Morphological Characterization, Seed Physiology, Antimicrobial Properties, and Habitat Suitability of Two Plant Genera (*Ziziphus* Mill. and *Balanites* Del.) Co-occurring in Semi-Arid Ecosystems of Ethiopia

By:

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A Dissertation Submitted to the Graduate Programmes of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Plant Biology and Biodiversity Management

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Dedication

This dissertation work is dedicated to my family.

Statement of Author

I, **Mohammed Adefa Seid**, declare that this PhD dissertation, entitled "Morphological Characterization, Seed Physiology, Antimicrobial Properties, and Habitat Suitability of Two Plant Genera (*Ziziphus* Mill. and *Balanites* Del.) Co-occurring in Semi-Arid Ecosystems of Ethiopia," is my own original work. It is being submitted for the degree of Doctor of Philosophy in Plant Biology and Biodiversity Management at the College of Natural and Computational Sciences (CNCS), Addis Ababa University, Addis Ababa, Ethiopia. The work has not been submitted before for any degree or examination at any other university.

Mohammed Adefa Seid _____

Name of candidate Signature of candidate

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Acronyms

ANOSIM … Analysis of similarity
ANOVA … Analysis of variance
APG IV … Angiosperm Phylogeny Group IV
AUC … Area under the Receiver Operator Curve
BD … Bulk density
BRT … Boosted regression tree
CEC … Cation exchange capacity
CMIP6 … Coupled Model Inter-comparison Project Phase 6
CRD … Completely Randomized Design
FAO … The Food and Agriculture Organization of the United Nations
GAM … General additive model
GLM … Generalized linear model
HadGEM3-GC31-LL … The third Hadley Centre Global Environmental Model run in the Global Coupled Configuration 3.1
HC … Hierarchical clustering
IGAD … The Intergovernmental Authority on Development
IPCC … Intergovernmental Panel on Climate Change
ISRIC … International Soil Reference and Information Centre
MARS … Multivariate adaptive regression splines
PCA …. Principal component analysis
RDA ... Redundancy Analysis
RF … Random forests
ROC … Receiver operator characteristics
SDM … Species distribution model
SOC … Soil organic carbon content
SSP … Shared Socioeconomic Pathways
TSS … True Skill Statistic
SVM … Support vector machines

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Abstract

Despite their multipurpose nature and wide distribution, there is a lack of understanding regarding the morphological variability, seed physiology, antimicrobial properties, and habitat suitability of *Ziziphus* Mill. and *Balanites* Del. in Ethiopia. This study aimed to investigate the inter- and intra-specific morphological variability, seed viability and germination behavior, antibacterial properties, and habitat suitability of *Ziziphus* and *Balanites* in Ethiopia. To analyze the inter- and intra-specific morphological variability, the bark color, leaf shape, fruit shape, and spine condition were examined for specimens of *Balanites* (*n* = 123) and *Ziziphus* (*n* = 130). The data was analyzed using hierarchical clustering (HC) and redundancy analysis (RDA) ordination. To evaluate the effects of pre-sowing treatments on the seed germination of *Ziziphus* (i.e., *Z. spina-christi* and Z. *mucronata*) and *Balanites* (*B. aegyptiaca* and *B. rotundifolia)*, seeds were collected from many parts of Ethiopia, subjected to eight pre-sowing treatments, and planted in pots arranged in a CRD. The data was analyzed using germination percentage (GP), daily germination percentage (GD), germination time (GT), germination index (GI), and ANOVA at $p \leq 0.05$. To examine the antibacterial properties of *Ziziphus* and *Balanites*, ethanol and methanol extracts of leaves of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca,* and *B. rotundifolia* were tested against selected pathogenic bacteria using agar-well and broth serial macro-dilution. To predict the habitat suitability of *Ziziphus* and *Balanites* current and future (i.e., SSP2-45 and SSP5-85 of HadGEM3-GC31-LL) climates, ensemble SDMs were computed in R 4.1.3 using occurrence points of *Z. spina-christi* (*n* = 159), *Z. mucronata* (*n* = 101), *B. aegyptiaca* (*n* = 224), and *B. rotundifolia* (*n* = 80), and bioclimatic, soil, and landscape variables. HC indicated five and eight population clusters (morphotypes) of *Balanites* and *Ziziphus*, respectively. RDA indicated that the effects of Annual Mean Temperature (Bio01), Isothermality (Bio03), and Precipitation of Wettest Quarter (Bio16) on morphological variability s of *Balanites* are highly significant ($p \leq 0.001$). Similarly, the effects of Bio03, Temperature Seasonality (Bio04), Min Temperature of Coldest Month (Bio06), Mean Temperature of Warmest Quarter (Bio10), Precipitation of Driest Month (Bio14), and Precipitation of Driest Quarter (Bio17) are highly significant ($p \leq 0.001$) on the morphological variability of *Ziziphus*. One-way ANOVA for *Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia*) and two-way ANOVA for *Ziziphus* (i.e., *Z. spina-christi* and *Z. mucronata*) showed the presence of significant differences in GP, GD, GT, and GI among treatment groups at $p < 0.05$. The methanol extract of *Z. spina-christi* showed potency against *E. faecalis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *S. typhimurium*, and *K. pneumoniae*. The ethanol and methanol extracts of *B. aegyptiaca* showed inhibitory activities against *K. pneumoniae* and *S. typhimurium*. The ensemble SDM under the current climate predicted that about 8.6% of the areas of Ethiopia are suitable for *Z. spina-christi,* while 8.2% and 9.4% will be suitable under SSP2-45 and SSP5-85, respectively, in the years 2061–2080. Similarly, the ensemble SDM under the current climate predicts that about 6.4% of the areas of Ethiopia are suitable for *Z. mucronata*, while 5.5% and 5.7% will be suitable under SSP2-45 and SSP5-85, respectively. The ensemble SDM under the current climate predicted that about 8.6% of the areas of Ethiopia are suitable for *B. aegyptiaca,* while 8.8% and 9.5% will be suitable under SSP2-45 and SSP5-85, respectively. The ensemble SDM under the current climate predicted that about 3.1% of the areas of Ethiopia are suitable for *B. rotundifolia,* while 2.2% and 3.8% will be suitable under SSP2-45 and SSP5-85. The findings of this study would contribute to the selection and breeding, propagation, conservation, and sustainable use of *Ziziphus* and *Balanites* in Ethiopia.

Keywords: Antimicrobial Properties; *Balanites* Del.; Leave Extracts; Morphological Variability; Species Distribution Models; Seed Germination; *Ziziphus* Mill.

Chapter 1

1. General Introduction

1.1 Background and Justification

The dryland ecosystems cover nearly half of the global land surface, which harbors high biodiversity resources and support over two billion people (Kidane Georgis, 2014; Fu et al., 2021). Similarly, a large proportion of the landscape of Ethiopia (*ca*. 76–81%) is considered dryland that has abundant biodiversity resources supporting enormous communities (Mulugeta Lemenih & Demel Teketay, 2004; Kidane Georgis, 2014). Drylands are generally characterized by receiving an annual mean precipitation < 700 mm and aridity index (AI) < 0.65. Aridity index is the ratio of precipitation (P) to potential evapotranspiration (PEP). Four classes of drylands are known: hyper-arid (AI < 0.05, P < 25 mm), Arid (0.05 ≤ AI < 0.2, 25 ≤ P < 250 mm), Semi-arid (0.2 ≤ AI < 0.5, 250 ≤ P < 500), and dry sub-humid (0.5 ≤ AI < 0.65, 500 ≤ P < 700) (Doorenbos & Pruitt, 1977). The dryland ecosystem in Ethiopia has four main sub-systems: *hyper-arid, arid, semi-arid,* and *dry sub-humid* (Mulugeta Lemenih & Demel Teketay, 2004). It encompasses five forest vegetation types (Friis et al., 2010; Mengesha Asefa et al., 2020): dry evergreen montane forest, *Combretum-Terminalia* woodland, *Vachellia-Commiphora* woodland, desert and semi-desert scrubland, and lowland semi-evergreen forest (e.g., wooded grassland of the eastern Gambella region). In Ethiopia, the most distinctive plant species in dryland ecosystems include *Ziziphus* Mill., *Balanites* Del., *Vachellia spp.* (formerly known as *Acacia*), *Tamarix spp., Erythrina spp., Cordia spp., Ficus spp., Milicia spp., Cyathea spp., Olea spp.*, etc. Moreover, this area is crucial in agroecology as it cultivates high-value fruit trees, cereals, pulses, and tubers (Azene Bekele-Tesemma, 2007). However, the dryland ecosystems in this region are neglected and face an array of threats including rapid population growth, land degradation, biodiversity loss, recurrent drought, rainfall variability, and climate change (Efrem Garedew et al., 2009).

Ziziphus Mill. and *Balanites* Del. are known multipurpose shrubs or small trees with various uses and values such as food, forage, medicine, construction, etc. (Orwa et al., 2009). They are widely distributed in the semi-arid ecosystems of Ethiopia (Azene Bekele-Tesemma, 1993, 2007; Orwa et al., 2009). *Ziziphus* Mill. (Rhamnaceae) and

Balanites Del. (Balanitaceae) are plant genera that belong to the orders Rosales and Zygophyllales, respectively. The two orders are grouped in the same clade, named Fabid (APG IV, 2016).

The morphological traits of plants tend to vary depending on the heterogeneity of habitats in which they are living. Plant morphological changes, such as seed size and shape, leaf stomatal trichome density, and trichome structure, are crucial for the adaptation of plants to diverse microclimates (Soheili et al., 2022). Habitats vary due to several factors, where geological and climatic changes, together with biological and anthropogenic factors, are major deriving forces in the dynamics of habitat (Pescador et al., 2015). Morphological characterization of species needs to address questions related to the effects of physical and climatic variables in shaping the pattern of phenotypic divergence with geographic distribution. Similarities, due to the present distribution of *Balanites* and *Ziziphus* across extensive ranges of geological, climatic, biome, and socio-cultural environments (anthropological factors), high level of morphological variability are expected among the different species of *Ziziphus* and *Balanites*. Although the Flora of Ethiopia (Hedberg & Edwards, 1989) defined *Balanites* and *Ziziphus*, little attempt has been made to undertake computational and comparative morphological analyses, as well as assess the impact of environmental variables on morphological variability. Thus, analyses of inter- and intra-specific morphological variability of *Balanites* and *Ziziphus* was one of the first main research objectives of this study.

Seeds are the germplasm that function as dispersal and reproductive units of plants. They play a crucial role in ecological succession and the continuity of biodiversity (van der Maarel & Franklin, 2013). Tree seeds always exhibit some degree of dormancy, resulting in a delay and irregularity of germination in the nursery and even on forest floors (Schreckenberg et al., 2006). The seed viability test employs dormancy-breaking techniques in order to determine the best ways to increase seed germination capacity. Hence, understanding plant physiology and growth performance has an important tool in the context of identifying viable populations to enhance future conservation through afforestation. In this regard, no information was previously reported on the seed germination behavior of *Ziziphus* and *Balanites* species in Ethiopia under the different seed treatments. As a result, testing the seed

viability and germination performance of selected species of *Ziziphus* (i.e., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia*) under different pre-sowing treatments was one of the second main research objectives of this study.

Similarly, it is well known that secondary metabolites produced by plants can help plants develop strategies to outcompete their competitors and overcome environmental constraints. These compounds are biosynthesized under varying stress patterns and are therefore associated with the survival strategies of plants. Plant-derived natural products make up the highest proportion of the claimed and promoted natural products. Plants provide a huge array of secondary metabolites that underlie their importance in human nutrition and health and are the basis for their commercial value (Atanasov et al., 2015). The existing knowledge on the benefits of plants to humans came as a result of long-term intimacy or interaction with nature. Plants are recognized for their contribution to both traditional and modern medicine. In this regard, *Ziziphus* and *Balanites* are commonly used as medicinal plants by the local peoples in different cultural landscapes of Ethiopia (Mirutse Giday & Tilahun Teklehaymanot, 2013; Tilahun Teklehaymanot, 2017; Asmare Talie et al., 2018; Ali Zeynu et al., 2021). However, no in-vitro tests of the antimicrobial properties of *Ziziphus* and *Balanites* were reported in Ethiopia. Thus, evaluating the antibacterial properties of selected species of *Ziziphus* (i.e., *Z. spina-christi and Z. mucronata*) and *Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia*) was one of the third main research objectives of this study.

Climate change has many impacts on biodiversity, threatening populations and causing shifts in phenology and habitat ranges (Thuiller et al., 2010). Due to biological factors and physical limitations, species are unable to spread across all ecological ranges and suitable habitats. Hence, analysis of environmental data using geographical information systems (GIS) is vital to bringing new insight into this field of evolutionary biology (Kozak et al., 2008). The spatial distribution of species with the niche concept can be modeled using different mathematical algorithms with the help of software programs (e.g., R and GIS). In relation to this, conditions of plant physiology and growth performance explain the adaptive mechanisms of the species. In other words, elucidating species distribution with bioclimatic variables provides

robust evidence for conservation plans and sustainable use of plant resources by identifying potential suitable habitats (Start et al., 2018). However, no works on the habitat suitability for *Ziziphus* and *Balanites* under climate change in Ethiopia have been reported previously. Thus, predicting the habitat suitability of selected species of *Ziziphus* (i.e., *Z. spina-christi and Z. mucronata*) and *Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia*) under the climate change in Ethiopia was one of the fourth main research objectives of this study.

1.2 Statement of the Problem

Plant morphological changes and seed physiology (e.g., domancy) are crucial for the adaptation of plants to diverse microclimates (Soheili et al., 2022). Therefore, it is important to consider morpho-physiological adaptations to propagate, domesticate, and conserve species in response to climate change (Villeneuve et al., 2016). Recent research has demonstrated that seed dormancy, specifically different types such as physiological dormancy and physical dormancy, significantly impact plant survival and ecological dynamics (Wyse & Dickie, 2018). In other word, physiological traits can help to elucidate species distribution (Start et al., 2018). The transition of dormancy in response to changing environments has been linked to the diversification of seed plants, making it a prominent characteristic throughout their evolutionary history (Willis et al., 2014; Zhang et al., 2022). Furthermore, the morphology of seeds, including their size, is closely assocuated with dormancy strategies. Larger seeds are typically associated with non-dormancy in lineages with longer growing seasons, whereas smaller seeds with dormancy are favored in shorter growing seasons. This highlights the adaptive significance of dormancy in seasonal environments (Rubio de Casas et al., 2017). To fully comprehend species distribution patterns and evolutionary processes in plant communities, it is essential to understand the interplay between morphology, seed physiology, and environmental factors.

For the purpose of this dissertation work, two co-occurring genera (i.e., *Ziziphus* Mill. and *Balanites* Del.) were selected to represent claimed to provide multipurpose use such as food, forage, medicine, construction, fuel wood and incomes for rural communities (Saied et al., 2008; Orwa et al., 2009; Panghal et al., 2010; Mokgolodi et al., 2011; Abraha Teklay et al., 2013; Mirutse Giday & Tilahun Teklehaymanot, 2013; Tilahun Teklehaymanot, 2017; Asmare Talie et al., 2018; Ali Zeynu et al.,

2021). The two genera are widely distributed in tropical Africa, adapting to the dry and hot climates. *Balanites* belong to the family Balanitaceae, while *Ziziphus* is classified under the family Rhamnaceae, which in turn is classified under the orders Zygophylales and Rosales, respectively. These two Angiosperm Orders are placed in the same clade, namely Fabid (APG IV, 2016), which indicates the closer relationship between *Ziziphus* and *Balanites*. Therefore, the fact that the two species have many features in common and are potential sources multipurpose use calls for deeper studies to generate relevant data for future conservation and sustainable use. The present research focuses on the following four components: 1) Morphological characterization of *Ziziphus* Mill. and *Balanites* Del.; 2) Seed viability potential and germination performance of prominent species of *Ziziphus* (i.e., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia*); 3) Antimicrobial properties of prominent species of *Ziziphus* (i.e., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia);* and 4) Identify suitable habitat of prominent species of *Ziziphus* (i.e., *Z. spina-christi* and *Z. mucronata) and Balanites* (i.e., *B. aegyptiaca* and *B. rotundifolia*).

Despite the multipurpose uses of *Ziziphus* and *Balanites* for enormous communities in the semi-arid ecosystems of Ethiopia, the inter- and intra-specific morphological diversity, seed germination behavior, antimicrobial properties, and habitat suitability of both genera are not well studied and documented. Therefore, a cross-scale integration of knowledge combining the study of morphological variability, seed germination physiology, social-economic uses (e.g., antimicrobial properties), and habitat suitability modeling of species could reveal the interacting processes that determine specie distribution ranges (Talluto et al., 2016). In this regard, modeling species distribution (niche) with environmental data using geographic information systems (GIS) is essential to bring new insight into this field of conservation management of biodiversity (Kozak et al., 2008). Predicting the potential suitability and species' distribution in an area could be determined in response to the environmental conditions, which are represented by different GIS layers (Meron Awoke et al., 2021). Moreover, habitat suitability models provide a framework for understanding how environmental variables relate to species occurrences (Hirzel & Le Lay, 2008), which is crucial for predicting suitable conditions for plant growth and function (Belluau & Shipley, 2018). Similarly, understanding plant growth

performance and the underlying physiology elucidates the adaptive mechanisms. This approach is important to protect species in the context of identifying adaptive features and viable populations. Therefore, findings produced from such integrated approaches will provide robust evidence for conservation plans and sustainable use of plant resources by identifying potential suitable habitats.

1.3 Literature Review

Morphological characters and phenetic classification

Morphological (and anatomical) traits have been used to group and classify species, although the application of molecular markers (e.g., DNA) has increased in recent days (Pons et al., 2006; Clement & Donoghue, 2012). However, this tendency has led to a debate on the suitability of molecular markers over morphology, and vice versa, when studying the relationship among species (Spooner et al., 2009). Morphological traits allow the explanation of the observed character traits of species (e.g., plants) and help to make hypotheses on the adaptation and evolutionary processes of species (Knapp, 2001). Hence, morphological characters are important sources of data in the classical taxonomy and classification of organisms.

Phenetic classification is a numerical method of classification solely based on morphological characters or other visible traits of the organisms to classify, regardless of their evolutionary relationships. Traits ranging from morphological features such as leaf shape and venations to anatomical features such as vascular bundle structure are used in phonetic clssification. It was introduced in the late 1950s and early 1960s with the advent of computers (Sneath, 1984). In other words, phonetics, often called numerical taxonomy, is the technique of grouping organisms by overall similarity based on the available characters without hierarchical structure (Sokal, 1986). The classification of organisms using phenetic approaches involves the formation of inter- and intra-specific resemblances based on all accessible data from the organisms to be studied (Moss & Webster, 1970). By the 1990s, it had become rarely used except in particular subspecific and population studies.

Phenetic analyses of morphological data apply too many methods but can be generally classified into two types: those that generate tree-like diagram or dendrogram through clustering and others that generate scatter diagrams using more than one axis through

ordination (Sokal, 1986). A dendrogram is produced from a data matrix containing all available morphological data using proper coding procedures. Discrete (qualitative) morphological character states are easy to code, whether binary or multistate. The challenge comes when coding continuous (quantitative) morphological parameters. Continuous characters are potentially important inputs for phenetic (and phylogenetic) studies alleviating the possible potential limitations of discontinuous character (Wiens, 2004; Parins-Fukuchi, 2018). However, continuous morphological characters can be coded using different approaches, namely scaling by among-group variability, e.g., range-scaling and normalization (Thorpe, 1984), scaling by within-group variability (Thorpe, 1976, 1979), simple gap-coding, homogeneous-subset coding, and generalized gap-coding (Archie, 1985). The current study used a combination of scaling by both among-group and within-group variability, using range-scaling of the standard deviation (SD).

The phenetic approach is rather a comparatively straight-forward method of classification (Colless, 1967). On the contrary, cladistic approaches try to classify organisms based on hypothetical common ancestors (Camin & Sokal, 1965; Mayr, 1968), while the phyletic method (i.e., evolutionary) combines both phenetic and cladistic approaches (Mayr, 1969). As evolutionary classification is based on assumptions, it cannot be accurately verified (Colless, 1967), even with a moderately complete fossil record. Thus, phenetic classification will still remain an important approach in classification and taxonomy presently and in the future.

Seed germination biology: principles and methods

Plants have evolved a number of strategies to regulate seed germination to ensure successful seedling establishment under diverse conditions in the natural environment (Nonogaki, 2019). There are several reproductive adaptations in plants. The evolution of new reproductive structures is fundamental for the success of terrestrial plants (Bhattacharya & Medlin, 1998; Bareke, 2018). A seed is defined as a diploid mature ovule after fertilization (Reddy et al., 2022), which is surrounded by nutritive tissue (endosperm) that is enveloped by a protective covering (seed coat). In other words, seeds are the reproductive unit of seed plants, connecting the past and future (Bareke, 2018). They contain the genetic information of the past and the capability for its continuation in the future.

Seed germination is simply the emergence of radicles when favorable conditions are met (Bewley & Black, 1994; Zietsman & Botha, 1987). Seed germination involves three common physiological processes: *imbibition*, *respiration*, and *cell division*. Plants have developed different adaptive and evolutionary strategies to regulate seed germination, ensuring germination success and seedling establishment in diverse environmental conditions (Bewley et al., 2013; Nonogaki, 2019). Morphological adaptations of seed structure often play a crucial role in regulating germination (Bewley et al., 2013). Thus, it is important to understand the morphological changes of seed structures during imbibition that cause the emergence of radicles. Moreover, understanding the principle of seed germination also requires understanding the biochemical and molecular changes that induce seed dormancy (Nonogaki, 2019). In this regard, apart from the innate factors (i.e., genetic, physical, and physiological), studies have also implicated the importance of environmental factors (epigenetic) in regulating seed germination and dormancy (Nonogaki, 2014, 2017, 2019).

Seed dormancy, an adaptive strategy of seed plants, has played a vital role in the survival of seed plants over an era (Reddy et al., 2022). Seed dormancy generally involves physical and physiological dormancy. Dormancy is a well-known adaptation strategy for species occurring in areas of seasonally harsh climates, and even in moist tropical forests, dormancy occurs in pioneer and light-demanding genera (Reddy et al., 2022). Hence, dormancy enables the plants to survive as seed when they might die as seedlings. Plants undergo different dormancy strategies: physical, morphological, physiological, and combinational (Reddy et al., 2022). Physical dormancy results from seed coats and their impermeability to water. Morphological dormancy occurs when an embryo is underdeveloped but differentiated physiologically, dormant, and simply needs time to grow and germinate (Reddy et al., 2022). Physiological dormancy, which is the primary dormancy, uses germination-inhibiting hormones to prevent germination when the favorable environmental conditions required for germination are absent (Reddy et al., 2022). Physiological dormancy is a dormancy program initiated by either the embryo or the surrounding endosperm tissues.

