789, 2019.

[53] Nair, P. K. R.. Grand Challenges in Agroecology and Land Use Systems. Front. Environ. Sci., 2014, 2: 1-4.

[54] Paulson, S. Masculine and Feminine Conditions and Relations in the (Re)Production of Andean Silvopasture Systems. Earthscan Reader on Gender and Forests, C.J.P. Colfer, E. Marlène, B. Sijapati, Basnett, and S.S. Hummel, eds., Routledge, 2017.

[55] Pinheiro, F. M., Nair, P. K. R. Silvopasture in the Caatinga Biome of Brazil: A Review of Its Ecology, Management, and Development Opportunities. For. Syst., 2018, 27(1): 1-16.

[56] Chatterjee, N., Nair, P. K. R., Chakraborty, S., Nair, V. D. Changes in Soil Carbon Stocks across the Forest-Agroforest-Agriculture/Pasture Continuum in Various Agroecological Regions: A Meta-Analysis. Agric. Ecosyst. Environ., 2018, 266: 55-67.

[57] Correia, M. D., Menezes, R. S. C., Olinda, R. A. Modelagem Geoestatística Da Distribuição de Carbono Do Solo e Biomassa de Herbáceas Em Sistema Silvopastoril Na Região Nordeste Do Brasil. Rev. Bras. Biom., 2014, 31(2): 116-129.

[58] Costa, M. P., Schoeneboom, J. C., Oliveira, S. A., Viñas, R. S., de Medeiros, G. A. A Socio-Eco-Efficiency Analysis of Integrated and Non-Integrated Crop-Livestock-Forestry Systems in the Brazilian Cerrado Based on LCA. J. Clean. Prod., 2018, 171: 1460-1471.

[59] Carlson, F. A. Brazilian Agriculture: Prospects and Challenges. Agron. J., 1925, 17(11): 725-730.

[60] Pinheiro, F. M., Nair, P. K. R., Paulson, S., Nair, V. D., DeVore, J., Tonucci, R. G. An Innovative, Farmer Initiative of Silvopastoral Restoration in a Degraded Semiarid Caatinga Region of Brazil. 4th World Congress on Agroforesry. CIRAD, Montpellier, Fr, 2019, 708.

[61] Steffen, W., Richardson, K., Rockstrom, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., de Vries, W., de Wit, C. A., Folke, C., Gerten, D., Heinke, J., Mace, G. M., Persson, L. M., Ramanathan, V., Reyers, B., Sorlin, S. Planetary Boundaries: Guiding Human Development on a Changing Planet. Science (80-). 2015, 347(6223: 1259855-1259855.

[62] Oxfam. Left behind by the G20. Oxford, UK, 2012.

[63] Paulino, E. T. The Agricultural, Environmental and Socio-Political Repercussions of Brazil's Land Governance System. Land use policy, 2014, 36: 134-144.

[64] MDA. Plano Safra Da Agricultura Familiar 2012/2013. Brasília, DF, Brazil, 2012.

[65] Nunes, B., Bennett, D., Marques, S. Sustainable Agricultural Production: An Investigation in Brazilian Semi-Arid Livestock Farms. J. Clean. Prod., 2014, 64: 414-425.

[66] OECD. Gender Equality: A Key for Poverty Alleviation and Sustainable Development. SDC, 2003.

[67] Altieri, M. A., Toledo, V. M. The Agroecological Revolution in Latin America: Rescuing Nature, Ensuring Food Sovereignty and Empowering Peasants. J. Peasant Stud., 2011, 38(3): 587-612.

[68] Altieri, M. A. Agroecology: The Science of Natural Resource Management for Poor Farmers in Marginal Environments. Agric. Ecosyst. Environ., 2002, 93(1-3): 1-24.

[69] IAASTD. Agriculture at a Crossroads. Island Press, Washington, DC, 2009.

[70] Holt-Giménez, E. Campesino a Campesino: Voices from Latin America's Farmer to Farmer Movement for Sustainable Agriculture. Food First Books, Oakland, CA, 2006.

[71] Rosset, P. M., Sosa, B. M., Jaime, A. M. R., Lozano, D. R. Á. The Campesino-to-Campesino Agroecology Movement of ANAP in Cuba: Social Process Methodology in the Construction of Sustainable Peasant Agriculture and Food Sovereignty. J. Peasant Stud., 2011, 38(1): 161-191.

[72] Altieri, M. A., Koohafkan, P. Enduring Farms: Climate Change, Smallholders and Traditional Farming Communities. Third World Network (TWN) Penang, 2008.

[73] Apolinário, V. X. O., Dubeux, J. C. B., Lira, M. A., Ferreira, R. L. C., Mello, A. C. L., Santos, M. V. F., Sampaio, E. V. S. B., Muir, J. P. Tree Legumes Provide Marketable Wood and Add Nitrogen in Warm-Climate Silvopasture Systems. Agron. J., 2015, 107(5): 1915-1921.