The problem of seed dormancy can be broken using seed pre-sowing treatments that trigger germination. Pre-sowing treatments (seed pre-treatment) are different treatments used on seeds before sowing to enhance the vigor or capability of

germination (Sharma, 1994; Sharma et al., 2015). The most common and widely used pre-sowing treatments include mechanical (e.g., cutting or filling of hard impervious seed coat), soaking in cold water for 12, 24, or 48 hours, soaking in hot water of 50–75ºC for a few minutes and allowing to cool for 12 hours, alternate wetting and drying, chemical treatment of seeds for a few minutes (e.g., acids, citric acid, hydrogen peroxide, or sulfuric acid), hormonal treatment (e.g., Gibberellin-GA), priming, and seed coating and pelleting (Kumar, 2015; Hamad & Anwer, 2021; Reddy et al., 2022).

Medicinal plants and antimicrobial properties

Traditional medicine has been used by nearly 80% of the rural population in developing countries as a primary healthcare system (WHO, 2002; Devi et al., 2009). In this regard, medicinal plants constitute an effective source of both traditional and modern medicine. Medicinal plants have proven to be an important source of potentially therapeutic drugs. In this regard, antimicrobial agents of plant origin have greater therapeutic values against human infections of bacteria, fungi, and viruses (Salau & Odeleye, 2007). Therapeutic compounds derived from traditional medicinal plants have beneficial antioxidants and bioactive agents (Liu & Henkel, 2002; Philip et al., 2009). *Ziziphus* and *Balanites* are commonly used medicinal plants by the local people in different cultural landscapes in Ethiopia (Mirutse Giday & Tilahun Teklehaymanot, 2013; Tilahun Teklehaymanot, 2017; Asmare Talie et al., 2018; Ali Zeynu et al., 2021).

Plants have unlimited potential for synthesizing secondary metabolites, including flavonoids, phenolic acids, tannins, and others (Das et al., 2010). Plants synthesize secondary metabolites as defense tools against infectious microbes and herbivory (Barsi & Fan, 2005). Recently, the development of microbial resistance to the available antibiotics has inspired researchers to examine the antimicrobial properties of medicinal plants (Maoz & Neeman, 1998; Hammer et al., 1999). This in turn increases the acceptance of traditional medicine as an alternative form of healthcare (Maoz & Neeman, 1998; Hammer et al., 1999). So far, many efforts have been made to find new antimicrobial agents from various kinds of plant sources. In this regard, phytochemical screening and examination of medicinal plants may result in the discovery of novel therapeutics. Secondary metabolites are biosynthesized under

varying stress patterns and are, therefore, associated with the survival strategies of plants. They can help plants develop strategies to outcompete their competitors and overcome environmental constraints.

Extraction is the process of separating bioactive compounds from plants using selective solvents through standard procedures (Azwanida, 2015). Some of the widely used extraction methods for plant products include maceration, infusion, decoction, percolation, Soxhlet extraction, microwave-assisted extraction, and ultrasound-assisted extraction (Azwanida, 2015; Abubakar & Haque, 2020). Maceration, a method employed in the current study, is an extraction process in which coarsely powdered plant materials, such as leaves, stems, roots, or bark, are placed inside a container filled with liquid solvents (e.g., alcohol such as methanol or ethanol, water, ether, or chloroform), allowing them to stand at room temperature for a minimum of three days with regular shaking and filtration processes (Handa et al., 2008). Infusion (suitable for softer plant materials) and decoction (suitable for harder plant materials) are soaked in cold or boiled water and follow the same principle as maceration (Handa et al., 2008; Azwanida, 2015).

The antimicrobial properties of plant extract are commonly evaluated in vitro using several methods: diffusion methods such as agar disk-diffusion (Heatley, 1944), agar well diffusion method (Magaldi et al., 2004; Valgas et al., 2007), agar plug diffusion method, cross streak method, thin-layer chromatography (TLC)-bioautography, and dilution methods such as broth dilution and agar dilution methods, etc. (Balouiri et al., 2016). In this regard, the current study also employed agar-well diffusion methods of antimicrobial evaluation tests for selected species of *Ziziphus* and *Balanites*.

Species distribution models

Species distribution models (SDMs) are mathematical models that simulate species' distributions in reference to observed environmental variables (e.g., temperature, light, landscape, soil, etc.), making spatial predictions of the species in a given time (Elith & Leathwick, 2009; Elith & Franklin, 2017). Recently, SDMs have been steadily employed in ecological studies, such as habitat suitability studies, climate change impacts, etc. (Guisan & Zimmermann, 2000). They are widely used methods to predict the spatial and temporal distribution based on species occurrences and environmental variables that limit species habitats (Pearson et al., 2007). The SDMs

have variable performances (Nurhussen Ahmed et al., 2021), so the selection and implementation of models require great care to avoid model uncertainty that could mislead policymakers (Elith & Leathwick, 2009). Consequently, many studies used more than one model in comparison (Früh et al., 2018; Nurhussen Ahmed et al., 2021). Species distribution models are classified into three main groups: profile models (e.g., Bioclim), statistical regression models (e.g., general linear model), and machine-learning models (e.g., MaxEnt). "Bioclim" is one of the earliest species distribution “profile” models used in many ecological studies, including invasive species prediction (Booth et al., 2014). Some of the most commonly used SDMs are discussed hereafter.

Maximum Entropy (MaxEnt) is one of the most commonly used machine learning programs used in many studies of species distribution and niche modeling (Merow et al., 2013). MaxEnt was previously used to model the probability distributions of species. It identifies non-linear relationships between environmental parameters and geo-referenced sites (Li et al., 2022). The Generalized Linear Model (GLM) is one of the popular regression models that process and manage a non-linear data structure, which is suited for ecological studies (Guisan et al., 2002). GLM is based on parametric functions and is an extension of the classical linear regression method. It predicts the dependent (response) variable as a function of multiple independent (predictor) variables (Kienast et al., 2012). The Generalized Additive Model (GAM), an extension of GLM, is also a popular regression model and is extensively used in ecological studies for modeling non-normal distributions. The Multivariate Adaptive Regression Spline (MARS) is a non-linear and non-parametric regression designed for building numerous linear regression models across a range of independent parameters (Friedman, 1991). The Random Forest (RF) is a powerful machine learning species distribution model based on a combination of tree predictors (Zhang et al., 2019). It is the most frequently used algorithm, having an effective method of modeling species richness and density (Abdi, 2020). Similarly, the Boosted Regression Tree (BRT) is a non-linear machine learning model based on a combination of a relatively small number of trees to increase the performance of predictive variables (Elith et al., 2008) and has the capacity to process several predictors with high predictive accuracy (Gu et al., 2019). The Support Vector Machine (SVM) is a non-parametric algorithm developed from the theory of statistical learning in which the error involved with

sample size is minimized and the upper limit of the error involved in model generalization is narrowed (Huang & Wang, 2006). It can be used on small data sets as it is independent of any distributional assumptions or asymptotic arguments (Wilson, 2008). For this study, six models (i.e., GLM, GAM, BRT, RF, SVM and MARS) are computed and analyzed.

1.4 Research Questions

In this study, four research components with interrelated research questions are addressed, whose outcomes will contribute to the future conservation plan and sustainable use of the two plant genera. The first component addresses research questions such as (1) Are there inter- and intra-specific morphological variability s in *Ziziphus* and *Balanites*?, (2) What are the major morphological characters playing important contributions in the variability between and within the species of *Ziziphus* and *Balanites*?, and (3) What environmental variables do most significantly constrain the morphological variability between and within the species of *Ziziphus* and *Balanites*? For these reasons, it was hypothesized that *Ziziphus* and *Balanites* would show inter- and intra-specific morphological variability along bioclimatic and physical gradients.

The second component addresses questions such as (1) What are the types of pre-sowing treatments that could enhance the seed germination percentages of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*)?, and (2) How significant are the pre-sowing treatments in decreasing the germination time of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) compared to the untreated seeds? For these reasons, it was hypothesized that pre-sowing seed treatments could enhance the seed germination of *Ziziphus* and *Balanites*.

The third component addresses questions such as (1) What is the degree of antimicrobial potencies that *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) have on some pathogenic bacteria?, and (2) What are the MIC and MBC of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) have on some pathogenic bacteria? For these reasons, it was hypothesized that *Ziziphus* and *Balanites* species would show potency against common bacterial pathogenic strains.

The fourth component addresses questions such as (1) How large and where do the suitable habitats of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) occur in Ethiopia? (2) Will *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) change their distribution ranges under the changing climate (i.e., SSP2-45 and SSP5-85/HadGEM3-GC31-LL in 2061–2080) in Ethiopia?, and (3) What are the most important bioclimatic, soil, and landscape variables that play significant effects on the distribution of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) in Ethiopia? For these reasons, it was hypothesized that the *Ziziphus* and *Balanites* species would change their species distributions (i.e., either increase or decrease their areas of suitable habitats) under the changing climate.

1.5 Objectives

General objective

The general objective was to study the inter- and intra-morphological variability, seed viability, and antimicrobial properties of *Ziziphus* Mill. and *Balanites* Del., as well as identify habitat suitability of both genera under climate change in Ethiopia.

Specific objectives

This study was specifically designed:

1). To measure the morphological variability between and within the species of Balanites and *Ziziphus*,

2). To identify the main environmental factors determining the morphological variability between and within the species of *Balanites* and *Ziziphus*,

3). To identify the best pre-sowing treatments that enhance the seed germination of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca*, and *B. rotundifolia*,

4). To investigate in-vitro the susceptibility of selected pathogenic bacteria to the alcoholic leaves extracts of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca*, and *B. rotundifolia*,

5). To model the habitat suitability of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca*, and *B. rotundifolia* under the current and future (SSP245 & SSP585/HadGEM3-GC31-LL) climates in Ethiopia.

1.6 Significance of the Study

This study examined and analyzed the morphological variability in *Balanites* and *Ziziphus*, aiming to contribute to future selection, breeding, and conservation programs for these species in Ethiopia. Additionally, the study identified the most effective seed pre-sowing treatment to enhance seed germination for *Z. spina-christi*, *Z. mucronata*, and *B. aegyptiaca*, aiming to contribute to the effective propagation, conservation of these species. Furthermore, the study confirmed the antibacterial properties of *Z. spina-christi* and *B. aegyptiaca*, which are crucial findings for developing therapeutic drugs, particularly antibiotics, derived from these plants. Lastly, the study predicted the habitat suitability of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca*, and *B. rotundifolia* under climate change in Ethiopia. This information will play a significant role in the future conservation and sustainable utilization of these species under changing climatic conditions in Ethiopia.

1.7 Scope of the Study

This study primarily focuses on analyzing the morphological variability of *Ziziphus* Mill. and *Balanites* Del., evaluating their seed germination behavior and antibacterial activities, and modeling the habitat suitability of these Genera in Ethiopia. The study is geographically delimited to the semi-arid ecosystems of Ethiopia and partly to the arid ecosystems along the altitudes of 500 to 2,300 (-2,500) m.a.s.l. (**Figure 1**). The areas are where *Ziziphus* and *Balanites* widely occur (Hedberg & Edwards, 1989; Azene Bekele-Tesemma, 2007; Orwa et al., 2009). The average annual rainfall of semi-arid (- arid) areas of Ethiopia ranges from 200 to 700 mm (-1600 mm), while the aridity index (AI), according to Kidane Georgis et al. (2010) as calculated by the Penman method (Doorenbos & Pruitt, 1977), ranges from 0.05 to 0.5. Additionally, the mean annual temperature falls between 22–35°C.

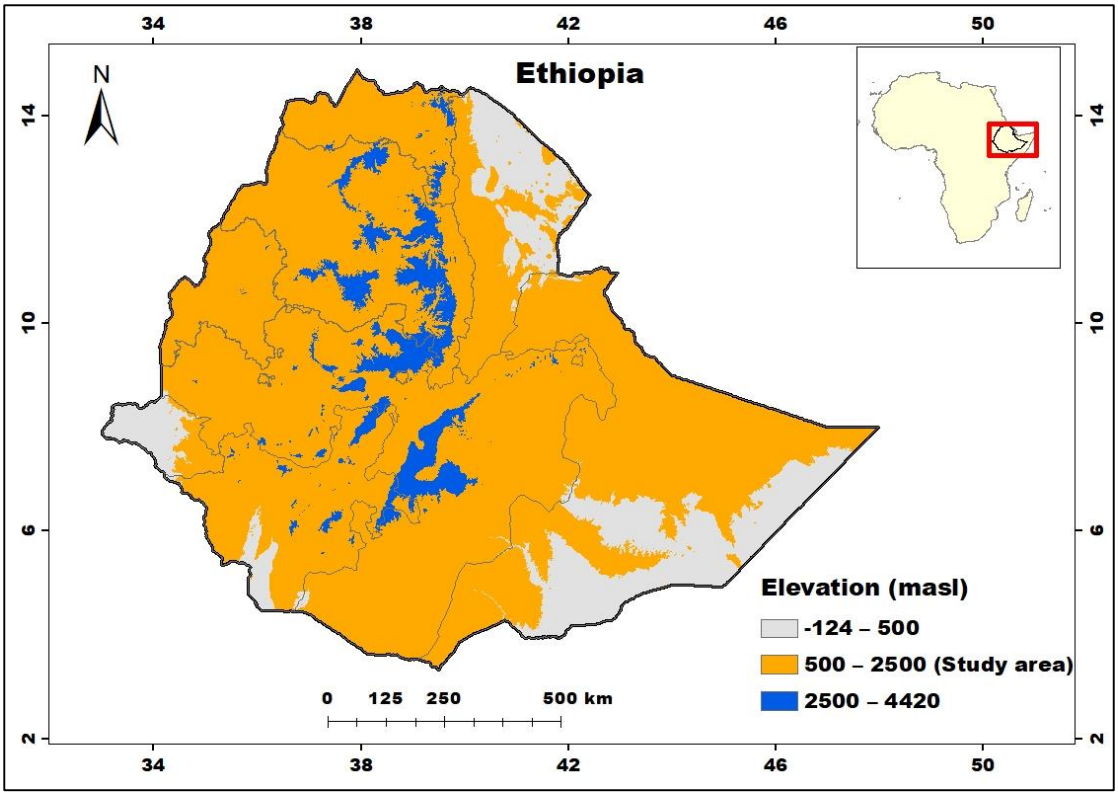

Figure 1. Map of Ethiopia showing the areas of the study with altitudinal ranges.

The arid and semi-arid areas in Ethiopia account for 50–55% of the total area of Ethiopia (Mulugeta Lemenih & Demel Teketay, 2004). The semi-arid ecosystem covers about 27% of the landmass of Ethiopia (Melesse Temesgen et al., 2008). Both the arid and semi-arid areas in Ethiopia are characterized by high moisture stress (Melesse Temesgen et al., 2008) and food insecurity (IGAD & FAO, 1995). Thus, the scope of the study encompasses the lowland areas ("*Kolla*") with an altitude of 500 to 1,500 m.a.s.l. and the midland areas ("*Woina-Dega*") of Ethiopia with an altitude of 1,500 to 2,300 m.a.s.l. This includes the Ethiopian Rift Valley, the drylands of Northeastern, North-western, Northern, Southern, and Eastern Ethiopia, which included the *Vachellia-Commiphora* woodland and bushland proper, the *Vachellia* wooded grassland of the Ethiopian Rift Valley, and the *Combretum-Terminalia* woodland wooded grassland ecosystems of Ethiopia (Friis et al., 2010).

1.8 Limitations of the Study

One of the limitations of the study is that it did not include one of the rarest species of *Balanites*, namely *B. pedicellaris,* in the morphological characterization of *Balanites* because of the absence of voucher specimens in the herbarium and/or fresh samples not found during the field survey and specimen collections. Moreover, the sample size (i.e., specimens) of *Z. hamur* was small, as it appeared to have one of the rarest species of *Ziziphus* that was not easily found in the field. As a result, as many specimens as possible were not included in the morphological characterization of *Z. hamur*. This study did not include a phytochemical characterization or molecular elucidation of bioactive compounds from *Ziziphus* and *Balanites*. This could be considered one of the limitations of this study. The other possible limitation is that the species distribution models of *Ziziphus* and *Balanites* are based one global climate model, namely HadGEM3-GC31-LL. Moreover, due to its rarity, a smaller sample size of occurrence data was used to model the habitat suitability for *B. rotundifolia*. This is the other possible limitation of the study.

1.9 Structure of the Dissertation

This dissertation work is consisting of seven (7) chapters.

Chapter 1: Introduces the general background of the study and presents basic concepts and methods of phenetic classification, the biology of seed germination, antimicrobial properties of plants, and species distribution models, as well as their contributions to the conservation and sustainable utilization of species. Finally, it presents the overall research objectives and provides a description of the study area.

Chapter 2: Analyzes the morphological variability between and within the species of *Ziziphus* Mill. and *Balanites* Del. It addresses the questions of what major morphological characters play important roles in the variability between and within the species of *Ziziphus* and *Balanites.* It also addresses the question of what main environmental variables significantly constrain the morphological variability between and within the species of *Ziziphus* and *Balanites*. It reveals the presence of sufficient inter- and intra-specific variability among the species of *Ziziphus* and *Balanites* in Ethiopia. It discusses the different morphotype cluster groups of *Ziziphus* and *Balanites* in relation to the Flora of Ethiopia and other literature. Thus, it also

discusses the most important morphological characters that play a significant role in the variability among the species of *Ziziphus* and *Balanites*. It also reveals and discusses the effects of bioclimatic and landscape variables on the morphological variability between and within the species of *Ziziphus* and *Balanites*.

Chapter 3: Evaluates the seed germination performance of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) under different pre-sowing treatments. It addresses the question of what types of pre-sowing treatments could enhance the seed germination percentages of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*). It also addresses the question of how significantly the pre-sowing treatments affect the germination time of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) compared to the untreated seeds. It reveals and discusses how mechanical treatments (e.g., cracking and rubbing) and chemical treatments (e.g., soaking in cold water for one or two days, in hot water at 65–75 ^{o}C for a few minutes, and in 98% H_2SO_4 for a few minutes) of the seed stones significantly enhance the germination potential of *Z. spina-christi* and *Z. mucronata*. It also reveals and discusses how chemical treatments (e.g., soaking in 98% H_2SO_4 and hot water at 65–75 ^{o}C for a few minutes) of the seeds significantly enhance the germination potential of *B. aegyptiaca*. One of the manuscripts produced for this chapter is published in *Scientifica*, and the publication can be accessed at https://doi.org/10.1155/2023/5571489.

Chapter 4: Evaluates the antibacterial activities of ethanol and methanol extracts of the leaves of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca*, and *B. rotundifolia* against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using agar-well diffusion. The chapter reveals the potential inhibitory properties of *Z. spina-christi* and *B. aegyptiaca* against pathogenic bacteria. It shows and discusses that *Z. spina-christi* and *B. aegyptiaca* extracts showed clear potency at concentrations below 500 mg/mL against the tested bacteria.

Chapter 5: Addresses the species distribution models of *Ziziphus* (e.g., *Z. spina-christi* and *Z. mucronata*) and *Balanites* (e.g., *B. aegyptiaca* and *B. rotundifolia*) under the changing climate in Ethiopia. It predicts the habitat suitability of *Z. spina-*

christi and *Z. mucronata* using six modeling options (i.e., GLM, GAM, BRT, RF, MARS, and SVM). It estimates and discusses the suitable habitats of *Z. spina-christi* and *Z. mucronata* in Ethiopia based on the ensemble SDMs under the current and future (i.e., SSP2-45 and SSP5-85 of HadGEM3-GC31-LL) climates. The chapter discusses how bioclimatic variables, mainly pH, Precipitation of Driest Quarter (Bio17), Precipitation of Wettest Month (Bio13) and elevation for *Z. spina-christi*, and clay, sand, silt, and BD for *Z. mucronata*, affect their distribution. Moreover, the chapter discusses how bioclimatic variables, mainly Temperature Annual Range (Bio07) and Precipitation Seasonality (Bio15) for *B. aegyptiaca*, and Bio17 and Annual Precipitation (Bio12) for *B. rotundifolia*, affect their distribution. One of the manuscripts produced for this chapter is published in the *International Journal of Ecology*, and the publication can be accessed at https://doi.org/10.1155/2024/6656529.

Chapter 6: Presents the general discussion. It discusses the overall findings, their importance, and relevance in this field of study. Generally, it interprets and discusses the overall findings of the study in light of previously reported research reports on similar subject areas, emphasizing the importance of the research objects in the context of the study.

Chapter 7: Presents the general conclusions and recommendations. It summarizes the study, highlighting the implications and contributions of the overall findings of the study.

Chapter 2

2. Inter- and Intra- Specific Variability of Ethiopian *Balanites* Del. and *Ziziphus* Mill. Based on Morphological Traits

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Abstract

Morphological characters are important sources of data that provide reliable evidence for evolutionary studies. This project aimed at evaluating the inter- and intra-specific morphological variability s among Ethiopian species of *Balanites* and *Ziziphus*. Up to three individual plant specimens per population were collected between January 2019 and June 2022. Sampling was done at a distance of 5–10 km and 50–100 m off the roadside. Bark color, leaf shape, leaf texture, fruit shape, and spine condition were encoded and examined. Mean annual precipitation, soil, and landscape were extracted from WorldClim.v2, ISRIC, and FAO's terrain databases, respectively. Hierarchical clustering (HC) and principal component analysis (PCA) ordination in R 4.1.3 were used to analyze the data. Redundancy analysis (RDA) was computed to determine the effects of environmental factors on morphological traits. The HC resulted in five and eight population clusters of *Balanites* Del. and *Ziziphus* Mill., respectively. Analysis of similarity (ANOSIM) indicated the presence of significant dissimilarity among cluster groups of *Balanites* (p=0.001; R=0.763) and *Ziziphus* (p=0.001; R=0.938). PCA1 and PCA2 indicated that fruits shape (FrSh) and spine bearing condition (SpB) followed by flower merosity (FloM) are the most important characters contributing to variability among the species of *Balanites*. Whereas, leaf base symmetry (LBSym) followed by bark color (BarkCol), and leaf surface pubescence (LSurPub) for the species of *Ziziphus*. RDA indicated that the effects of Annual Mean Temperature (Bio01), Isothermality (Bio03), and Precipitation of Wettest Quarter (Bio16) on morphological variability are highly significant ($p\leq0.001$) while the effects of Temperature Annual Range (Bio07) and Tpi are very significant ($p\leq0.01^{**}$) in *Balanites*. Similarly, the effects of Bio03, Temperature Seasonality (Bio04), Min Temperature of Coldest Month (Bio06), Mean Temperature of Warmest Quarter (Bio10), Precipitation of Driest Month (Bio14), and Precipitation of Driest Quarter (Bio17) are highly significant ($p\leq0.001$) on the morphological variability of *Ziziphus* species, while Precipitation of Coldest Quarter (Bio19) have very significant contributions ($p\leq0.01$), and elevation and Tpi have significant contributions ($p\leq0.05$). Therefore, this study generally indicated the presence significant variability between and within the species of *Balanites* and *Ziziphus* in Ethiopia. Phylogenetic analyses using molecular data is recommended to further corroborate the importance of morphological data in elucidating classification and evolutionary history.

Keywords: *Balanites* Del.; Clustering; Morphometric; Ordination; Variability*: Ziziphus* Mill.

2.1 Introduction

Balanites Del. and *Ziziphus* Mill. belong to the families Balanitaceae and Rhamnaceae, respectively. The Orders (Zygophylales and Rosales, respectively), in which these families belong to, are placed in the same clade called Fabid (APG IV, 2016). *Balanites* is represented by nine species that are widely distributed in India and Burma, throughout Africa, Arabian Peninsula and the Jordan valley (Hedberg & Edwards, 1989). In Ethiopia, four species of *Balanites* (*B. aegyptiaca, B. glabra, B. rotundifolia* and *B. pedicellaris*) occur where *B. aegyptiaca* (L.) Del. is the dominant and widely distributed species. The genus *Ziziphus* consists of 170 species mainly distributed in the tropical and subtropical regions (Liu & Cheng, 1995). *Z. spina-christi, Z. pubescens, Z. mucronata, Z. hamur, Z. abyssinica* and *Z. mauritiana* occur in Ethiopia (Hedberg & Edwards, 1989). *Z. spina-christi* (L.) Willd. and *Z. mucronta* Willd. are the most widely distributed species. Species of the two genera are known for their diverse use: such as sources of medicine, cosmetic, food, feed, income generating and energy needs. Both genera are good sources of saponin being potentially important to detergent industry.

There had been an attempt of characterizing different populations of *Balanites* species which recognized infra-specific taxa. For example, Sands (2001) identified nine species and eleven intra-specific taxa of *Balanites* for specimens collected from Ethiopia, Tanzania, Somalia, Senegal, Congo, Zambia, Egypt and Saudi Arabia. This classification was based on spine length, leaflet shape, indumentum density of leaflet and flowers, pedicel length, and others (Sands, 2001; Hamada et al., 2022).

Plants show variability due to several factors that cause differences in their appearance. Environmental conditions such as variability in geological, climatic, biome and socio-cultural environments affect morphological and physiological traits of plants (Sultan, 1995; Robakowski et al., 2003; Pescador et al., 2015; Qi et al., 2020). They tend to adjust the expression of traits such as morphology, rate of growth, functions, and metabolism to persevere their adaptability in diverse environments (Bradshaw, 1965; Berli et al., 2013; Gratani, 2014; Qi et al., 2020). Species of *Balanites* and *Ziziphus* occur in semi-arid and arid ecosystem, which are highly dynamic and fragile systems experiencing extreme climate and environmental

fluctuations. Therefore, as a mechanism of adaptation high level of inter-and infra-specific morphological variability are exhibited by species of the two genera. Morphological characters are important sources of data to elucidate evolutionary histories (Carlquist, 1961; Radford et al., 1974; Stuessy, 1990; Judd et al., 2008; Ningrum & Chasani, 2021), and thus, useful to infer relationships among taxa. Such morphology-based relationships among and within species can be detected through phenetic analyses and reconstruction of phenograms based on morphological characters (Ningrum & Chasani, 2021).

The Flora of Ethiopia (Hedberg & Edwards, 1989) delineated species of *Balanites* and *Ziziphus* using morphological features. However, little attempt has been done to undertake computational and comparative analyses to show closely related species as well as impact of different environmental variables on the morphological variability. Therefore, the general aim of this study was to evaluate the inter- and intra-specific morphological variability, and reconstruct the phenetic relationships among the species of *Balanites* and *Ziziphus* in Ethiopia. Therefore, the study also aimed at identifying main morphological characters playing the major role to variability among species of *Balanites* and *Ziziphus*, as well as identifying main environmental factors determining the morphological variability among species of *Balanites* and *Ziziphus*.

2.2 Materials and Methods

Study area

The study is delimited to the Ethiopian Rift Valley, the drylands of Northeastern, Northwestern, Northern, Southern and Eastern Ethiopia (**Figure 2**). It includes the *Vachellia-Commiphora* woodland and bushland proper, *Vachellia* wooded grassland of the Rift Valley; and *Combretum-Terminalia* woodland and wooded grassland ecosystems of Ethiopia (Friis et al., 2010).

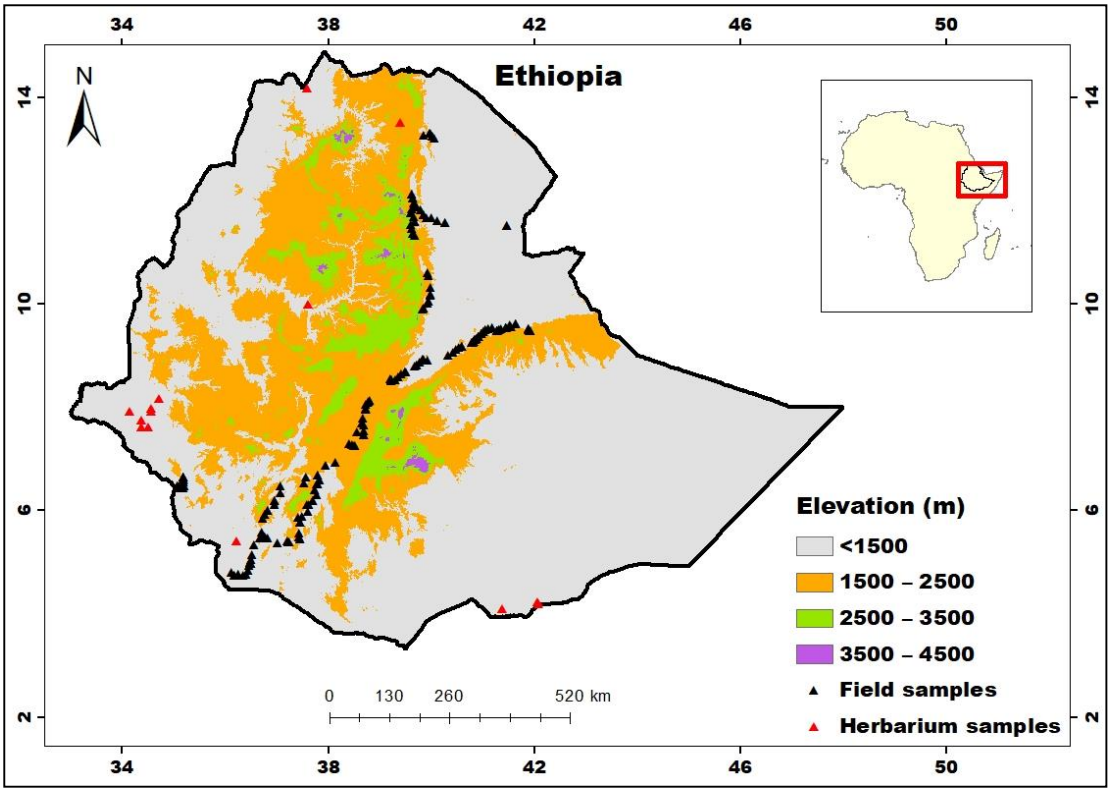

Figure 2. Map of the study area and sampling sites with 239 field and 14 herbarium samples representing 123 and 130 specimens of Balanites and Ziziphus, respectively.

Sampling, processing, and identification of plant specimens

Although *Balanites* is recognized as an evergreen plant, it may shed foliage when the effects of the dry season become pronounced (Wickens, 1976), and start flowering during the onset of dry season (Orwa et al., 2009). Depending on the pattern of precipitation (unimodal or bimodal), flowering and fruiting often occur once or twice a year (Wickens, 1976). *Ziziphus*, co-occurs with *Balanites*, is assumed to follow similar pattern of phenology. Therefore, plant specimen collection was done in two phases: (i) between January 2019 and October 2020 (collections during this period was made by Dr. Tigist Wondimu for the purpose of phylogeography study by herself), and (ii) between January and June 2022. Sampling and collection of plant specimens for populations of individual species was done over 50 meters off the roadsides in every 5–10 kilometers (**Figure 3**); and up to 3 sample specimens were collected in 5m spacing to represent individual populations.

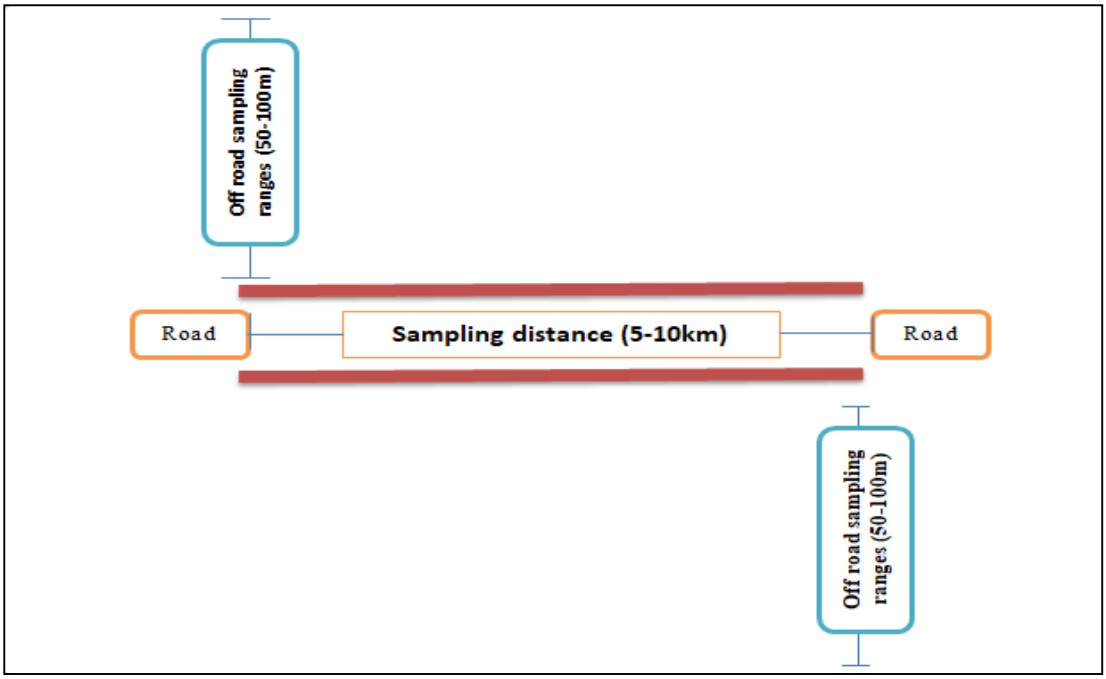

Figure 3. An illustration depicting the method of sampling plant specimens off the roadsides along the road.

Species such as *Z. pubescens*, *Z. hamur* and *B. pedicellaris* were not found during the field visits. Therefore, morphometric data for *Z. pubescens* and *Z. hamur* species were extracted from herbarium voucher specimens. But no data was extracted for *B. pedicellaris* due to the specimen conditions. Moreover, for species with smaller sample size (e.g., *Z. abyssinica*), additional herbarium samples were examined to obtain representative data. Plant specimens collected in the field were pressed and transported to the National Herbarium of Ethiopia (ETH), Addis Ababa University (AAU). Specimens were then processed, identified and labeled in consultation with previously collected herbarium specimens and the Flora of Ethiopia (Hedberg & Edwards, 1989), and were deposited in the National Herbarium for morphological characterization and further phenetic analysis.

Characters selection, measurement and coding procedure

Characters were selected carefully and rigorously to allow validity for character selection and analyses following Kraus (1988), Hufford and Dickson (1992), and Hoot et al. (1994). Accordingly, characters that have discontinuous variations (i.e., discrete) within the study group were selected and examined (Archie, 1985; Thiele, 1993; Strait et al., 1996). Some morphological characters show discrete or binary states, which are relatively straightforward to code while others are less clearly

defined and show intermediate states (Stevens, 1991). This is especially true for continuous data values that can be used to develop binary or multistate character codes (Archie, 1985). In this study, five morphological character groups for *Balanite*s (i.e., growth habit, spine, leaves, flower, and fruit) and four morphological character groups for *Ziziphus* (i.e., growth habitat, spine, bark, and leaf) were used (**Annex 1**) for phenetic analyses and classification following Hedberg and Edwards (1989), Sands (2001) and Islam and Simmons (2006). Only mature plant parts were used to generate the morphological data. A micro-morphological character such as leaf pubescence type and margin type were examined using a magnifying hand lens and manuals of leaf morphology and anatomy. Leaf length, width, petiole length, inter-node length and spine length were measured using a Vernier caliper, and the average values representing the given specimen (or the population) were taken for coding purpose. For discontinuous characters, the states that occur most often in each specimen were taken as the states representing a given population. Therefore, an arbitrary character state delimitation (i.e., binary and/or multistate) was used to assign numerical codes to the different character state observed (Wiens, 2001). For continuous characters, following scaling by among-group and within-group variability, range-scaling using standard deviation was employed for coding (Thorpe, 1984). Thus, a discretized continuous character was employed for characters such as leaf length, leaf width, petiole length, inter-node length and spine length (**Annex 1**). The naming of the terminal taxa or operation taxonomic units (OTUs) (i.e., populations) were defined by the population names (i.e., initial of the genus name and the three consecutive letters from specific epithet, e.g., *B.aeg* for *B. aegyptiaca*) followed by the location of the sampling (district (or collection number), zone and region) and order number (1, 2, 3, etc.) in reference to the district from which the samples were collected (**Annex 2**). Thus, a data matrix comprised of both the discrete and discretized continuous morphological data was produced to generate the dendrogram.

Environmental variables

Moreover, the present values of 42 environmental variables, namely bioclimatic variables (i.e., Bio01–Bio19), elevation, solar radiation (Srad), and topographic index (Tpi); and soil properties at 30-60cm depth (e.g. proportions of soil clay, silt and sand particles in g/100g (%), and soil pH, Cation Exchange Capacity (CEC) in cmol(c)/kg,

soil organic carbon content (SOC) in g/kg and bulk density in kg/dm³); and landscape variables (e.g. aspects and slopes) were extrapolated from the WorldClim version 2.1 climate data (Fick & Hijmans, 2017), the International Soil Reference and Information Centre - ISRIC World Soil Information (Poggio et al., 2021) and FAO's terrain database (Fischer et al., 2008), respectively. Variables sharing the higher variance (i.e., >80% total variance) were identified using PCA as recommended by Manly (1994) and Cruz-Cárdenas et al. (2014), and less collinear ones ($r \leq 0.6$) were selected (De Marco & Nóbrega, 2018).

Data analysis

A total of 123 specimens of *Balanites* (101 for *B. aegyptiaca*, 19 for *B. rotundifolia*, and 3 for *B. glabra*) representing 100 populations, and 130 specimens of *Ziziphus* (67 for *Z. spina-christi*, 21 for *Z. mauritania*, 26 for *Z. mucronata*, 6 for *Z. abyssinica*, 7 for *Z. pubescens*, and 3 for *Z. hamur*) representing 114 populations were examined (**Annex 2**). Analysis of data and interpretation of findings were based on the data matrix (discrete and discretized continuous morphological data). The inter- and intra-specific morphological variability among the species of *Balanites* and *Ziziphus* were analysed using agglomerative hierarchical clustering with "*Euclidean*" distance and "ward. D2" methods in R 4.1.3. The degree of morphological dissimilarity among population clusters was also determined using box-potting. In box-plotting R value closer to 1 normally indicated that the presence of high dissimilarity among cluster groups, while when closer to 0 implied an even distribution of high and low ranks within and between groups (Clarke & Gorley, 2001). In other words, the closer the R value is to 1, the more similar the populations within clusters are to each other and dissimilar to the populations in other clusters. When the sum of intra-cluster distance gets shorter, then the sum of inter-cluster distance gets longer for a given dataset. As the rule of thumb introduced by Lepš and Šmilauer (2003) the Detrended Correspondence Analysis (DCA) was computed on environmental data to determine the ordination type to employ. The length of the first DCA axis was found to be less than three (<3) indicating a homogeneous dataset from dryland ecosystem for which linear ordination methods (e.g., RDA) are suitable to determine the gradients of morphological changes among the sampled populations. Hence, principal component analysis (PCA) ordination (unconstrained and indirect gradient analysis) was also computed in R 4.1.3 to determine the main morphological parameters contributing to

the morphological variability among the populations of *Balanites* and *Ziziphus*. Moreover, constrained Redundancy Analysis (RDA) was performed to determine the effects of environmental factors on the morphological variability among the populations of *Balanites* and *Ziziphus*.

2.3 Results

Morphological variability between and within the species of Balanites and Ziziphus

The hierarchical clustering indicated five possible cluster groups (morphotypes) consisting of different populations of *Balanites* species (**Figure 4**, **Figure 5**). Cluster-1 included 3 populations of *B. glabra*, Cluster-2 consisted of 24 populations of *B. aegyptiaca*, Cluster-3 consisted of 54 populations of *B. aegyptiaca*, Cluster-4 includes 14 populations of *B. rotundifolia*, and the remaining five populations of *B. rotundifolia* placed in Cluster-5.

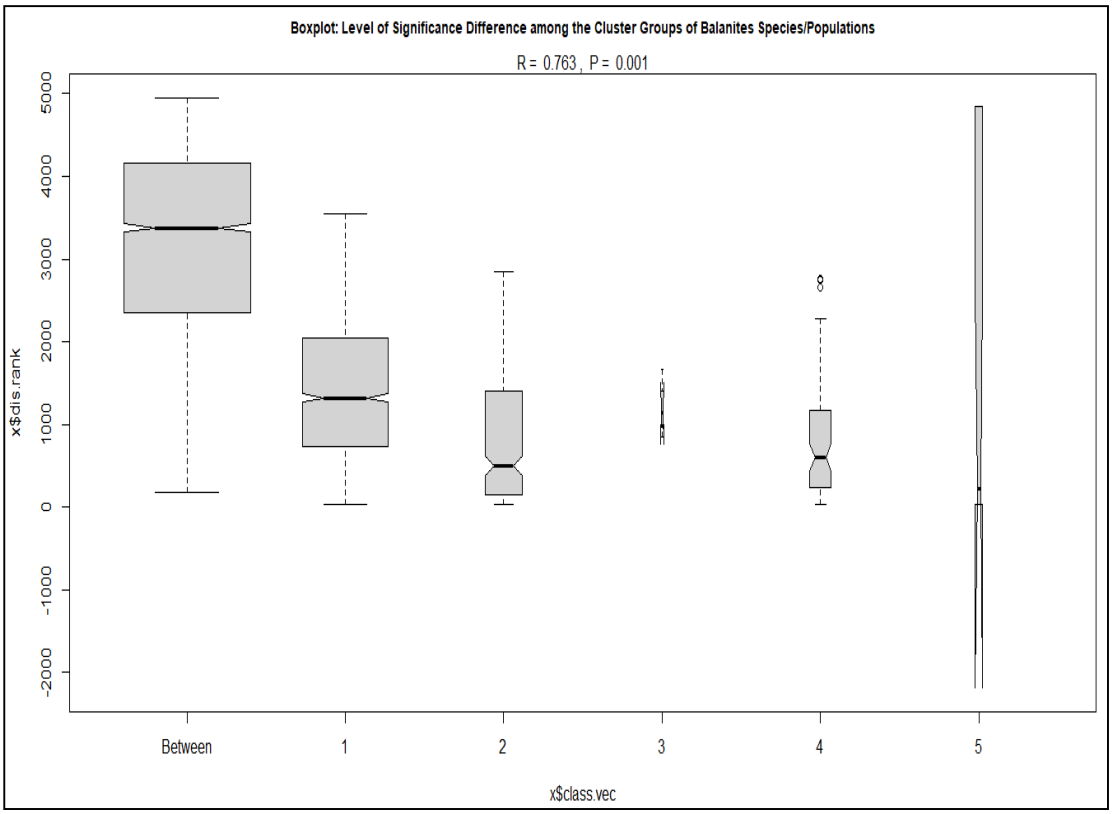

Figure 4. A boxplot showing the dissimilarity ranks between and within classes in *Balanites* populations (Number of permutations=999, *p*=0.001).

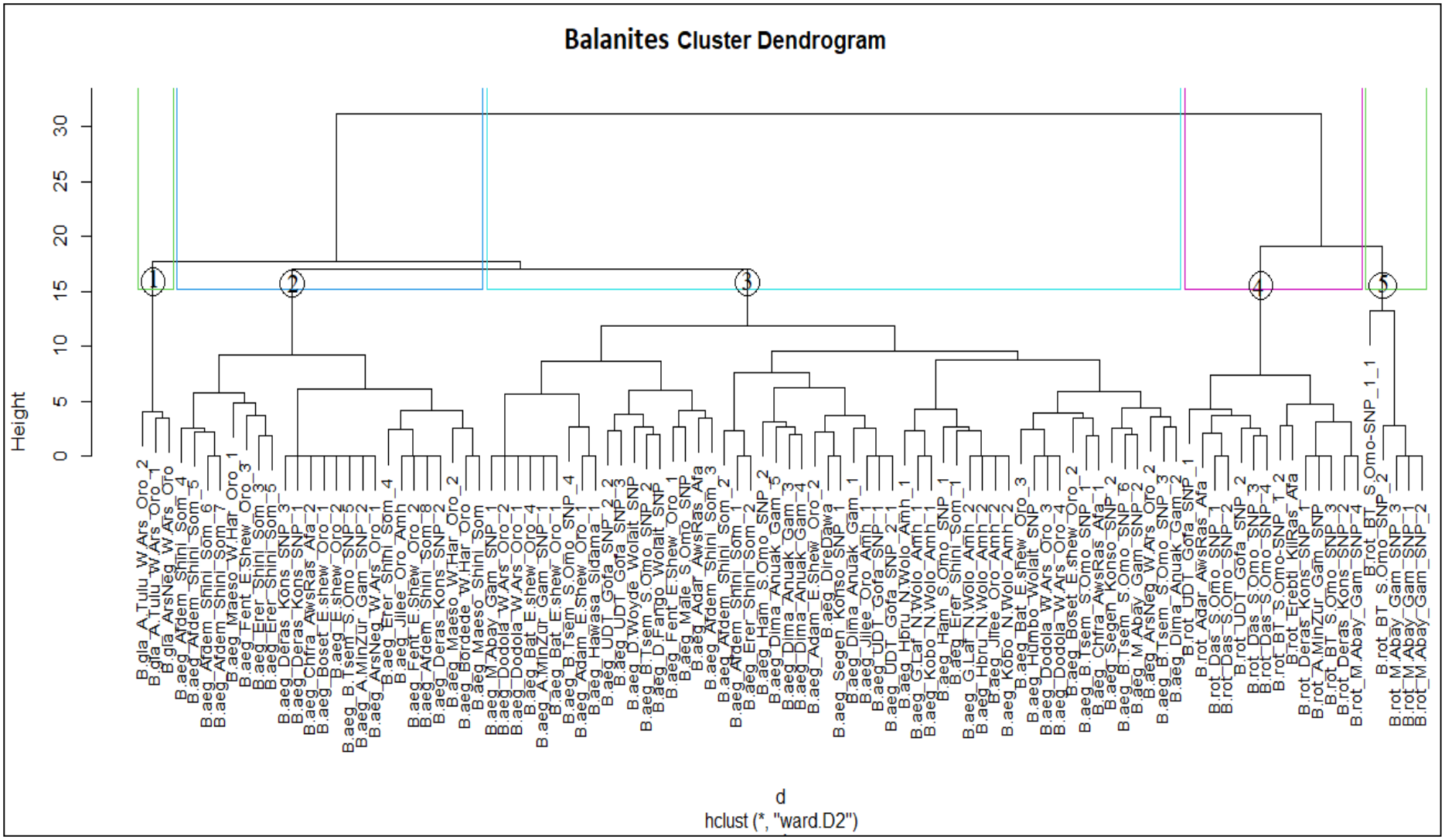

Figure 5. Hierarchical clustering of the populations of *Balanites* species based on dissimilarity matrix of scaled data using Euclidean distances and Wards.D2 method.