[74] UNFCCC. Adoption of the Paris Agreement. 2016.

[75] Brazil. Intended Nationally Determined Contribution: Towards Achieving the Objective of the United

 DOI: https://doi.org/10.30564/jrb.v2i3.2102

Nations Framework Convention on Climate Change. 2015.

[76] Escobar, H. Brazilian President Attacks Deforestation Data. Science (80-), 2019, 365(6452): 419-419.

[77] Tollefson, J. Tropical Trump'sparks Unprecedented Crisis for Brazilian Science. Nature, 2019, 572: 161-162.

[78] Abessa, D., Famá, A., Buruaem, L. The Systematic Dismantling of Brazilian Environmental Laws Risks Losses on All Fronts. Nat. Ecol. Evol., 2019, 3(4): 510-511.

[79] United Nations. Convention on Biological Diversity, 1992.

[80] MMA. National Biodiversity Strategy and Action Plan, Brasília, DF, Brazil, 2017.

[81] IPCC. Climate Change 2014: Synthesis Report. Geneva, Switzerland, 2014.

[82] Simon, M. F., Grether, R., Queiroz, L. P., Skema, C., Pennington, R. T., Hughes, C. E. Recent Assembly of the Cerrado, a Neotropical Plant Diversity Hotspot, by in Situ Evolution of Adaptations to Fire. Proc. Natl. Acad. Sci., 2009, 106(48): 20359-20364.

[83] IBGE. Censo Agropecuário 2006. Rio de Janeiro, RJ, Brazil, 2009.

[84] Picasso, V. D. The 'Biodiversity-Ecosystem Function Debate': An Interdisciplinary Dialogue between Ecology, Agricultural Science, and Agroecology. Agroecol. Sustain. Food Syst., 2018, 42(3): 264-273.

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ARTICLE

Capacitance Characteristics of *Pinus densata*, *Pinus tabuliformis*, *Pinus yunnanensis and the hybrids Pinus tabuliformis* × *Pinus Yunnanensis*

Fengxiang Ma[1] Kangyi Lou[2] Xiaoyang Chen[3, 4] Yue Li[3*]

1. College of Science, Beijing Forestry University, Beijing, 100083, China
2. Department of Renewable Resources, 442 Earth Sciences Building, University of Alberta, Edmonton, Alberta, T6G 2E3, Canada
3. College of Biological Science and Biotechnology, Beijing Forestry University, Beijing 100083, China
4. College of Forestry, South China Agricultural University, Guangzhou, 510642, China

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ABSTRACT

We employed capacitance to evaluate the kinship and interspecific variation of homoploid hybrid conifer *Pinus densata, P. tabuliformis, P. yunnanensis* and artificial hybrids of *P. tabuliformis* (maternal parent) and *P. yunnanensis* (paternal parent) which were cultivated and selected in the common garden experiment. By measuring capacitance spectra under different voltage frequencies, we could differentiate different germplasms based on the electrical response. We aims to demonstrate that *P. densata* as the hybrid of *P. tabuliformis* and *P. yunnanensis* based on the capacitance values of the species, and to provide new evidence to the previously known biological evidence, as well as and the parental effect on the hybrids. Our results revealed that capacitance values between the species are significantly different in the spectra where *P. yunnanensis* positioned at the lowest and *P. densata* was much higher than all other species, indicating that *P. densata* had possessed a great capacity to store electrical energy. The capacitance spectra of *P. densata* and the artificial hybrid are not similar, which rejected our hypothesis. Both of the capacitance values of *P. densata* and the hybrids were closer to *P. tabuliformis* than to *P. yunnanensis*, which shows that the maternal influence was stronger than the paternal influence. Correlation analysis on the relationship between capacitance and fitness-related characteristics showed that capacitance is negatively correlated to mortality rate, and positively correlated with second-year survival rate. High capacitance values of *P. densata* and some of the hybrids reveal their superior adaptability to harsh environment in the Tibet Plateau. We concluded that capacitance as a new indicator for plant fitness and evolution evidence of homoploid hybrid conifers.

**Corresponding Author:*
Yue Li,
College of Biological Science and Biotechnology, Beijing Forestry University, Beijing 100083, China;
Email: 843193777@qq.com

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1. Introduction

Electrical properties such as resistance, electrical impedance and capacitance are traditionally important tools in medical pathology [1]. Recently, these electrical measurements have been used to study the structure of various organic and inorganic materials [2-4], seedling resistance to temperature [5-6], and water content of plant tissues [7-8]. In identifying kinship between species and seeking evolutionary evidence, molecular biological method is a fundamental one; however, it is usually complicated and time-consuming regarding lab analysis. Developing a convenient and rapid physical method that can be applied in the field to complement biological methods would be helpful to assist scientists study species relationships in the field.