Analysis of similarity (ANOSIM) indicated the presence of statistically high dissimilarity among cluster groups of *Balanites* at *p*=0.001 (R=0.763) (**Figure 4**). *B. aegyptiaca* and *B. rotundifolia* formed two clusters each (**Figure 5**). In particular, the hierarchical clustering grouped *B. aegyptiaca* and *B. rotundifolia* into two population clusters each with highly significant inter-clusteral dissimilarities in terms of morphometric variables at *p*=0.001 (R=0.763). Cluster-2 in figure 5 comprised of populations of *B. aegyptiaca* possessing shorter and narrower leaves with abaxial subglabrous and adaxial glabrous surface, and denser and longer spine complexity; while Cluster-3 comprised of populations of *B. aegyptiaca* possessing longer and broader leaves with abaxial glabrous and adaxial glabrous surface, and less dense and shorter spine complexity.

The hierarchical clustering for *Ziziphus* populations formed eight possible clusters (morphotypes) containing different populations of *Ziziphus* (**Figure 6**, **Figure 7**). The six species analyzed in this study are grouped in eight clusters where *Z. spina-christi* (Cluster-3 and 4) and *Z. mauritania* (Cluster-2 and 5) form two distinct clusters with statistically significant inter- and intra-clusteral dissimilarities (R = 0.938, *p*=0.001).

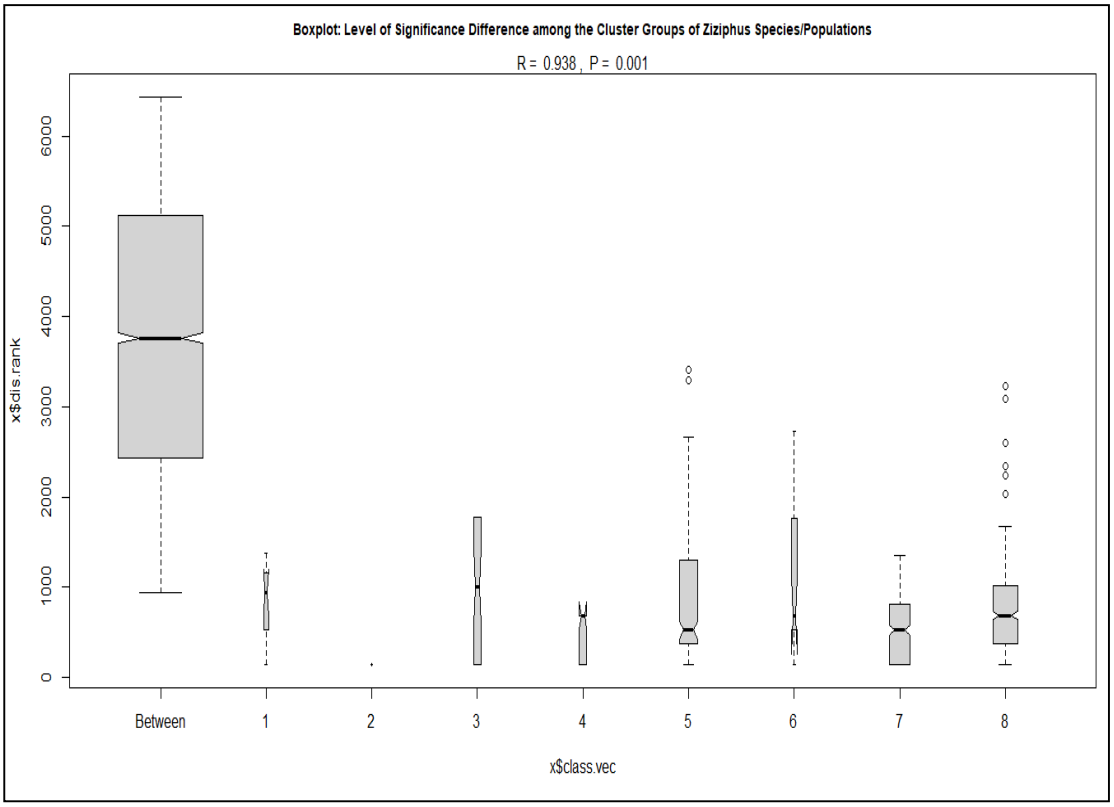

Figure 6. A boxplot showing the dissimilarity ranks between and within classes in *Ziziphus* populations (Number of permutations=999, *p*=0.001).

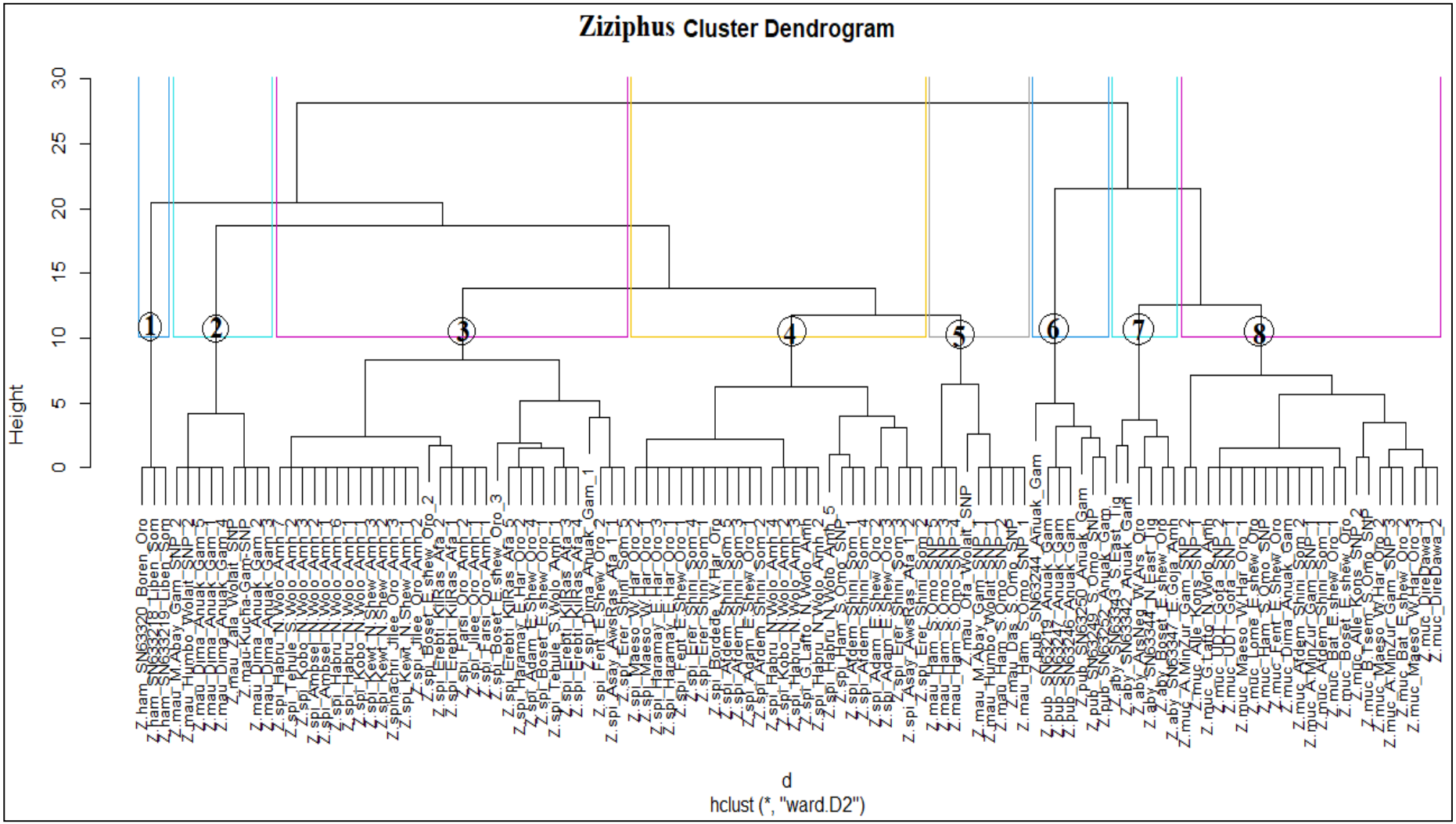

Figure 7. Hierarchical clustering of the populations of *Ziziphus* species based on dissimilarity matrix of scaled data using Euclidean distances and Wards.D2 method.

Cluster-3 in figure 7 comprises of populations of *Z. spina-christi* possessing mainly shorter and narrower and ovate leaves with abaxial glabrous and adaxial glabrous surface, while Cluster-4 comprises of populations of *Z. spina-christi* possessing predominantly longer and broader and elliptical leaves with abaxial subglabrous and adaxial glabrous surface. Each of the remaining species represented by distinct cluster. Moreover, the inter- and intra-cluster dissimilarities, or ANOSIM, indicated the presence of significantly high dissimilarity among cluster groups (**Figure 7**). Hence, the ANOSIM statistics appeared to have higher R value (R=0.938, *p*=0.001). The two clusters of *Z. spina-christi* (Cluster-3 and 4) and *Z. mauritania* (Cluster-2 and 5) also show high inter-clusteral dissimilarities (R=0.938, *p*=0.001).

PCA for morphological variability between and within Balanites and Ziziphus

The bivariate correlations between the morphometric parameters are indicated in figure 8 below.

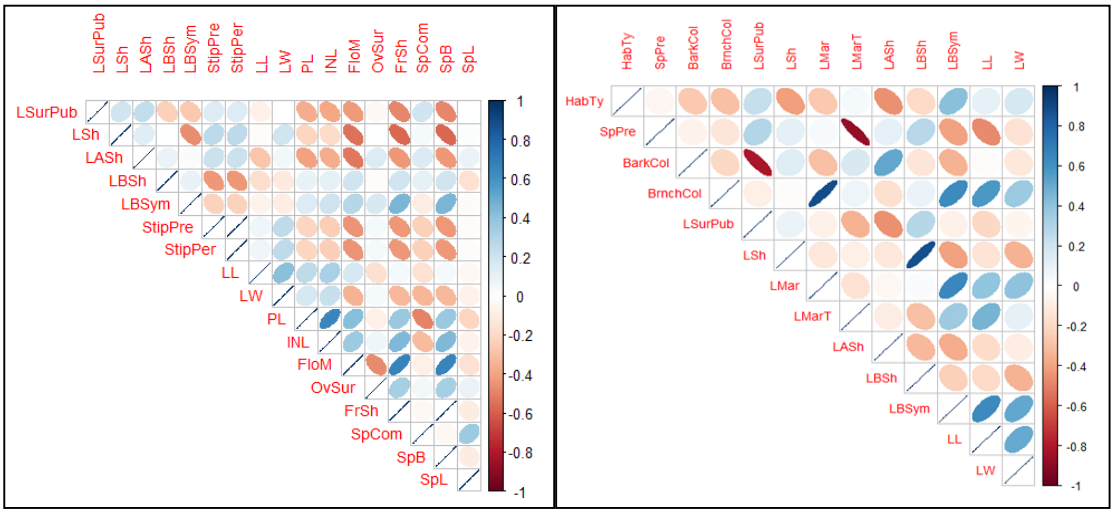

Figure 8. The bivariate correlation using R "ellipse" (r= -1 to 1) between most important morphometric parameters clustering the populations of *Balanites* (Left) and *Ziziphus* (Right).

In the figure 8, the degrees of correlation are presented by colored circles, in which their color represents the direction of correlation (positive correlation in blue and negative correlation in red). The intensity of the colors (e.g., very light blue, intermediate blue and deep blue) show the degree of correlations (i.e., 0 to 1).

PCA for *Balanites* populations (**Figure 9**) showed that the first five highly contributing variables in PCA1–PCA2 include FrSh (10%), SpB (10%), FloM (9%), StipPer (7.5%), and StipPre (7.5%). The PCA for *Ziziphus* populations indicated that leaf base symmetry (LBSym) was very important parameters followed by bark color (BarkCol), and leaf surface pubescence (LSurPub), while the least contributions were obtained from leaf shape (LSh) (**Figure 9**).

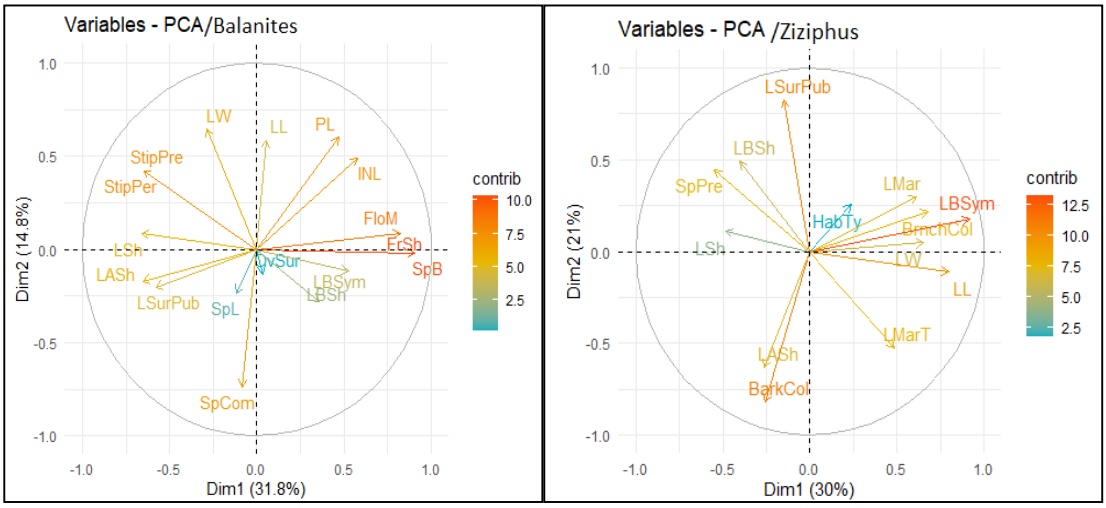

Figure 9. Variance contribution of morphometric parameters under PCA1–PCA2 of the dataset towards the variability of the populations of *Balanites* (Left) and *Ziziphus* (Right).

RDA for morphological variability between and within Balanites and Ziziphus

Out of the 42 environmental variables tested, the effects of Bio01, Bio03, and Bio16 on morphological variability of *Balanites* are highly significant ($p \leq 0.001$) while the effects of Bio07 and Tpi are very significant ($p \leq 0.01$) (**Table 1**).

Table 1. RDA for only significant environmental variables effecting the morphological variability of *Balanites* (permutations=999, p≤0.05).

	Df	***Sums of Sqs.***	***R^2***	***F***	***Pr(>F)***
Bio01	1	0.126	0.107	16.001	0.001***
Bio03	1	0.113	0.096	14.39	0.001***
Bio04	1	0.017	0.014	2.115	0.112
Bio07	1	0.040	0.034	5.10	0.008**
Bio16	1	0.100	0.085	12.68	0.001***
Bio17	1	0.0056	0.0047	0.71	0.511
Tpi	1	0.054	0.0456	6.86	0.003**
AspectClE	1	0.009	0.008	1.17	0.276
Sand	1	0.005	0.004	0.69	0.512
pH	1	0.004	0.003	0.53	0.64
Residual	60	0.7	0.59		
Total	99	1.18	1.0		

(Highly significant '***' if $p \leq 0.001$, Very significant '**' if $p \leq 0.01$, Significant '*' if $p \leq 0.05$)

In table 1 above, Bio01 = Annual Mean Temperature, Bio03 = Isothermality (Bio2/Bio7), Bio04 = Temperature Seasonality (Standard Deviation), Bio07 = Temperature Annual Range (Bio5-Bio6), Bio16 = Precipitation of Wettest Quarter, Bio17 = Precipitation of Driest Quarter, Tpi = Topographic index, AspectClE = Aspect: East: $45^{\circ} < \text{aspect} \leq 135^{\circ}$).

Similarly, the effects of Bio03, Bio04, Bio06, Bio10, Bio14, and Bio17 are highly significant ($p \leq 0.001$) on the morphological variability of *Ziziphus* species, while Bio19 has very significant contributions ($p \leq 0.01$), and elevation and Tpi have significant contributions ($p \leq 0.05$) to the morphological variability among *Ziziphus* (**Table 2**).

Table 2. RDA for only significant environmental variables effecting the morphological variability of *Ziziphus* (permutations=999, p≤0.05).

	Df	***Sums of Sqs.***	***R^2***	***F***	***Pr(>F)***
Bio02	1	0.008	0.002	0.31	0.783
Bio03	1	0.367	0.068	12.92	0.001***
Bio04	1	0.449	0.083	15.84	0.001***
Bio06	1	0.443	0.082	15.643	0. 001***
Bio10	1	0.198	0.037	6.97	0. 001***
Bio14	1	0.336	0.062	11.85	0.001***
Bio17	1	0.247	0.045	8.71	0.001***
Bio19	1	0.216	0.04	7.62	0.002**
Elevation	1	0.11	0.02	3.88	0.019*
Tpi	1	0.083	0.015	2.9	0.042*
AspectClS	1	0.045	0.008	1.57	0.226
CEC	1	0.021	0.004	0.73	0.530
Residual	101	2.86	0.53		
Total	113	5.39	1.0		

(Highly significant '***' if $p \leq 0.001$, Very significant '**' if $p \leq 0.01$, Significant '*' if $p \leq 0.05$)

In table 2 above, Bio02 = Mean Diurnal Range (Mean of monthly (max temp - min temp)), Bio06 = Min Temperature of Coldest Month, Bio10 = Mean Temperature of Warmest Quarter, Bio14 = Precipitation of Driest Month, Bio19 = Precipitation of Coldest Quarter, AspectClS = Aspect: South: 135˚ < aspect ≤ 225˚, CEC = Cation Exchange Capacity.

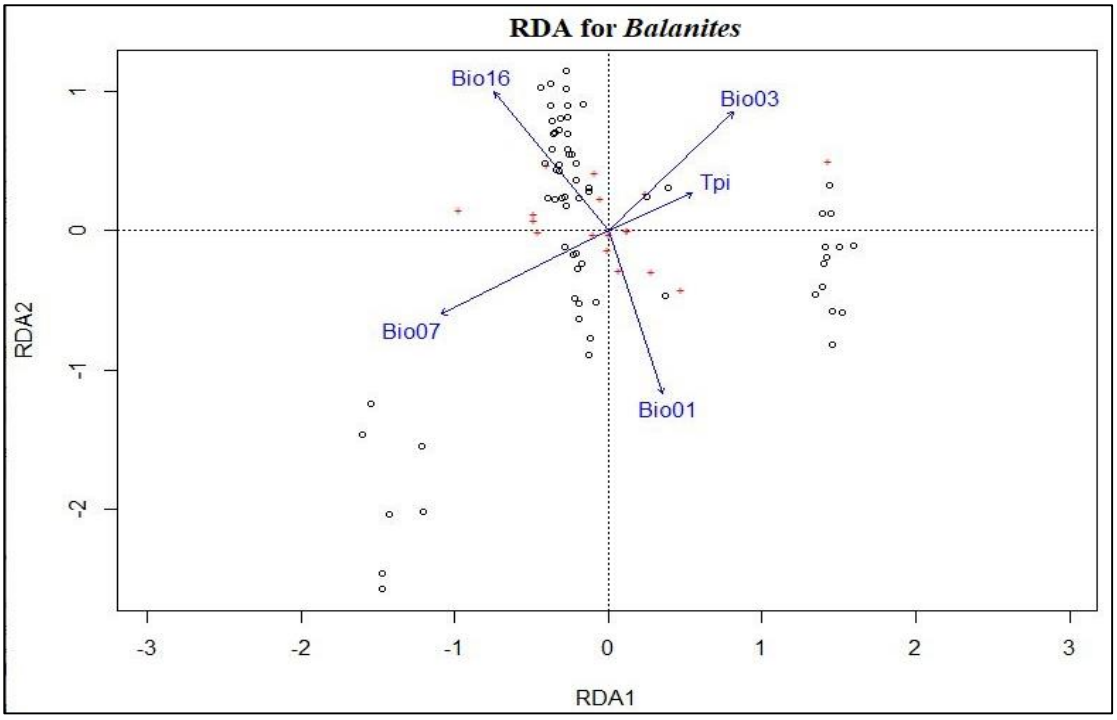

Figure 10. A constrained RDA biplot of the significant environmental variables, and the populations of *Balanites* species.

Figure 10 above and figure 11 below illustrated the constrained and direct gradient RDA analysis of environmental variables that constrained the morphological variability among the populations of *Balanites* and *Ziziphus* species, respectively. The circular dot plots are constrained scatterplots (clusters) of the populations of the different Balanites and Ziziphus species based on morphological variability.

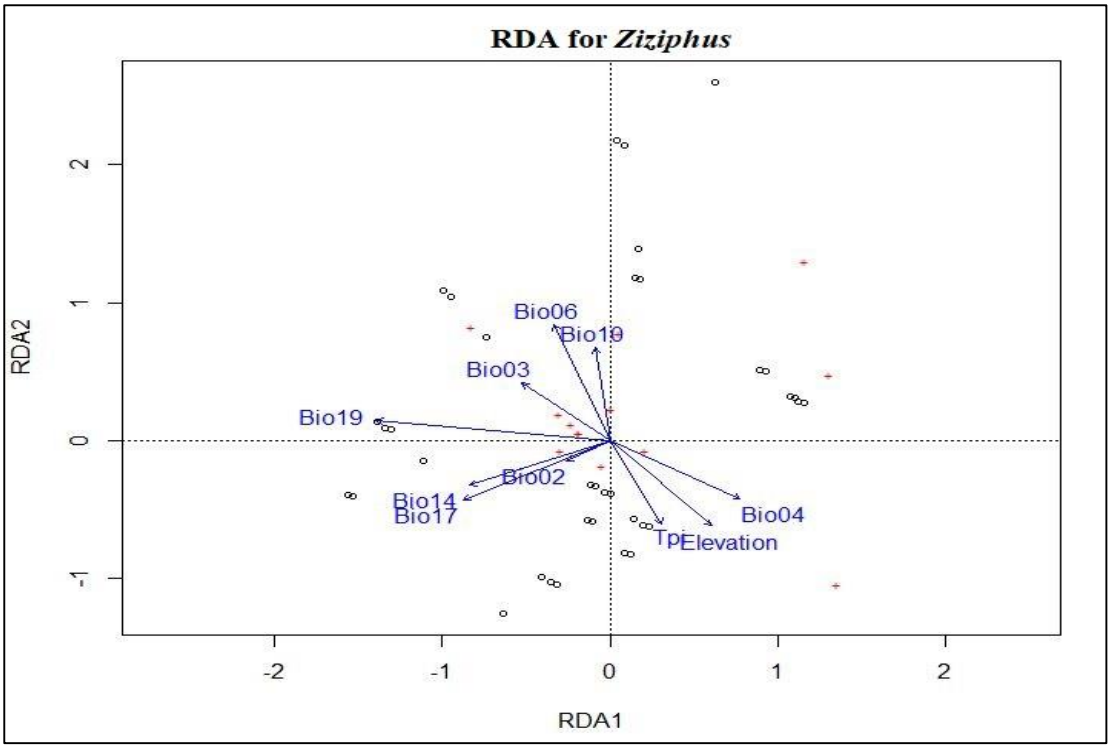

Figure 11. A constrained RDA biplot of the significant environmental variables, and the populations of *Ziziphus* species.