Capacitance is a basic electrical property that describes the charge storage capability of the conductor system. It is defined as the ratio of the total charge on one conductor to voltage [9]. A capacitor can store energy but also consume part of the energy dependent on the applied voltage. Therefore, the capacitor model is a parallel connection of both capacitor and resistor elements. In theory, there is an electric field between conductors of unequal charge potentials, leading to accumulation of charges, electric field energy, and the capacitance effect [10-11]. A plant sample can also be considered as a parallel connection of a capacitor element and a resistor element due to its tissue materials and structures.

After applying alternating current (AC) to a plant tissue, the same amount of opposite charges gather at both ends of the sample, and electric field energy then establishes in the sample. After electricity is removed, if the charge continues to stay in the sample, the sample is considered to be capable of storing charges or electric field energy. Therefore, measuring capacitance and evaluating the electrical storage capacity could potentially reveal the inner properties of plant tissues.

Previous molecular biological studies had indicated that *Pinus densenta* is a hybrid species originated from *P. tabuliformis* and *P. yunnanesis* [12]. In this study, we intended to use capacitance as a complementary method to study such kinship between the three species. We hypothesized that the artificial hybrids of *P. tabuliformis* and *P. yunnanesis* and *P.densata* would have very similar capacitance values. By treating the seedlings of *P. densata, P. yunnanensis, P. tabuliformis*, and artificial hybrids of *P. densata and P. yunnanensis* with AC field treatment, we aimed to detect the differences of their capacitance on a spectral level by applying AC under a range of frequencies and conduct correlation analysis between capacitance and growth adaptability index to understand the genetic variation within the same species and interspecific kinship, providing the first example for using capacitance as an empirical tool in studying plant kinship and parental effects.

2. Materials and Methods

2.1 Materials

The artificial hybrids of *Pinus tabuliformis and P. yunnanensis* were cultivated in a seed orchard in Ningcheng, Heilihe, Inner Mongolia. The maternal parents were six *P. tabuliformis* clone, and pollen of the paternal parent *P. yunnanensis* was collected from five *P. yunnanensis* in Yunnan, Kunming Province. Thirty hybrids were obtained, including 12 hybrids meeting the basic requirements of field trials. The 12 hybrids were marked as No. 401, 402, 403, 404, 405. 406, 407, 408, 409, 410, 411, and 413). To compare variation between the hybrids and the other species, open-pollinated seeds from the six female clones were used as *P. tabuliformis* samples and mixed seeds from the five pollen trees were used as *P. yunnanensis* samples. Mixed seeds from *P. densata* populations were used as *P. densata* samples. The P. densata samples were collected in Linzhi, Tibet, a natural distribution area where no other coniferous species were distributed.

2.2 Site Description and Field Experiment

The study site is located in a nursery of Department of Resources and Environment, Tibet Animal Husbandry College in Town of Bayi, Linzhi County (93°25′E, 29°50′’N, 2900 m). The area features a plateau-humid monsoon climate zone, with an annual minimum temperature of -5.3 ℃ , maximum temperature of 22.1 °C, and annual average temperature of 8.5 °C. The annual precipitation is 654.1 mm concentrated between April and October; average annual sunshine hours is 2022.2 hours, and frost-free period is 180 d or more.

Plants were cultivated by seeding beds with the length of 5 m (east-west), width of 1.1 m, and height of 10 cm. The experimental design follows a randomized complete block design with four replications. Each block consists of 12 plots each housing one of the 12 artificial hybrids and the three plots that house the three species. There are 10 plants in each hybrid family plot and 20 plants in each individual species plot. The field planting spacing and management conditions were consistent among all plots.

2.3 Measurement of Capacitance

The special electrodes were connected to an impedance

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analyzer (TH2828S) made by China Electronics Co. in Changzhou, Jiangsu Province. An open/short circuit correction was conducted to the impedance analyzer prior to measurement. After the adjustment, two seedlings were selected from the artificial hybrids, *P. tabuliformis, P. yunnanensis* and *P. densata,* and the measurement was conducted on needle leaves of each seedling without removing them from the seedlings. Each leaf was applied with a voltage of 10 mV, and capacitance values were measured at 53 frequencies between 1 and 100M Hz to develop capacitance spectra. We found that between 1-120 Hz, the spectra curve is more volatile than those at other frequencies, indicating physiological changes in plant cells. Therefore, we selected 1-120 Hz as the measurement range. We randomly sampled healthy needles of *P. tabuliformis, P. yunnanensis, P. densata* and the hybrid species to measure their capacitance. During the measurement, we avoided physical damage to samples and ensured the stability of the measured results by a non-destructive sampling method. To eliminate the influence of changing atmospheric temperature and light over time, every plant was measured in the morning, at noon and in the afternoon, and the average of three measurements was taken as the final measurement value.

3. Results

3.1 Capacitance Spectra of the Interspecific Hybrids

With increasing frequency, the capacitance of most hybrids gradually increased, and the overall change was small (except for No. 408) without any large fluctuation (Figure 1). When the voltage frequency was at 20 Hz, the capacitance gap between various hybrids is very clear (Figure 1), indicating the variation in physiological properties of different genotypes of the hybrids. The capacitance of the hybrids tended to slightly decrease between the low frequency span (5-10 Hz) and increased gradually when the frequency increases from 10 to 120 Hz. Notably, the spectra of No.408 and 404 positioned significantly higher than the rest, and the maximum capacitance values are 800 and 500, respectively (Figure1). The rest of hybrids do not have significant change and the gaps between each sample remain relatively constant. The two genotypes have stronger energy storage and discharge capacity for electric field than the other hybrids.

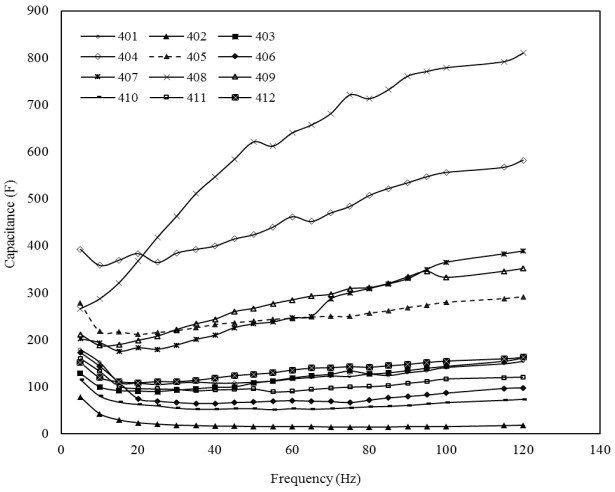

Figure 1. Capacitance of 12 hybrids with changing frequencies

In order to more directly compare the variation of capacitance among artificial hybrids and the other three species in the following analysis, we added all the capacitance values of each sample and calculated the average value for the four types of species. Among the hybrids, No.408 and 404 clearly showed significantly higher capacitance than the others (Figure 2a). Among the four species, *P. densata* has the highest capacitance, followed by the hybrid and *P. tabuliformis*, and the capacitance of *P. yunnanensis* is less than 100.

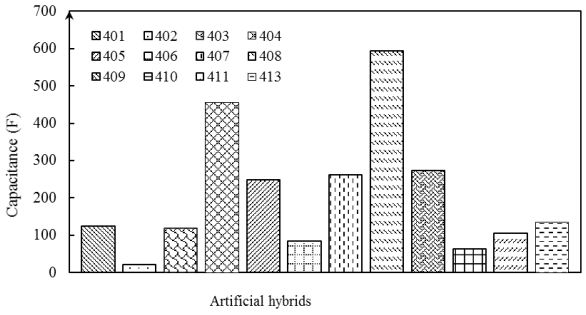

Figure 2a

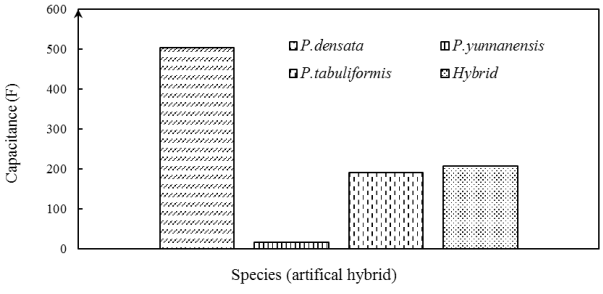

Figure 2b

Figure 2. (a) Average capacitance values of 12 hybrids across frequencies; (b) Capacitance of *Pinus densata, P. tabuliformis* and *P. yunnanensis* and the artificial hybrid

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3.2 Capacitance Spectra of Artificial Hybrids and the Three Species