2.4 Discussions

Clusters formed as a result of morphometric parameter analyses indicated the existence of significant variability among species of *Balanites* and *Ziziphus*. This was supported by analysis of similarity (ANOSIM). The variability among the species of *Balanites* and *Ziziphus* is constrained by both temperate and precipitation-related variables, elevation, and topographic index. The populations of *B. aegyptiaca* and *B. rotundifolia* species form two clusters (Cluster-2 and 3 and Cluster-4 and 5, respectively). The two population clusters of *B. aegyptiaca* are differentiated mainly in their leaf width and pubescence (indumentum) and spine length and density. This finding supports the Flora of Ethiopia (Hedberg & Edwards, 1989) which identified and documented two varieties of *B. aegyptiaca* in Ethiopia; *B. aegyptiaca* var. *pallida* and *B. aegyptiaca* var. *aegyptiaca.* The first one is mainly characterized by having leaflet indumentum usually sparse and/or early glabrescent or minutely puberulous, and the later one is characterized by leaflet indumentum densely tomentellous. Hence, Cluster-2 is likely associated with *B. aegyptiaca* var. *pallida*, while Cluster-3 is likely associated with *B. aegyptiaca* var. *aegyptiaca*. Sands (2001) recognized five varieties

of *B. aegyptiaca* (i.e., *B. aegyptiaca var. aegyptiaca*, *B. aegyptiaca var. quarrei, B. aegyptiaca var. ferox, B. aegyptiaca var. pallida, B. aegyptiaca var. tomentosa*) for specimens collected from Ethiopia, Tanzania, Somalia, Senegal, Congo, and Zambia based on leaflet indumentum, flower positions (either at spineless or spinous node), leaflet shapes, pedicels length, petiole length, spine length, pedicels and calyx indumentum. Hence, Sands (2001) identified *B. aegyptiaca var. pallida* as the unique variety to Ethiopia. The discovery new and additional varieties by Sands (2001) is possibly due to the fact that specimens were examined from wider ecological ranges across East African countries. Another study by Kamal et al. (2020) reported some distinct populations of *B. aegyptiaca* in Sahelo-Sudanian zones possessing heavy and big fruits, long leaflets and petioles, and large sized spines. Similarly, Hamada et al. (2022) identified one and two varieties of *B. aegyptiaca* from Makkah (Saudi Arabia) and Egypt, respectively, using a comparative taxonomic study of the species based on morphological parameters such as leaflet shape, leaflet indumentum density, petiole length, etc. They indicated that the petiole length, leaflet length/width ratio, leaflet shape and apex, and leaflet indumentum played the profound contributions in the differentiation of *B. aegyptiaca* in Egypt and Saudi Arabia. In the present study, characters mainly leaf width and pubescence (indumentum) and spine length and density play a key role in differentiation of the two conspicuous population clusters of *B. aegyptiaca*. This demonstrated that *B. aegyptiaca* is widely distributed and dispersed in wider ecological ranges in semi-arid and arid ecosystems and show variability driven by ecological adaptation.

The findings in present study agree with the two varieties of Z. *spina-christi* (i.e., Z. *spina-christi* var. *mitissima* Chiov. and Z. *spina-christi* var. *microphylla* Hochst. ex A. Rich.) mentioned by the Flora of Ethiopia (Hedberg & Edwards, 1989). As the result, cluster-3 is seemingly related with Z. *spina-christi* var. *mitissima*, while cluster-4 is seemingly related with Z. *spina-christi* var. *microphylla*. Generally, Z. *spina-christi* var. *mitissima* is known by its whitish branchlets and larger leaves (approximately 3–9 cm long and 1–4 cm wide). The leaves have an ovate to lanceolate shape, with acute tip and prominent veins that run from the base to the tip. The lateral leaf veins are not very noticeable, and the leaf margins are slightly crenate. On the contrary, Z. *spina-christi* var. *microphylla* is characterized by being very bushy with brown-reddish branchlets, smaller leaves (approximately 1.5–3.5 cm long), mostly ovate-ellipsoid

leaf shapes, a rounded leaf apex, one central vein and conspicuous lateral veins, and an almost entire leaf margin. Similarly, Almalki and Alzahrani (2018) reported the occurrence of two varieties (*Z. spina-christi* var. *spina-christi*, *Z. spina-christi* var. *microphylla*) in Saudi Arabia. Moreover, Zarei et al. (2021) also indicated the presence of higher genetic diversity within *Z. spina-christi* was observed compared to the two other *Ziziphus* species (*Z. mauritiana* and *Z. nummularia*). Hedberg and Edwards (1989) also suggested that the wider presence and dispersion of *Z. spina-christi* and the occurrence of several morphotypes of *Z. spina-christi* could also be associated with the fact that the fruits are edible, and the stones are often dispersed by the local people and travellers. Dispersal of plant units by different vectors has a fundamental contribution in plant succession and co-existence by avoiding intraspecific competition, avoiding inbreeding and predation (Dirzo & Dominguez, 1986), and so affects the level of gene flow (Young et al., 1996), and influences local adaptations or speciation (Harrison & Hastings, 1996).

The current study also indicates for the presence of possible taxonomic ranks below species level under *Z. mauritania*. The first group (Cluster-2) is generally characterized with oval (round) leaf shape, round to ovate leaf-apex and oval (round) leaf-base, while the second group (Cluster-5) possesses an elliptical leaf shape, acute leaf-apex and cuneate to acute leaf-base. The dissimilarity between Cluster-2 & Cluster-5 of *Z. mauritania* is significant as illustrated by boxplot dissimilarity ranks ($R = 0.938$, $p=0.001$). *Z. mauritania* is reported to be high priority native fodder and fruit tree or shrub species in Afar and Somali regions (Wubalem Tadesse et al., 2012). The interactions with human and livestock likely increase the dispersal of seeds across a wider distances and ecological ranges contributing for its succession by avoiding intraspecific competition, avoiding inbreeding and predation. However, no taxonomic ranks under *Z. mauritania* are described by the Flora of Ethiopia (Hedberg & Edwards, 1989). The possible presence of taxonomic ranks below species level under *Z. mauritania* can be further verified following molecular approach.

The PCA1–PCA2 of morphometric parameters indicated that fruit (ovary) shape (FrSh) and leaves (or flowers) bearing spines (SpB) or naked are very important parameters playing for morphological diversity among *Balanites* followed by flower merosity (FloM). This finding fundamentally agrees with the Flora of Ethiopia

describing that the presence of SpB, followed by FrSh, naked or hairy ovary surface (OvSur), and FloM as the basal characters that played the profound effects to the dichotomous key and classification of the Genus *Balanites* (Hedberg & Edwards, 1989). The PCA1-PCA2 of morphometric parameters indicated that leaf base symmetry (LBSym) was very important parameters playing for morphological diversity among *Ziziphus* followed by bark color (BarkCol), and leaf surface pubescence (LSurPub). Overall, leaf morphological characters were important for classification of the Genus *Ziziphus*. This finding partly agrees with the Flora of Ethiopia in which the presence and absence of spines (SpPre) followed by the presence and absence of leaf marginal tooth (LMarT), the degree of leaf surface pubescence, branchlets color (BrnchCol), leaf shape (LSh), and leaf-base symmetry constituted the basal characters that played the profound effects to the dichotomous key and classification of the Genus *Ziziphus* (Hedberg & Edwards, 1989). In this regard, empirical studies also highlighted that leaf morphology and anatomy were highly varied plant part of angiosperms and proposed as most used as a classical data source to delineate groups in plant taxonomy (Carlquist, 1961; Radford et al., 1974; Stuessy, 1990; Judd et al., 2008).

RDA for environmental variables showed that Bio01, Bio03, Bio07, Bio16 and Tpi are very important variables determining the morphological variability among the populations of *Balanites* species. Karbstein et al. (2020) reported that the variability of morphology-related functional traits of plants were generally correlated to the gradients of the habitat slope, aspects and soil factors. RDA for environmental variables rendering the morphological variability among the populations of *Ziziphus* species revealed that Bio03, Bio04, Bio06, Bio10, Bio14, Bio17, Bio19, elevation and Tpi are very important variables determining the morphological variability among the populations of *Ziziphus* species. Generally, in response to abiotic environmental gradients, plant species steadily show an intra-specific variability and species abundance (Pescador et al., 2015).

2.5 Conclusions

The findings of this study revealed the presence of significant inter- and intra-specific variability among the species of *Balanites* and *Ziziphus* in Ethiopia. The study also indicated the presence of at least two distinct groups (morphotypes) for *B. aegyptiaca*

and *B. rotundifolia* as well as *Z. spina-christi* and *Z. mauritania* each in Ethiopia. The morphological variability among *Balanites* is mainly determined by mainly fruit (ovary) shape, presence of naked or leaves (or flowers) bearing spines, and flower merosity. The morphological variability among *Balanites* is also constrained by environmental variables mainly Bio01, Bio03, Bio07, Bio16 and Tpi. The variability of *Ziziphus* is generally determined by mainly leaf base symmetry, bark color, and leaf surface pubescence, which in turn are constrained by Bio03, Bio04, Bio06, Bio10, Bio14, Bio17, Bio19, elevation and Tpi. *Balanites* such as *B. rotundifolia*, *B. glabra* and *B. pedicellaris;* and *Ziziphus* such as *Z. mucronata*, *Z. mauritania*, *Z. hamur*, *Z. abyssinica* and *Z. pubescens* were observed less abundant and less frequent in the study area. Hence, special consideration should be given to these species as well, and a targeted conservation strategy should be implemented to promote their population density in their natural habitats.

In this regard, it is essential to consider innovative ways of maintaining the genetic quality of these species by domesticating them into a wider scale of ecological and social environment. So, potential stakeholders including biodiversity conservation institutes, forest and environmental institutes, forest and seed enterprise, and other forest-based enterprises can consider these findings when implementing conservation works related to *Balanites* and *Ziziphus* in Ethiopia. Moreover, this study also recommends further investigations on the genetic diversity of the genera using molecular approaches to support the congruence of the findings of phenetic analysis based on morphological characters. Overall, this study is expected to make important contributions in understanding the morphological diversity and phenetic relationships among *Balanites* and *Ziziphus* in Ethiopia.

Chapter 3

3. Enhancement of Seed Germination of *Ziziphus spina-christi* (L.) Willd. and *Ziziphus mucronata* Willd. (Rhamnaceae), *B. aegyptiaca* (L.) Del. and *B. rotundifolia* (Tiegh.) Blatt.) using Different Pre-Sowing Treatments in Ethiopia

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Abstract

The stony endocarp and impermeable seed coat of *Ziziphus*, as well as the fleshy mesocarp of *Balanites*, inhibit the seed germination of both genera. The present study aimed at evaluating the effects of different pre-sowing treatments on seed germination of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia*. Seeds of each species were collected Central and Southern Rift valley and Northeast Ethiopia. The seeds were thoroughly mixed, and homogenized to create representative samples. Seeds of each *Ziziphus* species divided into cracked and uncracked seed stones (n = 1152 each) and *Balanites* species (n = 864 each) were subjected to different pre-sowing treatments, and planted in pots arranged in a CRD. The mean germination percentage (GP), mean daily germination (GD), mean germination time (GT) and mean germination index (GI), One-way ANOVA (for *Balanites*) and two-way ANOVA (for *Ziziphus*) and THSD were computed. Two-way ANOVA for *Ziziphus* generally showed that there exists a significant mean difference among treatment groups under all other germination parameters at p<0.05. The THSD for *Z. spina-christi* showed that the highest GP was achieved by cracked seed stones treated with *CW48h* (52.7±6), *Ctrl* (48.5±3), *CW24h* (46.5±5.7), and *RuSP* (41±12.1) that is significantly different at p<0.05, while the highest GI was recorded by cracked seed stones treated with *CW48h* (1.3±0.2) that is significantly different at p<0.05. THSD test for *Z. mucronata* indicated that the highest GP, along with highest GI, was achieved by cracked seed stones soaked with *98HSO20m* (28.5±6) that is significantly different at p<0.05. One-way ANOVA showed the presence of significant GP, GD, GT, and GI among treatment groups at p<0.05 under both *Balanites* species. THSD for *B. aegyptiaca* showed that seeds soaked with *98HSO10m* and *98HSO20m* have the highest GPs (87±8.8 and 82±10.2, respectively) and highest GIs (1.04±0.09 and 1.01±0.08, respectively) that are significant at p<0.05. However, no treatment group was observed to enhance the germination of *B. rotundifolia* compared to the control. Overall, the findings of this study will contribute potential seed enhancement technologies for *Ziziphus* and *Balanites*, with greater implications for the propagation, conservation, and sustainable utilization of the species in the agricultural and pastoral communities of Ethiopia.

Keywords: *Balanites;* Dormancy; Enhancement; Germination; Treatments; *Ziziphus*

3.1 Introduction

Most tree species including the study plants of this research propagate with seeds. Yet, knowledge about information that hinders or accelerates the rate of seed germination is little known. Tree seeds often exhibit dormancy, which causes delays and irregularities of germination in nurseries and forest floors (Maiden et al., 1990; Oyewole & Adedamola, 2015). Understanding the seed physiology of plants can enhance conservation efforts through afforestation. Thus, knowledge of seed germination behavior is crucial for successful utilization and conservation planning (Zobel & Talbert, 2003). In this regard, effective propagation and seedling establishment are the basic requirements for sustainable management for species with notable seed dormancy profiles. For that purpose, application of pre-treatments has a significant impact on seed germination as they enhance water absorption, stimulate germination performance, and promote seedling vigor. Wild fruit trees play an important role in the livelihood of rural people in sub-Saharan Africa and are sources of nutrition for the local population (Schreckenberg et al., 2006). Hence, understanding the seed physiology of plants can enhance future conservation through afforestation

Ziziphus and *Balanites* are known multipurpose shrub or tree with various uses and values (Orwa et al., 2009), and widely distributed in arid and semi-arid ecosystems of Ethiopia (Bekele-Tesemma, 1993, 2007; Orwa et al., 2009). *Ziziphus* is a multipurpose shrub or tree with orthodox seed storage behavior, distributed in arid and semi-arid ecosystems of Ethiopia (Azene Bekele-Tesemma, 1993, 2007; Orwa et al., 2009). Members of the genus *Ziziphus* are usually cross-pollinated and propagated by seed (Vashishtha & Pareek, 1979; Sudhersan & Hussain, 2003; Saied et al., 2008; El Maaiden et al., 2020). Similar to other tropical and subtropical species, *Ziziphus* species have been reported to have issues with seed dormancy, which slows down uniform seed germination in nurseries (Schmidt, 2000; Wondwossen Gebretsadik, 2013). The establishment of *Ziziphus* plantations is generally hindered by poor germination and seedling emergence due to a stony endocarp and seed dormancy related to the impermeability of the seed coat. This can be addressed through the gradual softening of the seed coat or by scarification or acid treatment to free the seeds from the pericarp (Schmidt, 2000; El-Siddig et al., 2001; Saied et al., 2008).

Additionally, mechanical removal of the endocarp and soaking of kernels in water has been significantly improving seed germination for several *Ziziphus* species, including *Z. mauritania* and *Z. abyssinica* (Krishnan & Kulasekaran, 1984; Murthy & Reddy, 1989; Sheoran et al., 2018).

The seeds of *Balanites* are generally highly variable among sources in weight and morphology (Elfeel & Warrag, 2011), as well as in germination capacity (Elfeel, 2010, 2012). Different seed treatments, such as cold and hot water, were reported to enhance the germination of *B. aegyptiaca* (Hall & Walker, 1991). Fresh seeds of *B. aegyptiaca* are believed to germinate readily without treatments, and the presence of mesocarp is thought to delay the seed germination of *B. aegyptiaca* (Elfeel, 2012). The seed storage behaviour of *B. aegyptiaca* is orthodox in that seed viability can be maintained for two or even several years in air-dry storage at cool temperatures or hermetic storage at 3°C with 6–10% moisture contents (Orwa et al., 2009). The species can be propagated by seedlings (seeds) using direct sowing, as well as by applying pre-sowing treatments to maximize the germination percentage (Azene Bekele-Tesemma, 1993, 2007; Orwa et al., 2009). Different seed treatments, such as cold and hot water, have been reported to enhance the germination of *Balanites* (Hall & Walker, 1991; Zarad et al., 1998; Schmidt & Jøker, 2000). *B. rotundifolia* is a very drought-resistant tree, even more so than *B. aegyptiaca* (Beentje, 1994; Ruffo et al., 2002). The fruits of *B. rotundifolia* are brittle and smooth on the outside, with a fibrous and oily endocarp with high moisture content (Ruffo et al., 2002). *B. rotundifolia* is the less-studied *Balanites* in terms of its seed germination behaviour compared to *B. aegyptiaca*. However, direct sowing of the seed (or fruits) without applying treatments has previously been suggested (Ruffo et al., 2002).

However, there is no documented information on the seed germination behaviour of Ethiopian species of *Ziziphus* and *Balanites* using different pre-sowing treatments (i.e., combining both mechanical and chemical treatments). Hence, extensive studies are yet to be conducted for populations of *Z. spina-christ*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia* in other geographical areas, including Ethiopia. In this regard, documentation of the seed germination behaviors of species is very important for successful utilization or conservation plans (Zobel & Talbert, 2003). Therefore, this study was aimed at investigating the seed germination responses of *Z. spina-*

christ, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia* under different pre-sowing treatments so as to identify the best seed treatment techniques suitable to maximize the seed germinations of the species. Therefore, it was hypothesized that pre-sowing seed treatments could enhance seed germination of *Z. spina-christ*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia*.

3.2 Materials and Methods

Seed collection and processing

Seed collection was done between February 2022 and February 2023. Seed samples were collected from the Central and Southern Rift valley and Northeast Ethiopia (**Figure 12**).

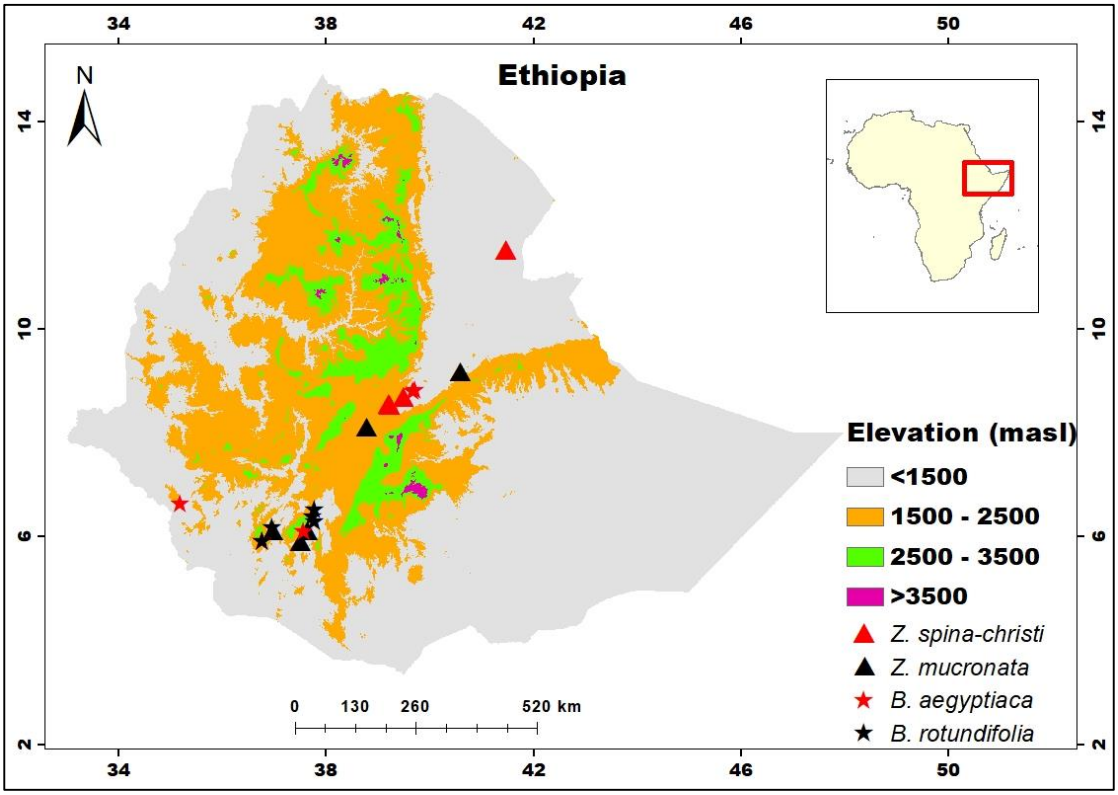

Figure 12. Map of Ethiopia showing seed collection sites *Z. spina-christ*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia*.

Hence, the seed for *Z. spina-christi* from Asayita district in Afar region (i.e., 41.470555°E, 11.526388°N), Adama Zuria district-1 and 2 (i.e., 39.20774°E, 8.54799°N; 39.2293°E, 8.53156°N) and Boset district (i.e., 39.489722°E, 8.6775) in Oromia region; and *Z. mucronata* from Arba Minch Zuria district-1 and 2 (i.e., 37.62743°E, 6.12204°N; 37.492368°E, 5.894401°N) and Ubadeberetsehay district

(i.e., 36.9503°E, 6.11977°N) in SNNPR, Boset district-2 (i.e., 38.77472°E, 8.105833°N) and Maeso district (i.e., 40.58287°E, 9.17381°N) in Oromia. Moreover, the seeds of *B. aegyptiaca* were collected from Boset district-1, 2 and 3 in Oromia region (i.e., 39.21644°E, 8.54042°N; 39.35609E, 8.59369°N; 39.4755°E, 8.6767°N), Ubadeberetsehay district-1 (i.e., 37.06422°E, 6.47314°N) and Arba Minch Zuria district (i.e., 37.56822°E, 6.036998°N) in Southern Nations, Nationalities and Peoples Region (SNNPR), and Dima district (i.e., 35.18337°E, 6.64147°N) in Gambella region; *B. rotundifolia* from Mirab-Abaya district-1, 2 & 3 (i.e., 37.697543°E, 6.181298°N; 37.710982°E, 6.199166°N; 37.732482°E, 6.232607°N) and Ubadeberetsehay district-2 and 3 (i.e., 36.76659°E, 5.92465°N; 36.9647°E, 6.19399°N) in SNNPR.