Capacitance (average value of all samples) of the hybrids linearly decreased as voltage frequency increased from 5 to 10 Hz and slowly increased with the increasing voltage frequency afterwards (Figure 3). Capacitances of *P. densata* were relatively high and increased linearly when the voltage frequency increased from 5 to 35 Hz, and the capacitance decreased gradually as the voltage frequency increased afterwards, indicating that 35 Hz was the saturation point for *P. densata*. The capacitances of the hybrids were higher than the parental species *P. tabuliformis* and *P. yunnanensis*. With voltage frequency increasing from 80 to 120 Hz, the capacitance of *P. yunnanensis* decreased continuously, indicating that the storage capacity of *P. yunnanensis* was the smallest of all. The capacitance spectra of *P. tabuliformis* feature a spoon-shaped curve. When the voltage frequency was between 5 and 20 Hz, the capacitance slowly decreased, and at 20 Hz it reached the lowest point then rose quickly between frequencies of 70 and 120 Hz. The *P. yunnanensis* has the lowest electrical storage capacity, and storage capacity of the hybrid species was higher than their parental species when the voltage frequency is between 5 and 65 Hz. The capacitances of the hybrids reached the maximum at the frequency of 65 Hz, showing a superior storage capacity than its parental species *P. tabuliformis* and *P. yunnanensis*. After 65 Hz, storage and discharge capacity of the hybrids was larger than *P. yunnanensis* but smaller than *P. tabuliformis*. However, among all the species, the storage capacity of *P. densata* was much higher than that of the hybrids and their parental species. Among the four spectra, the hybrids and *P. densata* were closed to *P. tabuliformis,* showing that maternal influence was stronger than the paternal influence. However, the spectra of the hybrids and *P. densata* are not close to each other.

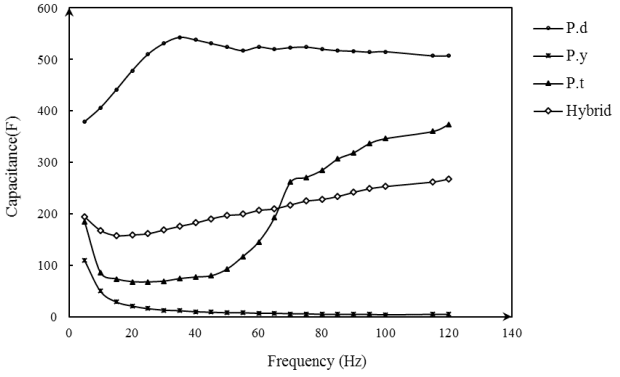

Figure 3. Capacitance spectra of *Pinus densata, P. tabuliformis and P. yunnanensis* and the hybrid

3.3 Modelling of Capacitance and Cluster Analysis of Capacitance

The capacitance spectra of *P. densata, P. tabuliformis, P. yunnanensis* and the artificial hybrids were modeled by the following equation.

$$C = af^3 + bf^2 + cf + d$$

where C is the capacitance of a sample, *a, b, c,* and *d* are species related parameters, and *f* is voltage frequency.

The capacitance varies with the frequency, and *P. yunnanensis* and the artificial hybrids had the best-fit with R^2 value close to 1. The R^2 value for *P. densata* and *P. tabuliformis* were 0.92, 0.83, respectively (Figure 4).

In order to more precisely present the data and simplify the model, each spectrum was segmented and individually modelled. For *P. densata* and *P. tabuliformis*, R^2 value was close to 1 (Figure 5a, b, c and d). *Pinus yunnanensis* was also modelled by segments. Segmented modeling for *P. densata* can be described as follow:

$$C_{P.d} = -1322f^2 + 10.812f + 31836 \quad (\mathbf{5}\,Hz \le f \le \mathbf{55}\,Hz), \quad R^2 = 0.99$$

$$C_{p.d} = -0.0022f^2 + 0.1068f + 525.3 \quad (\mathbf{60}\,Hz \le f \le \mathbf{120}\,Hz) \quad R^2 = 0.92$$

Cluster analysis using arithmetic averages were conducted for *P. tabuliformis*, *P. yunnanensis, P. densata* and artificial hybrids by grouping the samples based on a capacitance level of 0.4. The samples were clustered into five categories including one hybrid (No. 404) and *P. densata* as the first group, six hybrids (No.413, 403, 401, 411, 402, 410) and *P. yunnanensis* as the second group, three hybrids (No. 405, 407 and 409) and *P. tabuliformis* were clustered into the third group, and two additional hybrids (No. 408 and 406) were the other two groups.

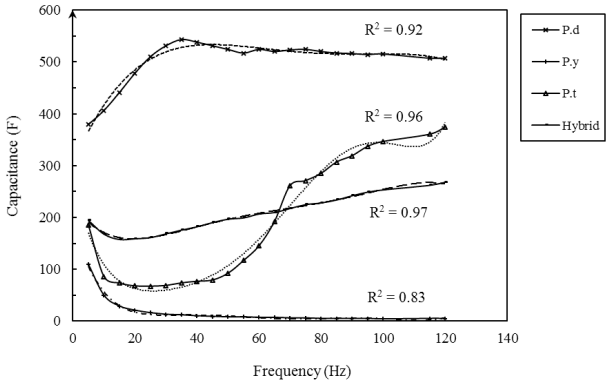

Figure 4. Capacitance modelling of *Pinus densata* (P.d), *P. tabuliformis* (P.t) and *P. yunnanensis* (P.y) and the hybrid of *P. tabuliformis* and *P. yunnanensis* (Hybrid)

Cluster analysis showed that the capacitance can clearly distinguish the species (Figure 6). It showed that the

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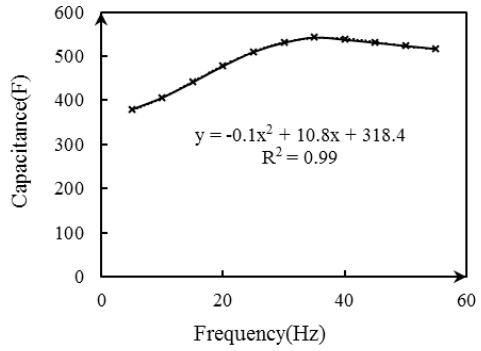

Figure 5a

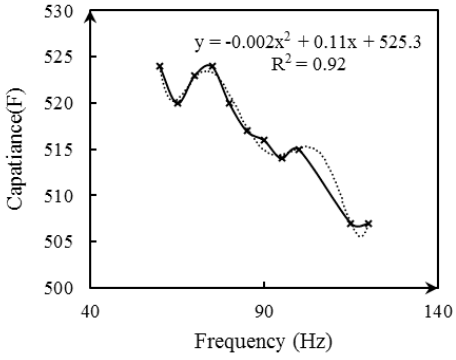

Figure 5b

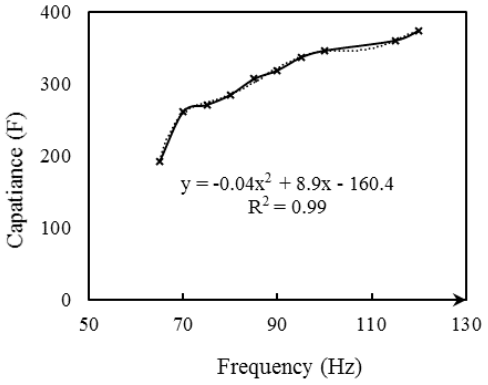

Figure 5c

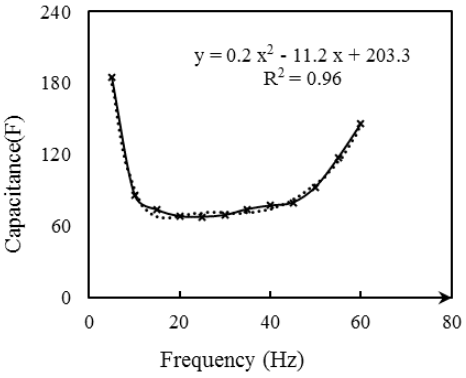

Figure 5d

Figure 5. (a) Capacitance modelling of *Pinus densata* at frequency between 10-50 Hz; (b) Capacitance modelling of *Pinus densata* at frequency between 50-120 Hz; (c) Capacitance modelling of *Pinus tabuliformis* at frequency between 5-60 Hz; (d) Capacitance modelling of *Pinus tabuliformis* at frequency between 60-120 Hz

hybrids have particular inclinations to maternal or paternal side in the kinship, instead of being evenly affected by each parent. Hybrid No.408 and 404 again have a closer position towards *P. densata*, in accordance with their capacitance values. The use of capacitance as an indicator for kinship among species may be related to inherent electrical properties of the basic genetic material sources.

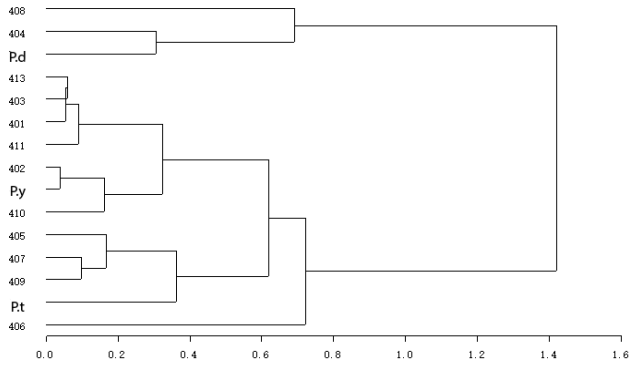

Figure 6. Capacitance clustering analysis of *Pinus densata* (P.d), *P. tabuliformis* (P.t) and *P. yunnanensis* (P.y) and the artificial hybrid (Hybrid)

3.4 Capacitance and Growth Adaptability Index

The water content and charged ions and particles are higher in seedlings in good growth conditions than that in poor conditions. Under external electric field, different capacitance of different plants might be due to their growth conditions. Therefore, we analyzed the correlation between capacitance and growth index in order to isolate the impact from growing environments. The growth adaptability index and capacitance data were measured at the same time.