Seeds were collected from conspicuous superior (mother) trees of *Z. spina-christi* and *Z. mucronata.* About 30-40 seeds per mother tree (about 200 seeds per population) were collected and mixed up to represent the population. The seeds were collected from different populations of each species and thoroughly mixed up and homogenized to represent the species. The homogenized seeds were further processed, cleaned, and purified from inert and unwanted materials (**Figure 13, Figure 14**). The seeds were dried and the moisture content (MC) was adjusted to 4.65% for *Z. mucronata*, 5.1% for *Z. spina-christi*, 6.0% for *B. aegyptiaca* and 9% *B. rotundifolia*, and checked using the moisture meter (KERN, DBS60-3 German-made moisture tester).

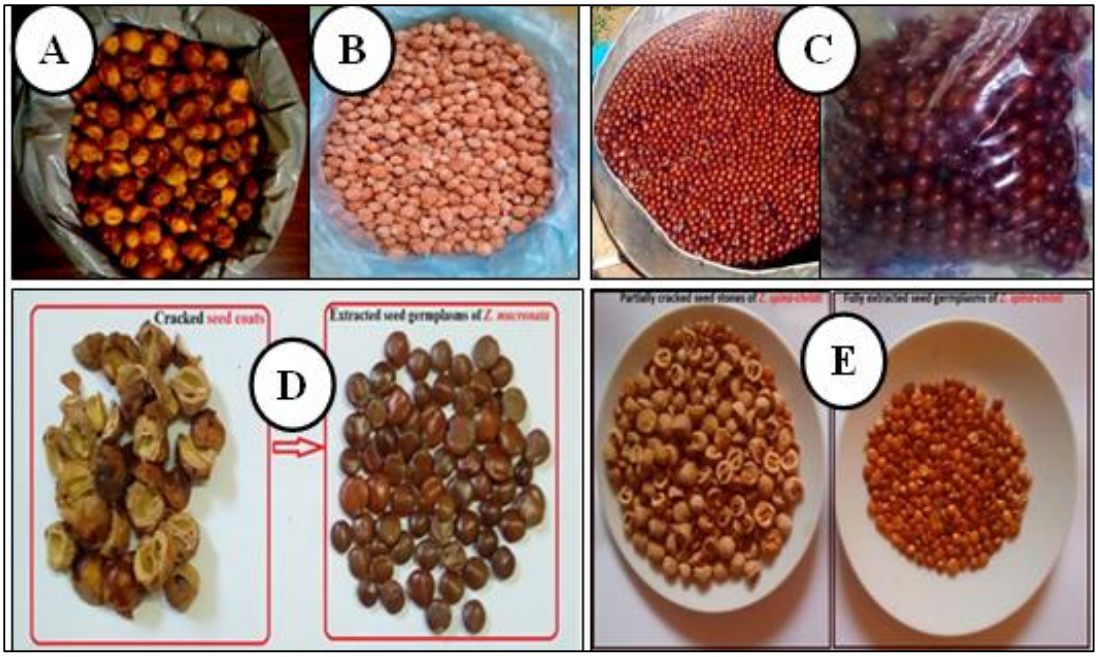

Figure 13. The fruits of *Z. spina-christi* (A), seed stones of and *Z. spina-christi* (B), and fruits of *Z. mucronata* (C), and cracked and extracted seed germplasms of *Z. mucronata* (D) and Z. spina-christi (E).

Figure 14. Kernels (seeds) of *B. aegyptiaca* after the fruits' mesocarps are removed (A); Fuits of *B. rotundifolia* (B), and kernels (seeds) of *B. rotundifolia* after the fruits' mesocarps are removed (C).

Preparations of seed treatments and experimental layout

The seeds of each *Ziziphus* species were divided into cracked and uncracked seed stones (*n* = 1152 each) and *Balanites* (*n* = 864 each) subjected to different pre-sowing treatments. Each *Ziziphus* species was subjected to 16 treatments: seed coat nature (2 levels, i.e., cracked and uncracked) and pre-sowing treatments (8 levels) with 4 replications each having a total of 64 treatment units. However, each *Balanites* species was subjected to exactly 8 pre-sowing treatments with 4 replications each having a total of 32 treatment units (**Table 3**). For this study, seed pre-sowing treatments were prepared based on literature and ISTA protocols (Hall & Walker, 1991; Schmidt, 2000; Elfeel, 2012; Hassen et al., 2005; ISTA, 2005; Orwa et al., 2009; Wondwossen Gebretsadik, 2013; Salami et al., 2019). The International Seed Testing Association (ISTA) recommends four replicates of 100 seeds each for standard germination tests, but there should not be fewer than 100 seeds in replicates of 25 or 50 seeds (ISTA, 1976, 2018, 2023). All experimental activities were carried out in the nursery house of the Ethiopian Forestry Development (EFD), Addis Ababa, Ethiopia.

Table 3. Lists of pre-sowing seed treatments employed to the seeds of *Ziziphus* and *Balanites* species.

No	Seed treatment types	No	Seed treatment types
1	Control, i.e., received no prior seed treatment (*Ctrl*)	5	Soaking with hot normal water at 65°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C (*HW65d*)
2	Rubbed with sandpaper (*RuSP*)	6	Soaked with hot normal water at 75°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C (*HW75d*)
3	Soaked with cold normal water for 24 hours at room temperature of 25°C (*CW24h*)	7	Soaked with 98% H_2SO_4 for 10 minutes and subsequent rinsed with water (*98HSO10m*)
4	Soaked with cold normal water for 48 hours at room temperature of 25°C (*CW48h*)	8	Soaked with 98% H_2SO_4 for 20 minutes and subsequent rinsed with water (*98HSO20m*)

Each experiment was laid out in a CRD layout (**Figure 15**) following the random tables of Gomez and Gomez (1984). The germinations were done in the germination substrate of topsoil, manure, and sand with a ratio of 3:2:1, respectively. The mixture

was placed in polyethylene plastic pots with 16 cm diameter. Generally, *B. aegyptiaca* has fruits with lengths ranging from 2 to 4 cm and widths of 1.5 to 2.5 cm, while *B. rotundifolia* has fruits with lengths ranging from 2 to 3 cm and widths of 2 to 2.5 cm. So, fruits (seeds) were sown in each pot with horizontal orientation of the seed stalk while fully covering the fruits (seeds) with the substrate (~2.5 cm depth). The sown seeds (or fruits) were then covered with transparent polythene sheets to optimize and regulate the moisture and temperature (**Figure 15**).

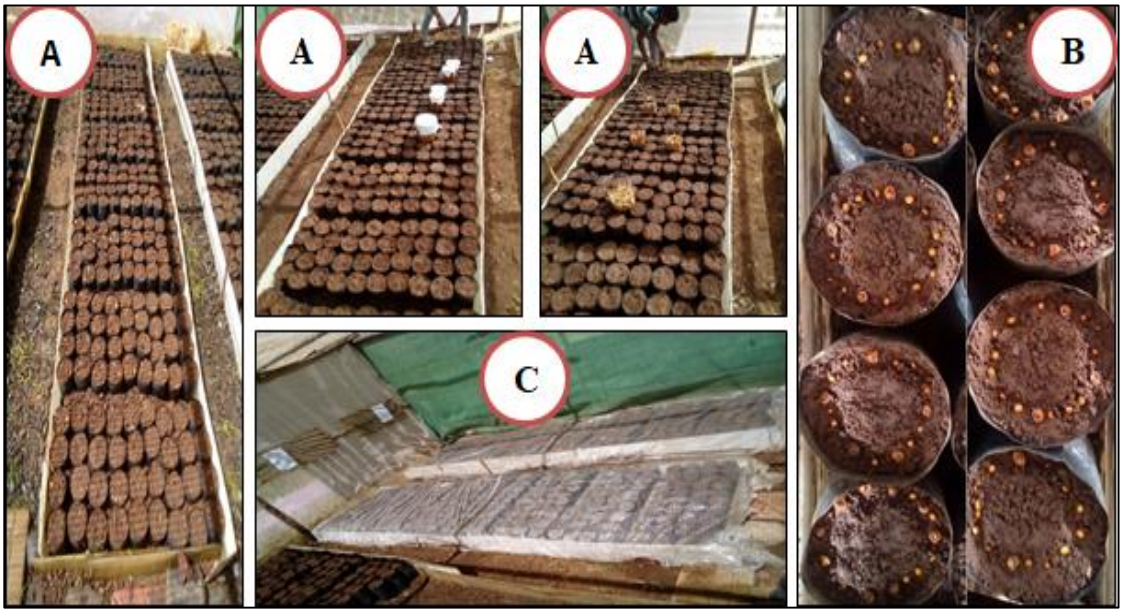

Figure 15. The nursery beds and the arrangement of pots, sown seeds, and sown seeds covered with transparent polythene sheet.

In the figure 15 above are nursery pots sown with uncracked seed stones (A), nursery pots sown with cracked seed stones and extracted seed germplasms of *Ziziphus* species (B), and the nursery beds with sown seeds covered with transparent polythene sheet for moisture regulation (C). The seeds were then sown on each pot and covered with a transparent plastic polythene sheet to optimize and regulate the moisture and temperature. The germination duration for cracked and uncracked seed stones of *Z. spina-christ* lasted for 4 weeks and 3 months; and 6 weeks and 3 months for cracked and uncracked seed stones of *Z. mucronata*, respectively. Moreover, the test duration lasted six weeks for *B. aegyptiaca* and three weeks for *B. rotundifolia*. Germination counting was taken starting from the first week of germination observation after sowing, every two days for two to three months, based on the species, until no new seedlings emerged for two successive weeks' laps. In this study, a seed was

considered germinated when the radicle emerged (Bewley & Black, 1994; Zietsman & Botha, 1987).

Statistical data analysis

The data analysis was based on untransformed seed germination data (i.e., raw data). The dataset was checked with histogram and has a normal distribution. The nineteen germination measurements (or parameters) were generated of which four parameters, namely mean germination percentage (GP), mean daily germination percentage (GD), mean germination time (GT), and mean germination index (GI) were analysed and presented for this study. The GP, which is an estimate of the germinability of the population of seeds, was calculated as follows:

$$\mathbf{GP} = \frac{\sum_{\mathbf{i=1}}^{\mathbf{k}} \boldsymbol{ni}}{\mathbf{N}} \boldsymbol{x}\ \mathbf{100} \quad \text{…………………………………..…………..….…….. } (1)$$

Where; n_i = number of seeds germinated in the i^{th} time, and N= total number of seeds used.

The GD, which is the mean number of seeds germinated per day (i.e., the number of seeds germinated daily relative to the maximum number of germinated seeds), was also calculated following Adams and Farrish (1992):

$$\mathrm{GD} = \frac{\mathrm{CP}}{\mathrm{Tn}} \text{………………………………………...………..………..….. } (2)$$

Where CP = final cumulative germination percentage and T_n = total number of intervals required for final germination.

Following Ellis and Roberts (1981), the GT, a measure of the rate and time-spread of the germination, of each treatment was calculated using the formula:

$$\mathbf{GT} = \frac{\sum(\mathbf{n} \times \mathbf{d})}{\mathbf{N}} \text{ ……………………………………..………...…………………..…. } (3)$$

Where "n" = number of seeds germinated on each day, "d" = number of days from the beginning of the test, and "N" = total number of seeds germinated at the termination of the experiment.

Furthermore, the GI, which is a measure of the percentage and speed of germination, was calculated following the following formula (AOSA, 1983):

$$GI = \frac{\text{No.of germinating seeds}}{\text{days of first count}} + \cdots + \frac{\text{No.of germinating seeds}}{\text{days of final count}} \quad \ldots\ldots\ldots (4)$$

The higher values for this measure indicate a greater rate of germination (Benech-Arnold et al., 1991).

Moreover, one-way (for *Balanites*) and two-way ANOVA (for *Ziziphus*) were computed to see the level of significance difference among the treatments means for the germination parameters at $p \leq 0.05$. Moreover, Tukey's Honestly Significant Difference (THSD) was computed to assess the significance of difference between pairs of treatments at $p \leq 0.05$ (Steel et al., 1997), allowing all possible pairwise comparisons while keeping the family-wise error rate low.

3.3 Results

Seed germination tests of Z. spina-christi using different treatments

Germination tests of *Z. spina-christi* for cracked and uncracked seed stones showed that cracked seed stones remarkably germinated much faster than the uncracked seed stones (**Figure 16, Figure 17**, **Annex 4**).

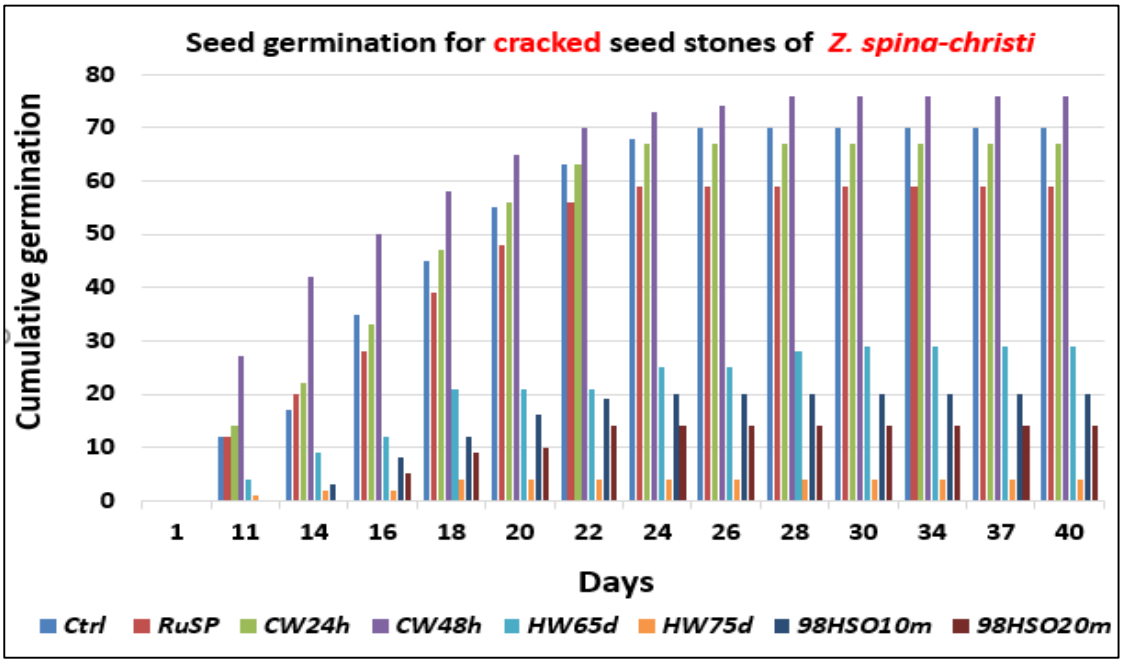

Figure 16. The cumulative germination of cracked seed stones of *Z. spina-christi* different treatments over days.

In the figure 16 above, *Ctrl*: Control, *RuSP*: Rubbed with sandpaper, *CW24h*: Soaked in cold water for 24 hours at room temp temperature of 25°C, *CW48h*: Soaked in cold water for 48 hours at room temperature of 25°C, *HW65d*: Soaked in hot water at 65°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C, *HW75d*: Soaked in hot water at 75°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C, *98HSO10m*: Soaked in 98% H_2SO_4 for 10 minutes and subsequent rinsed with water, and *98HSO20m*: Soaked in 98% H_2SO_4 for 20 minutes and subsequent rinsed with water.

The shortest germination duration (< 30 days) was recorded by cracked seed stones regardless of treatment types compared to uncracked seed stones (> 3 months). No new germination was observed in all treatment types for cracked seed stones of *Z. spina-christi* while new germinations were observed up to 3 months for uncracked seed stones of *Z. spina-christi* (**Figure 17**).

For cracked seed stones of *Z. spina-christi*, the highest cumulative germination (i.e., 76) was recorded by *CW48h*, followed by *Ctrl* (70), and *CW24h* (67) (**Figure 16**). However, for uncracked seed stones of *Z. spina-christi*, the highest cumulative germination (i.e., 21) was achieved by *98HSO20m*, followed by *CW48h* (20), and *98HSO10m* (19) (**Figure 17**).

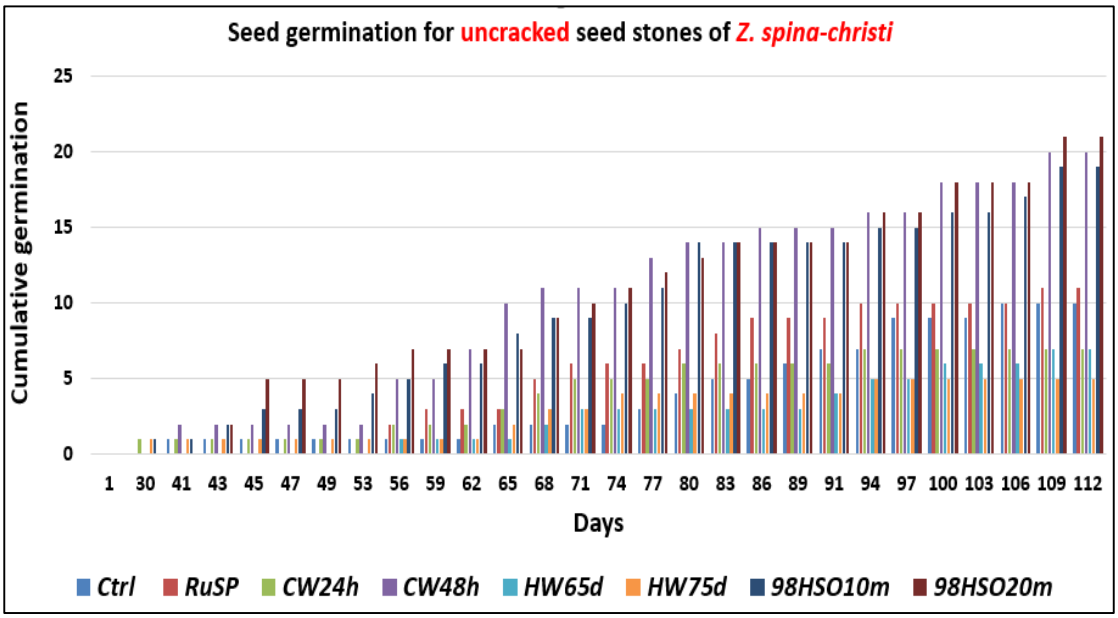

Figure 17. The cumulative germination of uncracked seed stones of *Z. spina-christi* different treatments over days.

The two-way ANOVA for the germination of *Z. spina-chrsiti* showed that, except among pre-sowing treatments and the interactions factor (presowing treatment and cracking of seed stones) of the GT, there exist a significant mean difference among treatment groups under all other germination parameters (i.e., GP, GD, and GI) at $p<0.05$ (**Table 4**).

Table 4. Two-way ANOVA of the mean significance difference among treatment groups for their effect on GP, GD, GT, and GI of *Z. spina-christi*.

	Df	*Sum Sq.*	*Mean Sq.*	*F-value*	*Pr(>F)*
			GP		
Pre-sowing	7	12163	1738	38.95	<2e-16***
Cracking	1	17424	17424	390.55	<2e-16***
Pre-sowing: Cracking	7	11957	1708	38.29	<2e-16***
Residuals	48	2141	45		
			GD		
Pre-sowing	7	7.385	1.055	50.38	<2e-16***
Cracking	1	15.035	15.035	717.91	<2e-16***
Pre-sowing: Cracking	7	7.383	1.055	50.36	<2e-16***
Residuals	48	1.005	0.021		
			GT		
Pre-sowing	7	2668	381	1.127	0.36
Cracking	1	43436	43436	128.407	3.6e-15***
Pre-sowing: Cracking	7	1520	217	0.642	0.719
Residuals	48	16237	338		
			GI		
Pre-sowing	7	3.115	0.445	49.13	<2e-16***
Cracking	1	6.126	6.126	676.32	<2e-16***
Pre-sowing: Cracking	7	3.132	0.447	49.40	<2e-16***
Residuals	48	0.435	0.009		

The THSD test for *Z. spina-christi* showed that the highest GP was achieved by cracked seed stones treated with *CW48h* (52.7±6), followed by *Ctrl* (48.5±3), *CW24h* (46.5±5.7), and *RuSP* (41±12.1) that were significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (**Table 5**). But no significant GP difference was observed among *CW48h, Ctrl*, *CW24h* and *RuSP* at $p\leq0.05$. The highest GD was recorded by cracked seed stones treated with

CW48h (1.90±0.22), except with cracked seed stones of the *Ctrl* (1.75±0.1) and *CW24h* (1.68±0.21), that is significantly difference at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (**Table 5**). In the table, the values denoted with the same alphabet (s) along the vertical column ($\bar{x}$±SD) of each germination parameter are not significantly different at $p\leq0.05$, and vice versa.

Table 5. Summary of GP, GD, and standard deviation (SD) of cracked and uncracked seed stones of *Z. spina-christi* under different pre-sowing treatments.

Pre-sowing treatments	**GP (%)**		**GD (%)**	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	48.5 ± 3^{a}	7 ± 8.0^{bc}	1.75 ± 0.1^{ab}	0.06 ± 0.08^{d}
RuSP	41 ± 12.1^{a}	7.8 ± 4.6^{bc}	1.48 ± 0.43^{b}	0.07 ± 0.04^{d}
CW24h	46.5 ± 5.7^{a}	5 ± 4.69^{bc}	1.68 ± 0.21^{ab}	0.04 ± 0.04^{d}
CW48h	52.7 ± 6^{a}	14 ± 2.24^{bc}	1.90 ± 0.22^{a}	0.12 ± 0.03^{d}
HW65d	17 ± 0.0^{b}	5 ± 4.69^{bc}	0.60 ± 0.0^{c}	0.04 ± 0.04^{d}
HW75d	2.7 ± 3.7^{c}	3.5 ± 3.3^{bc}	0.10 ± 0.14^{d}	0.03 ± 0.03^{d}
98HSO10m	14 ± 0.0^{bc}	13.3 ± 2.87^{bc}	0.50 ± 0.0^{c}	0.12 ± 0.02^{d}
98HSO20m	9.5 ± 3.0^{bc}	14.5 ± 6.14^{bc}	0.35 ± 0.1^{cd}	0.13 ± 0.06^{d}

Table 6. Summary of GT, GI, and standard deviation (SD) of cracked and uncracked seed stones of *Z. spina-christi* under different pre-sowing treatments.