To compare the capacitance and growth adaptability indexes (seeding height, survival rate, capping rate, germination rate, and mortality rate) of the species, we added capacitance value together regardless of the frequencies and calculated the average values for all indexes. The growth indexes were normalized to compare capacitance or electrical storage capacity and growth condition (Figure 7a). The capacitance of *P. densata* was consistent with its second-year growth height. The capacitance of *P. tabuliformis, P. yunnanensis* and the hybrid were consistent with their second-year seedling

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survival rates, and the growth index of the hybrid and *P. tabuliformis* were the same. n addition to germination rate, mortality rate and capacitance were positively correlated for *P. densata*, *P. tabuliformis* and the hybrid (Figure 7b).

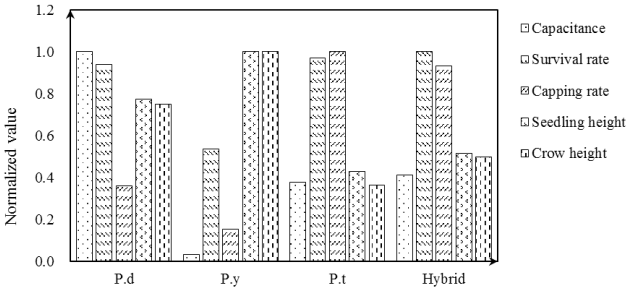

Figure 7a

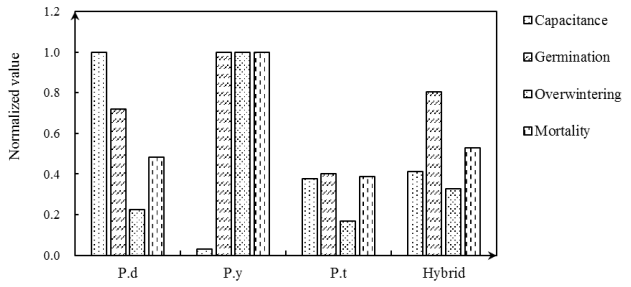

Figure 7b

Figure 7. (a) Normalized capacitance value and growth indexes of *Pinus densata*, *P. tabuliformis and P. yunnanensis* and the artificial hybrid; (b) Normalized capacitance value and mortality rate of *Pinus densata*, *P. tabuliformis* and *P. yunnanensis* and the artificial hybrid

The correlation coefficients between capacitance and second-year seedling height, second-year seedling crown height, and germination rate are negative (Table 1). Capping of height is an adaptation strategy for a seedling to store energy under low temperature and low photoperiod during winter. Capacitance and the capping rate was negatively correlated (Table 1), which is in agreement with previous findings [13]. In this study, capacitance is negatively correlated with mortality rate during winter and positively correlated to second-year survival rate.

4. Discussion

The fluids between cells can be regarded as electrolyte [14] and when different current of low voltage was applied to plant tissues, the current was conveyed by the fluids. When the frequency of the current applied increases, part of the current will get into the cell membrane and conducted through the cell membranes of plant tissues [15]. The change of capacitance value of *P. densata, P. tabuliformis*, *P. yunnanensis* and the hybrid as the frequency changes indirectly reflects the properties of membranes. *Pinus densata* and the hybrids are likely to inherit more dielectric properties of membranes from the material parent *P. tabuliformis*.

Photosynthesis is the process to transfer light into chemical energy (formation of organic matter) by plants. When turning light energy into electrical energy, electrons are transferred between the two photosystems systems, PS Ⅱ and PS Ⅰ. Physiological function of photorespiration is to consume excess energy [15]. When the tested sample was applied to an electric field, the storage and consumption of capacitance are two indicators of electric field energy storage and loss, respectively. The capacitance of *P. densata* was the largest, indicating that *P. densata* had the largest capacity to store charges or electrical energy. It is known that visible light is an electromagnetic wave, and sunlight can be considered as field energy with a frequency range of 480-680 MHz [10]. When the frequency was greater than 15 Hz, the decline in the capacitance was the lowest in *P. densata*, indicating that the energy loss in *P. densata* was the lowest. Ma et al [13] also reported that he energy storage capacity of *P. densata* is much larger than *P. yunnanensis*, *P. tabuliformis* and the hybrids, and its energy consumption is the smallest among them.

The relationship between seedling growth indexes and capacitance describes the linkage between the biological properties and their dielectric properties. The capacitance and growth and adaptability index were similar between *P. densata, P. tabuliformis* and the hybrid, again indicating that maternal effect was greater than the paternal influence. This is consistent with previous findings, which showed that the characteristics of *P. densata* are similar to *P. tabuliformis* and *P. yunnanensis* but closer to that of *P. tabuliformis* [16]. High capacitance of *P. densata*

Table 1. Correlation analysis between growth parameters and capacitance

	Germination rate	mortality rate	2nd year survival rate	2nd year capping rate	2nd year seedling height	2nd year crown height	2nd year mortality rate
Capacitance	-0.189	-0.595**	0.537**	-0.072	-0.075	-0.076	-0.243

Notes:
** P <0.05, ** P <0.01*

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indicates high energy storage within the seedling and better adaptability to harsh environments, resulting in high survival rate and low mortality rate. *Pinus densata* has been reported to have a higher photosynthetic rate and a higher reproductive adaptation at high altitudes than its parental species [17], achieving long-term survival under the harsh natural environment in Tibet, and eventually being able to evolve [18]. Two genotype of the first generation hybrids showed very close capacitance values as *Pinus densata*, and we could speculate that during natural selection, due to strong adaptability of the two, eventually they are able to survive and adapt to the environment.

5. Conclusions

We conclude that capacitance, a measure of energy storage capacity, can be used as a complementary indicator for studying genetic variation, adaptation ability to harsh environment, and kinship among species. In this study, we provide evidence to support that some particular genotypes of the artificial hybrid have a potential to evolve into *P. densata*, and the maternal parent has a stronger influence on the energy storage capacity of the hybrids. In addition, the ability of *P. densata* to adapt to harsh environment at a high altitude can be elaborated from its superior capacitance values over its parental species. This study opens a new perspective of using electrical parameters as a tool to study plant characteristics and provide complementary evidence of evolution and species kinship.

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References

[1] Dong, X. Recent Progress and Challenges in the Study of Bioimpedance. Chinese Journal of Biomedical Engineering, 2008, 27(5): 641-643.

[2] Ackmann, J.J., Seitz, M.A. Methods of complex impedance measurements in biological tissues. CRC Critical Review in Biomedical Engineering, 1984, 11(4): 281-311.

[3] Macdonald, J.R. Impedance spectroscopy: emphasizing solid materials and systems. New York: John Wiley& Sons, Inc, 1992.

[4] Schwan, H. The practical success of impedance techniques from an historical perspective. In: Riu PJ, Rosell J, Bragós R, Casas Ó, eds. Electrical bioimpedance methods: applications to medicine and biotechnology. Annals of New York Academy of Sciences, 1999, 873: 1-12.

[5] Hao, Z., Zhang, G., Li, Y. Physiological and Biochemical Indexes and Electrical Impedance Spectroscopy of Betula platyphylla Seedlings under Heat Stress. Journal of Acta Botanica Boreali-Occidentalia Sinica, 2010, 9: 1844-1851.

[6] Meng, Y., Zhang, G, Li, X., Gao, Z. Effect of NaCl Stress on Electrical Impedance Spectroscopy Parameters and Membrane Permeability of Moso Bamboo (*Phyllostachys edulis*) Seedling Leaves. Journal of Wuhan Botanical Research, 2010, 28(3): 341-346.

[7] Guo, W., Wu, L., Wei, Y. Influence of water loss on physiological and electrical properties of plants. Journal of Northwest A & F University (Nat. Sci. Ed.), 2007, 35(04): 185-188.

[8] Zhu, Z., Bao, Y. Study of the moisture content of soybeans on dielectric property of soybean. Journal of Zhejiang University (Agric.& Life Sci.), 2005, 31(02): 220-224.

[9] Xie, C., Rao, K. Electromagnetic Field and Wave. Beijing: Higher Education Press, 2006.

[10] Zhao, K., Chen, X. Electromagnetism. Beijing: Higher Education Press, 2003.

[11] Qiu, G. Circuit. Beijing: Higher Education Press, 2006.

[12] Gao, J., Wang, B., Mao, J.F., Ingvarsson P., Zeng, Q.Y., Wang, X.R. Demography and speciation history of the homoploid hybrid pine Pinus densata on the Tibetan Plateau. Molecular Ecology, 2012, 21(19): 4811-4827.

[13] Ma, F., Chen, X., Li, Y. Study on loss spectrum parameters in *Pinus densata* Masters and artificial hybrid of *P.tabuliormis Carrière* × *P. yunnanensis Faranch*. Journal of Forest and Environment, 2016, 36(01): 1-6.

[14] Pan, R. Plant physiology. Beijing: Higher Education Press, 2004.

[15] Grimnes S., Martinsen O. Bio-impedance and Bioelectricity Basics. London, UK: Academic Press, 2000.

[16] Kuan, C. Fundamental features of the distribution of Coniferae in Sichuan. Journal of Systematics and Evolution, 1981, 19(4): 393-407.

[17] Ma, F., Xu, T., Chen, L. Zhang, X., Zhao, C. Functional Stability of Photosystem Ⅱ in the Diploid Hybrid Species (*Pinus densata*) under Low Temperature Stress. Journal of Acta Botanica Boreali-Occidentalia Sinica, 2011, 31(6): 1174-1179.

[18] Mao, J., Li, Y., Liu, Y., Hao, L., Wang, X. Cone and seed characteristic of *Pinus densata* and their adaptive fitness implications. Journal of Plant Ecology, 2007, 31(02): 291-299.

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