Pre-sowing treatments	**GT (days)**		**GI (%/day)**	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	17.4 ± 0.6^{bc}	80.8 ± 19.3^{a}	1.1 ± 0.1^{b}	0.0 ± 0.0^{e}
RuSP	16.9 ± 0.7^{c}	74.9 ± 6.6^{a}	0.9 ± 0.3^{b}	0.0 ± 0.0^{de}
CW24h	16.8 ± 0.6^{c}	48.1 ± 32.8^{abc}	1.1 ± 0.1^{b}	0.0 ± 0.0^{de}
CW48h	15.6 ± 0.7^{c}	73.8 ± 7.4^{a}	1.3 ± 0.2^{a}	0.1 ± 0.0^{de}
HW65d	18.5 ± 0.3^{bc}	64.3 ± 44.6^{ab}	0.4 ± 0.0^{c}	0.0 ± 0.0^{e}
HW75d	8.1 ± 9.5^{c}	54.1 ± 39.4^{abc}	0.1 ± 0.1^{cde}	0.0 ± 0.0^{e}
98HSO10m	18.2 ± 1.3^{bc}	74.5 ± 7.9^{a}	0.3 ± 0.0^{cd}	0.1 ± 0.0^{de}
98HSO20m	18.6 ± 0.1^{bc}	76.6 ± 13.0^{a}	0.2 ± 0.1^{cde}	0.1 ± 0.0^{de}

The shorter GT under the germination test of cracked seed stones was observed by cracked seed stones treated with *HW75d* (8.1±9.5), followed by *CW48h* (15.6±0.7), *CW24h* (16.8±0.6), and *RuSP* (16.9±0.7) that were significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones. But no significant GT difference was observed among *HW75d, CW48h, Ctrl,*

CW24h and *RuSP* at $p \leq 0.05$. Furthermore, the highest GI was recorded by cracked seed stones treated with *CW48h* (1.3±0.2) that is significantly different at $p < 0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (**Table 6**).

Seed germination tests of Z. mucronata using different treatments

The seed germination tests for *Z. mucronata* under different treatments showed no new germinations after six weeks (45 days) for most cracked seed stones (**Figure 18**, **Annex 5**). However, the uncracked seed stones of *Z. mucronata* required longer germination duration (> 3 months) yet with lower germination percentages (**Figure 18**).

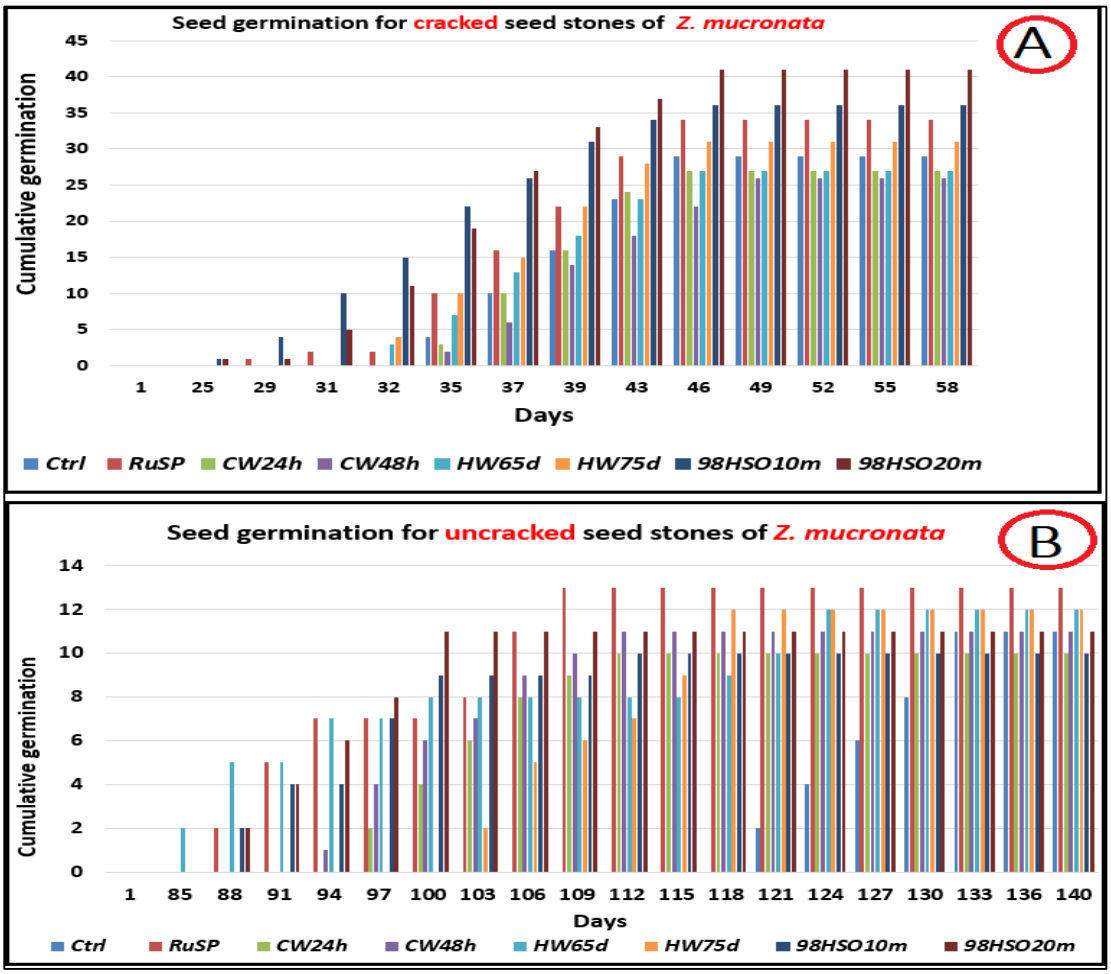

Figure 18. The cumulative germination of cracked seed stones (A) and uncracked seed stones (B) of *Z. mucronata* different treatments over days.

The highest cumulative germination record (i.e., 41) was attained by cracked seed stones treated with *98HSO20m,* followed by *98HSO10m* (36), and *RuSP* (34). Generally, however, the germination of uncracked seed stones of *Z. mucronata* was very low (i.e., < 20) regardless of treatment types (**Figure 18**) even if the highest possible total germination record (i.e., 13) was scored by *RuSP,* followed by *HW65d* and *HW75d* (**Figure 18**). The two-way ANOVA for the germination of *Z. mucronata* showed that there exists a significant mean difference among treatment groups, having interaction effects, under all germination parameters (i.e., GP, GD, GT and GI) at $p<0.05$ (**Table 7**). The test involves two factors, i.e., the pre-sowing treatments with eight levels, and cracking factor with two levels (i.e., cracked and uncracked seed stones).

Table 7. Two-way ANOVA of the mean significance difference among treatment group for their effect on GP, GD, GT, and GI of *Z. mucronata*.

	Df	*Sum Sq.*	*Mean Sq.*	*F-value*	*Pr(>F)*
			GP		
Pre-sowing	7	397	57	3.856	0.00210**
Cracking	1	8860	8860	602.560	< 2e-16***
Pre-sowing: Cracking	7	391	56	3.800	0.00234**
Residuals	48	706	15		
			GD		
Pre-sowing	7	0.119	0.017	4.890	0.00032***
Cracking	1	3.730	3.730	1070.895	< 2e-16***
Pre-sowing: Cracking	7	0.119	0.017	4.883	0.0003***
Residuals	48	0.167	0.003		
			GT		
Pre-sowing	7	1950	279	22.07	5.27e-13***
Cracking	1	68572	68572	5432.85	< 2e-16***
Pre-sowing: Cracking	7	1334	191	15.10	3.08e-10***
Residuals	48	606	13		
			GI		
Pre-sowing	7	0.0278	0.0040	8.045	1.92e-06***
Cracking	1	0.5166	0.5166	1045.179	< 2e-16***
Pre-sowing: Cracking	7	0.0275	0.0039	7.944	2.22e-06***
Residuals	48	0.0237	0.0005		

The THSD test indicated that the highest GP was achieved by cracked seed stones of *Z. mucronata* treated with *98HSO20m* (28.5±6) that is significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (**Table 8**), except with cracked seed stones treated by *98HSO10m* (25±4.9), *RuSP* (23.5±3), and *HW75d* (21.2±2.8). Similarly, the highest GD was

achieved by cracked seed stones of *Z. mucronata* treated with *98HSO20m* (0.7±0.1) that is significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (**Table 8**), except with cracked seed stones treated by *98HSO10m* (0.6±0.1) and *RuSP* (0.6±0.1). The shortest GT was scored by cracked seed stones treated with *98HSO10m* (35.1±1) and *98HSO20m* (36.7±1.7) that is significantly different at $p<0.05$ compared to the other treatment groups under uncracked seed stones, but not significant at $p\leq0.05$ compared to the other treatment groups under cracked seed stones (**Table 9**). Furthermore, the highest GI was achieved by cracked seed stones treated with *98HSO20m* (0.3±0.0) and *98HSO10m* (0.3±0.0) that is significantly different at $p<0.05$ compared to the other treatment groups under both cracked and uncracked seed stones (**Table 9**). The values denoted with the same alphabet (s) along the vertical column ($\bar{x}$±SD) of each germination parameter are not significantly different at $p\leq0.05$, and vice versa.

Table 8. Summary of GP, GD, and standard deviation (SD) of cracked and uncracked seed stones of *Z. mucronata* under different pre-sowing treatments.

Pre-sowing treatments	**GP (%)**		**GD (%)**	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	20.0±2.4^{b}	7.8±2.4^{c}	0.5±0.1^{c}	0.1±0.0^{d}
RuSP	23.5±3.0^{ab}	9.0±3.5^{c}	0.6±0.1^{ab}	0.1±0.0^{d}
CW24h	18.7±2.3^{b}	7.0±1.2^{c}	0.5±0.1^{c}	0.1±0.0^{d}
CW48h	18.0±1.1^{b}	7.8±3.9^{c}	0.4±0.0^{c}	0.1±0.0^{d}
HW65d	18.5±3.3^{b}	8.3±4.5^{c}	0.5±0.1^{c}	0.1±0.0^{d}
HW75d	21.2±2.8^{ab}	8.3±2.1^{c}	0.5±0.1^{c}	0.1±0.0^{d}
98HSO10m	25±4.9^{ab}	7.0±1.2^{c}	0.6±0.1^{ab}	0.1±0.0^{d}
98HSO20m	28.5±6.0^{a}	7.8±2.4^{c}	0.7±0.1^{a}	0.1±0.0^{d}

Table 9. Summary of GT, GI, and standard deviation (SD) of cracked and uncracked seed stones of *Z. mucronata* under different pre-sowing treatments.

Pre-sowing treatments	**GT (days)**		**GI (%/day)**	
	Cracked	*Uncra*cked	*Cracked*	*Uncra*cked
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD
Ctrl	40.4±0.4^{d}	127.3±1.3^{a}	0.2±0.0^{c}	0.0±0.0^{d}
RuSP	39.1±0.8^{d}	98.0±1.2^{c}	0.2±0.0^{c}	0.0±0.0^{d}
CW24h	40.0±0.6^{d}	103.1±3.2^{bc}	0.2±0.0^{c}	0.0±0.0^{d}
CW48h	39.0±0.6^{d}	102.3±3.6^{bc}	0.2±0.0^{c}	0.0±0.0^{d}
HW65d	38.8±1.5^{d}	98.1±11.4^{c}	0.2±0.0^{c}	0.0±0.0^{d}
HW75d	38.4±1.4^{d}	110.9±2.1^{b}	0.2±0.0^{c}	0.0±0.0^{d}
98HSO10m	35.1±1.0^{d}	96.6±5.3^{c}	0.3±0.0^{ab}	0.0±0.0^{d}
98HSO20m	36.7±1.2^{d}	95.0±2.4^{c}	0.3±0.0^{ab}	0.0±0.0^{d}

Germination of B. aegyptiaca

Figure 19 below illustrated the newly emerging germinate, and fully grown seedlings of *B. aegyptiaca*. After the six weeks of the germination period, no new germinates were observed in most of the treatments (**Figure 20**, **Annex 8**).

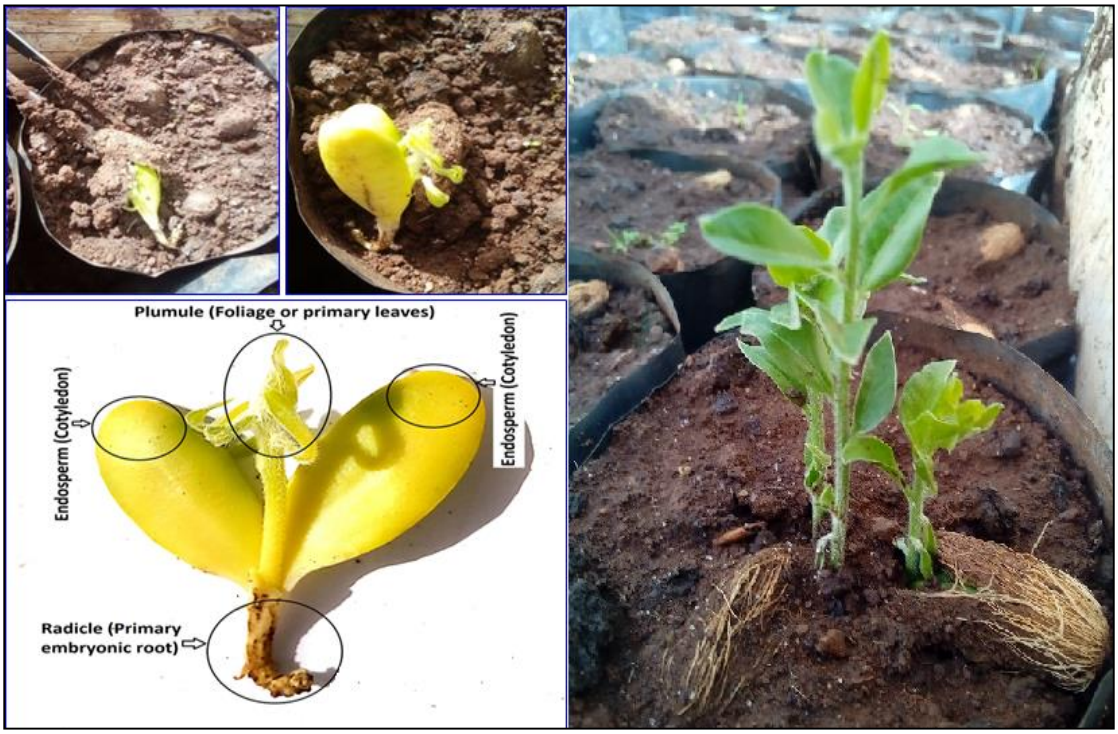

Figure 19. Illustration of newly emerging germinate, and fully grown seedlings of *B. aegyptiaca*.

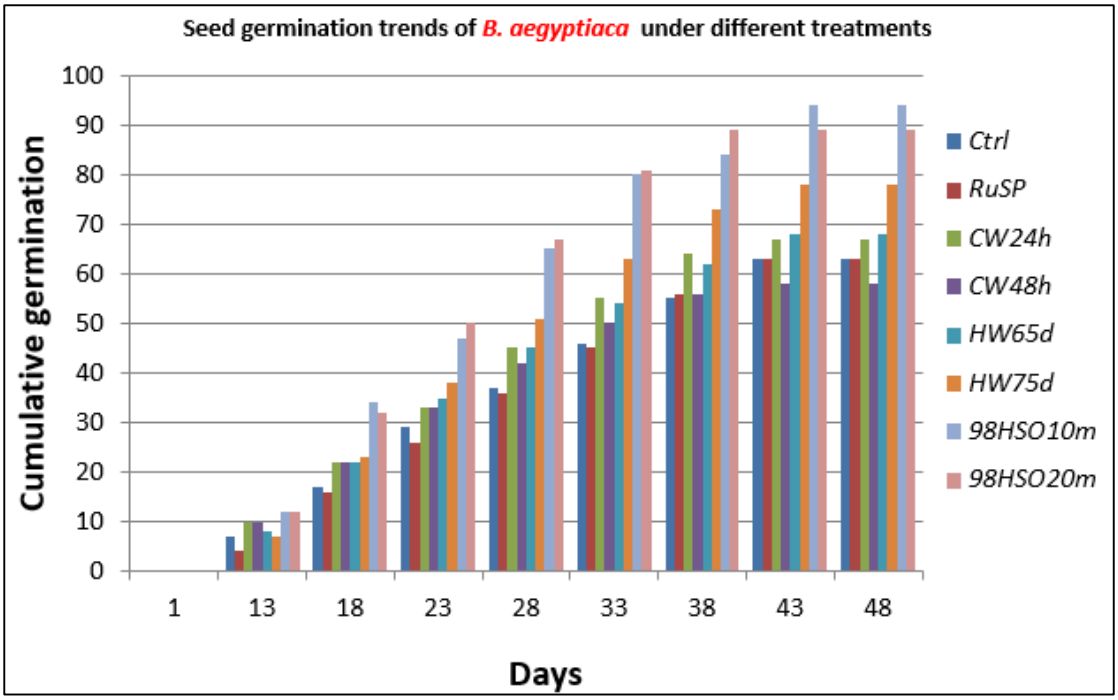

Figure 20. Cumulative germination of *B. aegyptiaca* under different treatments over days.

In the figure 20, *Ctrl*: Control, *RuSP*: Rubbed with sandpaper, *CW24h*: Soaked in cold water for 24 hours at room temperature of 25°C, CW48h: Soaked in cold water for 48 hours at room temperature of 25°C, *HW65d*: Soaked in hot water at 65°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C, *HW75d*: Soaked in hot water at 75°C for 10 minutes, and left to cool for 12 hours at room temperature of 25°C, *98HSO10m*: Soaked in 98% H_2SO_4 for 10 minutes and subsequent rinsed with water, and *98HSO20m*: Soaked in 98% H_2SO_4 for 20 minutes and subsequent rinsed with water.

The highest cumulative germination (94) was recorded by seeds soaked *98HSO10m*, followed by seeds soaked *98HSO20m* with 89 cumulative germinations, and seeds soaked in *HW75d* with 78 cumulative germinations. The lowest cumulative germination was recorded by seed soaked in *CW48h* with 58 cumulative germination percentage.

The one-way ANOVA also indicated that there exists significant mean variability among treatments groups for the different germination parameters (**Table 10**). In relation to this, the Tukey's HSD test showed that *98HSO10m* (87±8.8) and *98HSO20m* (82±10.2) have the highest GPs which are significant at $p<0.05$ compared

to the other treatment groups. Moreover, Tukey's HSD indicated that *98HSO10m*, *98HSO20m* and *HW75d* have the highest GDs (2%) that are significant at $p<0.05$ compared to the other treatment groups (**Table 11**). The Tukey's HSD for GT also showed that *98HSO20m* (24±2.2), *98HSO10m* (25±0.5) and *CW48h* (25±1.3) have the shortest GTs that are significant at $p<0.05$ compared to the other treatment groups (**Table 11**). Most importantly, the Tukey's HSD resulted that *98HSO10m* (1.04±0.09) and *98HSO20m* (1.01±0.08) have the highest GIs that are significant $p<0.05$ compared to the other treatment groups (**Table 11**).

Table 10. One-way ANOVA of the mean significance difference among treatment group for their effect on mean GP, GD, GT, and GI of B. aegyptiaca.

	Df	***Sum Square***	***Mean Square***	***F-value***	***Pr(>F)***
			GP		
Treatments	7	4162	594.6	10.52	5.38e-06 ***
Residuals	24	1356	56.5		
			GD		
Treatments	7	1.8053	0.25791	10.59	5.09e-06 ***
Residuals	24	0.5846	0.02436		
			GT		
Treatments	7	60.72	8.674	3.429	0.011 *
Residuals	24	60.72	2.530		
			GI		
Treatments	7	0.7313	0.10447	16.27	1.05e-07 ***
Residuals	24	0.1541	0.00642		

Table 11. Summary of GP, GD, GT, GI, and standard deviation (SD) of *B. aegyptiaca* under different pre-sowing treatments.

Treatments	**GP±SD (%)**	**GD±SD (%)**	**GT±SD (days)**	**GI±SD (%/day)**
Ctrl	58±1.9^{a}	1±0.0^{a}	28±2.5^{a}	0.65±0.06^{a}
RuSP	58±6.3^{a}	1±0.1^{a}	28±1.5^{a}	0.62±0.05^{a}
CW24h	62±6.3^{a}	1±0.1^{a}	26±1.7^{a}	0.74±0.06^{a}
CW48h	54±6.4^{a}	1±0.1^{a}	25±1.3^{b}	0.67±0.07^{a}
HW65d	63±10.0^{a}	1±0.2^{a}	26±0.9^{a}	0.73±0.14^{a}
HW75d	72±6.4^{a}	2±0.1^{b}	27±1.0^{a}	0.82±0.07^{a}
98HSO10m	87±8.8^{b}	2±0.2^{b}	25±0.5^{b}	1.04±0.09^{b}
98HSO20m	82±10.2^{b}	2±0.2^{b}	24±2.2^{b}	1.01±0.08^{b}

In table 11 above, the values denoted with the same alphabet (s) along the vertical column of each germination parameter are not significantly different at $p\leq0.05$, and vice versa.

Germination of B. rotundifolia

This study indicated that *B. rotundifolia* is characterized by faster seed germination behaviour compared to *B. aegyptiaca* (**Figure 21**, **Annex 9**).

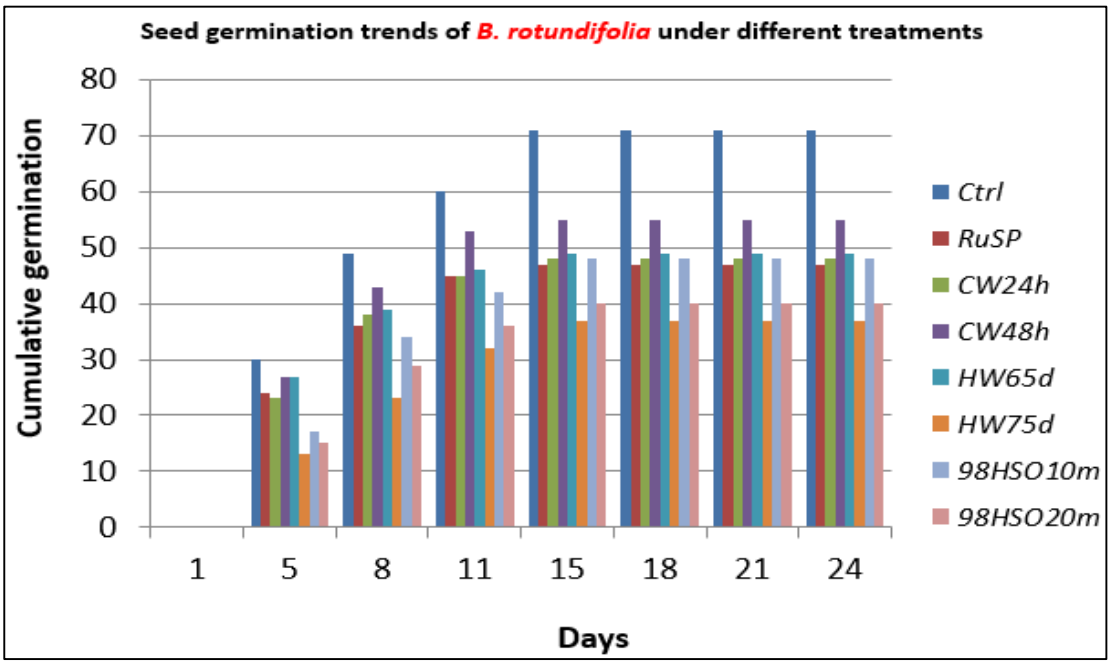

Figure 21. Cumulative germination of of *B. rotundifolia* under different treatments over days.

Figure 22 below illustrated the newly emerging germinate, and fully grown seedlings of *B. rotundifolia*.

Figure 22. A newly emerging germinate (Left), and fully grown seedlings of *B. rotundifolia* (Right).

The highest cumulative germination was recorded by the *Ctrl* (71), followed by *CW48h* (51). The one-way ANOVA also indicated that there exists significant mean variation among treatments groups for the different germination parameters (**Table 12**). However, THSD test showed that the control has the highest GP (66%±10.2) that is significant at $p<0.05$ compared to the other treatment groups (**Table 13**), followed by *CW48h* (51±14.0), while the lowest significant GP at $p<0.05$ was observed by *HW75d* (34±7.0).

Table 12. One-way ANOVA of the mean significance difference among treatment group for their effect on GP, GD, GT, and GI of *B. rotundifolia*.

	Df	***Sum Square***	***Mean Square***	***F-value***	***Pr(>F)***
			GP		
Treatments	7	2602	371.8	4.144	0.00406 **
Residuals	24	2153	89.7		
			GD		
Treatments	7	3.528	0.5039	4.105	0.00428 **
Residuals	24	2.946	0.1228		
			GT		
Treatments	7	9.053	1.2934	5.271	0.000957 ***
Residuals	24	5.889	0.2454		
			GI		
Treatments	7	4.446	0.6351	4.303	0.00328 **
Residuals	24	3.542	0.1476		

Overall, except for *HW75d* and *98HSO20m*, the GDs of the different treatments were found to be 2 germination percentage per day that are significant at $p<0.05$. The species tend to have a mean germination time (GT) of less than 10 days regardless of the treatment type applied. The THSD test for GT indicated that there exist no significant mean differences among treatment groups, except *HW75d*, at $p<0.05$. The Tukey's HSD test for GI also showed that the control has not significant different GI compared to the remaining treatment groups, except *HW75d* and *98HSO20m*, at $p<0.05$ (**Table 13**).

Table 13. Summary of GP, GD, GT, GI, and standard deviation (SD) of *B. rotundifolia* under different pre-sowing treatments.

Treatments	GP±SD (%)	SE	GD±SD (%)	SE	GT±SD (days)	SE	GI±SD (%/day)	SE
Ctrl	66±10.2^{a}	5.1	2±0.4^{a}	0.2	8.3±0.5^{a}	0.2	2.53±0.46^{a}	0.2
RuSP	44±4.7^{b}	2.3	2±0.2^{a}	0.1	7.4±0.6^{a}	0.3	1.81±0.28^{ab}	0.1
CW24h	44±9.1^{b}	4.5	2±0.3^{a}	0.2	7.3±1.1^{a}	0.5	1.83±0.28^{ab}	0.1
CW48h	51±14.0^{b}	7.0	2±05^{a}	0.3	7.3±0.1^{a}	0.1	2.11±0.57^{ab}	0.3
HW65d	45±1.9^{b}	0.9	2±0.1^{a}	0.0	7.2±0.2^{a}	0.1	1.93±0.11^{ab}	0.1
HW75d	34±7.0^{c}	3.5	1±0.3^{b}	0.1	8.6±0.2^{b}	0.1	1.25±0.23^{b}	0.1
98HSO10m	44±15.1^{b}	7.6	2±0.6^{a}	0.3	8.4±0.2^{a}	0.1	1.66±0.59^{ab}	0.3
98HSO20m	37±5.2^{b}	2.6	1±0.2^{b}	0.1	8.1±0.4^{a}	0.2	1.41±0.26^{b}	0.1

3.4 Discussions

The findings of this study revealed that seed germination of *Ziziphus* species (*Z. spina-christi* and *Z. mucronata*) is significantly affected by the method of pre-sowing seed treatment employed. In this regard, cracked seed stones showed a higher germination percentage and faster germination time in comparison to batches with uncracked seed stones, even when both batches were given a similar pre-sowing treatment. *Ziziphus* species generally possess hard seed stones (endocarp), a harder inner layer of fruits covering the seed germplasms, which can make it difficult for the embryo to receive the oxygen and moisture necessary for germination. Hence, it is important to apply mechanical and biochemical treatments to the seed (or fruit) in order to improve its ability to germinate in a shorter period of time. In other words, cracking (or breaking), nicking, and opening the harder seed stones are an effective way to improve the rate of germination. It is likely that the natural dormancy of *Ziziphus* seeds is reduced when the fleshy fruit part (mesocarp) is often eaten mainly by humans, birds, and primates. This exposes it to mechanical injuries, which in turn creates an environment conducive to germination by allowing oxygen and moisture to enter the seed's endosperm.

The application of extended soaking to seeds (or fruits) has been proven to facilitate the permeability of moisture and oxygen necessary for germination. In this study, the cracking of seeds stones along with soaking with chemical mainly water (cold and hot) significantly improved the mean germination percentage of *Z. spina-christi*. Saied et al. (2008) reported that cracking seed coats, scarification with sandpaper, and

soaking in acid generally improve the seed emergence of *Z. spina-christi*. A study report by Wondwossen Gebretsadik (2013) also showed that higher germination percentage was achieved from seeds treated with hot water at 85°C left to cool at room temperature (25°C) for 12 hours, and soaked with 98% H_2SO_4 for 30 minutes.

Similarly, batches with cracked seed stones of *Z. mucronata* tend to have about 6 weeks of germination duration whereas uncracked seed stones result in greater than 3 months of duration. In relation to this, though a very low germination percentage (<20%) was observed, soaking of cracked seed stones with 98% H_2SO_4 as well as rubbing with sandpaper improved the germination capacity of *Z. mucronata*. These findings agree with the report by Hassen et al. (2005) that scarification with sandpaper and soaking with Sulphuric acid for up to 20 minutes were the most effective techniques in breaking the seed dormancy of *Z. mucronata*. Moreover, rubbing with sandpaper and soaking in cold water for 48 hours are also suggested to improve the germination capacity of batches with uncracked stones of *Z. mucronata*. Similarly, Zietsman and Botha (1987) reported that only about l0% germination was observed for uncracked seed stones (endocarp) while more than 70% (<80%) germination for cracked seed stones and partly removed seeds. Azene Bekele-Tesemma (1993) also reported that cracking the seed stones and subsequently soaking them in cold water for at least six hours is suggested to improve the germination of *Z. mucronata*. In other study by Hassen et al. (2005), hot water treatment was also reported to reduce the seed germination of *Z. mucronata*, but not apparently observed in the current study. In this study, compared to *Z. spina-christi*, the seeds of *Z. mucronata* were generally observed to be less sensitive to water stress without exhibiting noticeable marks of soaking impairment even after an extensive soaking period. This is possibly an adaptation strategy for species adapted to arid and semi-arid ecosystems helping the seed dispersal along drainage lines after flash floods (Zietsman & Botha, 1987).

In the current study, some pre-sowing treatment techniques enhanced the germination capability of seeds of *B. aegyptiaca* compared to the control (i.e., seeds received no prior pre-sowing treatment). Generally, soaking the fruits (seeds) in 98% H_2SO_4 and water (hot or cold) enhances the germination percentages of *B. aegyptiaca*. In particular, seeds soaked with 98% H_2SO_4 for 10 and 20 minutes effectively broken the seeds dormancy and significantly improved the mean germination percentages (GP).

The soaking of fruits (seeds) with hot water for up to 10 minutes has also a promising effect on the germination of *B. aegyptiaca*. The fibrous endocarp of fruits needs to be digested to increase the inlet of moisture and oxygen to the embryo for germination to start. A study by Ahmed et al. (2017) reported that the highest germination percentage (>80%) was observed for seeds of *B. aegyptiaca* from Saudi Arabia soaked with cold distilled water for 24 hours. Similarly, a study by Elfeel (2012) in Saudi Arabia also reported that seeds soaked with cold water for 18 and 24 hours resulted in a higher germination percentage compared to seeds soaked for 48 hours. Similarly, soaking the seed in cold water for 24 hours is recommended by Azene Bekele-Tesemma (2007), and a germination rate of 50–70% can be attained. The observation of such variation in seed germination is likely due to the variability of the physical, physiological, and genetic quality of the seeds of the different populations of *B. aegyptiaca* distributed in wider ranges of ecological conditions. Therefore, the physical, physiological, and genetic differences among populations greatly affect their rate of water and chemical uptake by the seeds (Soltani et al., 2018, 2021).

Seed soaking duration affects embryonic physiological activities toward germination and ultimately determines the seed germination capacity, both negatively and positively (Saleem et al., 2014; Jamil, 2016). Increasing the soaking duration in both cold and hot water is very likely to decrease the seed germination percentage of *B. aegyptiaca* (Elfeel, 2012; Ahmed et al., 2017), which was also observed in our present study. Similarly, prolonged soaking of seeds in highly concentrated H_2SO_4 could cause damage in both the external and internal parts of the seeds, which ultimately damages the embryonic tissue, resulting in lower germination percentages (Aref et al., 2011; Ahmed et al., 2017). Overall, the optimal seed soaking duration required for attaining a better germination percentage varies with the species and the ecological conditions under which it evolved. Furthermore, apart from pre-sowing treatments, the sowing orientations of seeds in the soil substrate also affect the germination percentages. For instance, for *B. aegyptiaca*, the horizontal orientation of the seed stalk was recommended to attain higher germination (Elfeel, 2012), while Hall and Walker (1991) recommend the seed be sown vertically with the stalk end downwards.

In this study, the seeds treated with 98% H_2SO_4 were observed to reduce the germination time required for the seed populations to complete their germination. The

shorter the germination time, the shorter the nursery period, and the faster the species reach for plantation and forestry work. In the contrary, a study by Elfeel (2012) reported that seeds soaked with water for 18 and 24 hours as well as untreated seeds resulted in the shortest germination time compared to seeds soaked with water for 48 hours. According to Hall and Walker (1991) and Azene Bekele-Tesemma (2007), the germination of *B. aegyptiaca* generally occurs in 1–4 weeks, which also agrees with our present study. Overall, the current study indicated the potential of seed pre-sowing treatment technologies to enhance seed germination in *B. aegyptiaca,* which has greater implications for the conservation and sustainable utilization of the species in the semi-arid ecosystems of Ethiopia and beyond.

The germination of *B. rotundifolia*, however, was not enhanced by any treatment applied. This might be due to the morphology of the fruit and germplasm of *B. rotundifolia,* which has softer fruits and seed germplasm that are very likely injured by the pre-sowing treatments used, either mechanical or biochemical ones. However, for *B. rotundifolia,* having a good germination percentage (>60%) without pre-sowing treatments is very advantageous in terms of propagation and conservation strategies. Seed propagation without treatment application is by far the most cost-effective approach. The shorter germination time and faster germination rate observed by *B. rotundifolia* are the two additional advantages of the species for easy propagation and cultivation. Therefore, the application of treatments to the fruit (seed) of *B. rotundifolia* might not be necessary (Ruffo et al., 2002). The fruits of *B. rotundifolia* are brittle and smooth on the outside, with a fibrous and oily endocarp and high moisture content. This probably helped the species maintain the sufficient moisture required for germinating in dryland. That is why the species is a very drought-resistant tree, even more so than *B. aegyptiaca* (Beentje, 1994; Ruffo et al., 2002).

3.5 Conclusions

This study identified the best possible seed dormancy-breaking techniques for *Ziziphus* species (*Z. spina-christi* and *Z. mucronata*) and *B. aegyptiaca*. The coupling of mechanical and chemical treatments (e.g., cracking seed stone + soaking with water) generally enhance the germination of *Z. spina-christi* as well as *Z. mucronata*. It is, therefore, essential to first crack so as to open the seed stones, or totally extract the seed germplasms, and subsequent scarification (e.g., abrasion with sandpaper) and

soaking with biochemical reagents mainly water for 24 to 48 hours and/or using concentrated Sulfuric acid for 10 to 20 minutes. The study has also proven that *Z. spina-christi* can be easily propagated and planted, compared to *Z. mucronata*. In general, soaking of seeds in 98% concentrated H_2SO_4, hot water as well as in normal cold water proven to be effective methods to break the seed dormancy, improving the germination percentages, and reducing the mean germination time required to complete the germination. Yet, the untreated seeds of *B. aegyptiaca* also exhibited satisfactory germination (>50%), with implications of the high degree of regeneration and subsequent ecological succession of the species under the natural conditions of recurrent climate change and habitat fragmentation. However, no pre-sowing treatments applied enhanced the seeds germination of *B. rotundifolia*, where more than 60% of seed germination was attained without the application of pre-sowing treatments. This has an implication of the high degree of regeneration and subsequent ecological succession of *B. rotundifolia* under the natural conditions of recurrent climate change and habitat fragmentation. Therefore, both species can be generally considered as alternatives species for dryland rehabilitation and forestry development.

The findings of this study have greater implications for wider large-scale propagation and production of *Ziziphus* and *Balanites* in restoration and rehabilitations of dryland ecosystems as well as domestication and conservation of *Ziziphus* and *Balanites* in Ethiopia. Overall, the findings of this study will contribute to the propagation, conservation and sustainable utilization of *Ziziphus* and *Balanites* species in the agricultural and pastoral communities of Ethiopia.

Chapter 4

4. Evaluation of the Antibacterial Activities of Species from the Genus *Ziziphus* Mill. (*Z. spina-christi* (L.) Willd. and *Z. mucronata* Willd.) and *Balanites* Del. (*B. aegyptiaca* (L.) Del. and *B. rotundifolia* (Tiegh.) Blatt.) on Selected Pathogenic Bacteria

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Abstract

The genus *Ziziphus* and *Balanites* are important sources of traditional medicine in the rural communities of Ethiopia. This study aimed at evaluating the antibacterial activities of *Z. spina-christ, Z. mucronata, B. aegyptiaca*, and *B. rotundifolia*. The ethanol and methanol extracts of leaves were tested against *Staphylococcus aureus, Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using agar-well diffusion. The broth serial macro-dilution method was employed to determine the minimum inhibitory and bactericidal concentrations (MIC and MBC). The methanol extract of *Z. spina-christi* showed noticeable activity against *E. faecalis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *S. typhimurium*, and *K. pneumoniae*. Both solvents' extracts of *B. aegyptiaca* showed inhibitory activities against *K. pneumoniae* and *S. typhimurium*. The one-way ANOVA indicated that there exists significant mean variation among zones of inhibition caused by the different treatment groups (i.e., control and 500, 250 and 125 mg/ml extracts of *Z. spina-christi* and *B. aegyptiaca*) at $p \leq 0.05$. Tukey's HSD showed that, except compared with the control (i.e., Gentamicin), no mean significant difference is observed among the zones of inhibition resulted by 500, 250 and 125 mg/ml extracts of *Z. spina-christi* against *E. faecalis* and *P. aeruginosa* at $p \leq 0.05$. However, significant mean variations are observed between the effects of 500 mg/ml (14.3±2.1) and 125 mg/ml (6.7±0.6) as well between 250 mg/ml (11.7±0.6) and 125 mg/ml extracts of *Z. spina-christi* against *S. aureus* at $p < 0.05$. Similarly, significant mean variations are observed between the effects of 500 mg/ml (12.7±0.6) and 125 mg/ml (10.3±0.6) as well between 250 mg/ml (12.0±1.0) and 125 mg/ml of methanol extract of *B. aegyptiaca* against *K. pneumoniae* at $p < 0.05$. However, no mean significant difference is observed among the zones of inhibition resulted by 500, 250 and 125 mg/ml of ethanol extracts of *B. aegyptiaca* against *K. pneumoniae* as well by both methanol and ethanol extracts of *B. aegyptiaca* against *S. typhimurium*. The lowest MIC (15.625 mg/ml) and MBC (31.25 mg/ml) were recorded for *Z. spina-christi* against *E. faecalis*. *S. typhimurium* is a relatively susceptible bacterium to *B. aegyptiaca* (MIC = 125 mg/ml, MBC = 500 mg/ml) compared to *K. pneumoniae* (MIC = 250mg/ml, MBC = 500 mg/ml) under both ethanol and methanol extracts. This study verified the potential inhibitory properties of *Z. spina-christi* and *B. aegyptiaca* against pathogenic bacteria.

Keywords: Bacteria; *Balanites*; Extracts; Leave; Susceptibility; *Ziziphus*

4.1 Introduction

Traditional medicine is practiced and used by more than 80% of the rural communities in developing countries, including in Ethiopia, as a primary healthcare system (WHO, 2002; Devi et al., 2009). Traditional medicine is affordable and becomes highly accepted as an alternative form of healthcare in developing countries (WHO, 2002; Devi et al., 2009; Maoz & Neeman, 1998; Hammer et al., 1999). Recently, the developments of resistance by pathogenic microbes to the existing synthetic drugs have inspired researchers to investigate the antimicrobial properties of medicinal plants (Maoz & Neeman, 1998; Hammer et al., 1999). Hence, in recent days, the prospect of developing of new bioagents with antimicrobial properties becomes essential field of study in the field of medicinal chemistry (Salman et al., 2022; Zinad et al., 2023). Antimicrobial compounds originated from plants have great therapeutic potential against microbial pathogens such as bacteria and fungi (Salau & Odeleye, 2007). Notably, in this regard, many modern antibiotic drugs were developed from the alternative and complementary medicine (Fabricant & Farnsworth, 2001; Li-Weber, 2009; Yuan et al., 2016). For instance, plant-derived antibiotics such as morphine, quinine, aspirin, and quinidine were originally developed from traditional medicinal plants (Das et al., 2010; Khamkar et al., 2015). Therapeutic compounds derived from traditional medicinal plants have beneficial antioxidants and bioactive agents (Liu & Henkel, 2002; Philip et al., 2009). These include flavonoids, phenolic acids, tannins, and others (Das et al., 2010). These are merely the secondary metabolites of plants that provide defense tools to the plants against infectious microbes and herbivory (Barsi & Fan, 2005). The geographical location of the plant material has been observed to significantly influence the efficacy of plant extracts, as seen in various studies (Adhikari et al., 2020). In relation to this, the degree of antimicrobial properties of plants varies with the habitat heterogeneity, as environmental variables affect the process of biosynthesis and accumulation of bioactive compounds in the plant system.

Ziziphus and *Balanites* are commonly used medicinal plants by the local people in different cultural landscapes in Ethiopia. In Ethiopia, *Balanites aegyptiaca* is commonly used as a medicinal plant to treat pneumonia in the Ada'ar District of the Afar region (Mirutse Giday & Tilahun Teklehaymanot, 2013), treats wounds *(*Ali

Zeynu et al., 2021), treats infant sickness, herpes zoster, blackleg, breast cancer, lung infection, mumps, dysentery, and other diseases (Tilahun Teklehaymanot, 2017). *Balanites rotundifolia* is also reported to be used as a medicinal plant in the Ada'ar District of the Afar region (Mirutse Giday & Tilahun Teklehaymanot, 2013) and treats malaria *(*Ali Zeynu et al., 2021). *Ziziphus spina-christi* is commonly used as a medicinal plant to treat delayed placenta and pneumonia in the Ada'ar District of the Afar region (Mirutse Giday & Tilahun Teklehaymanot, 2013), treats Hemorrhage and placental retention (Asmare Talie et al., 2018), and heals Dandruff (Ali Zeynu et al., 2021). *Ziziphus mucronata* is an important multi-purpose plant species that has been used in African traditional medicine for years in the treatment of various human and animal infections (Mongalo et al., 2020). It is also widely used for skin and hair care, mostly by women in Northeastern and eastern Ethiopia. In addition to the above-mentioned uses, species of the two genera are known for their vital roles in securing food and forage, generating income and energy needs, and providing medicinal use for rural communities (Panghal et al., 2010). The fact that the two genera have many features in common, and are used as potential sources of input for the detergent industry, calls for deeper studies to generate relevant data for future conservation and sustainable use planning. Although *Ziziphus* and *Balanites* are reported for their traditional medicinal uses in Ethiopia, no *in-vitro* antimicrobial susceptibility tests have been reported so far. This study aimed at evaluating the anti-bacterial activities of *Z. spina-christ, Z. mucronata, B. aegyptiaca,* and *B. rotundifolia* against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, thereby verifying the medicinal importance of the study plants. Therefore, the objectives of this study were (i) to extract crude compounds from each plant species using alcoholic solvents (i.e., ethanol and methanol), (ii) to conduct an agar-well diffusion of anti-bacterial bioassay using MH agar media, and (iii) to measure the MIC and MBC of the study plants against the most susceptible bacterial strains. Therefore, it was hypothesized that *Z. spina-christ, Z. mucronata, B. aegyptiaca,* and *B. rotundifolia* species would show potency against common bacterial pathogenic strains.

4.2 Materials and methods

Plant samples collection

Young and healthy leave samples of the four plant species were collected from the Northern-Eastern, Central, and Southern Rift Valley of Ethiopia, including areas of the Western escarpment in Northeastern Ethiopia (**Figure 23**). The samples were collected between November and December 2022, mainly from localities and populations that have been previously surveyed and recorded as part of the thematic research under Dr. Tigist Wondimu as the principal investigator for studying the inter- and intra-morphological variability of *Balanites* and *Ziziphus* in Ethiopia. Hence, leaf samples for *B. aegyptiaca* were collected from Boset district in Oromia region (39.716388°E, 8.82833°N; 39.6725E, 8.8069444°N), Ubadeberetsehay district (36.76703°E, 5.92461°N) and Arba Minch Zuria district (37.57444°E, 6.1097222°N) in Southern Nations, Nationalities and Peoples Region (SNNPR), and Dima district in Gambella region (35.18337°E, 6.64147°N); and *B. rotundifolia* from Mirab Abaya (37.76833°E, 6.3075°N; 37.743611°E, 6.39888°N; 37.784444°E, 6.5252777°N) and Ubadeberetsehay (36.76659°E, 5.92465°N; 36.9647°E, 6.19399°N) district in SNNPR; *Z. spina-christi* from Asayita district in Afar region (41.470555°E, 11.526388°N), Adama Zuria district (39.20774°E, 8.54799°N; 39.2293°E, 8.53156°N) and Boset district (39.489722°E, 8.6775) in Oromia region; and *Z. mucronata* from Arba Minch Zuria district (37.62743°E, 6.12204°N; 37.492368°E, 5.894401°N) and Ubadeberetsehay district (36.9503°E, 6.11977°N) in SNNPR, Boset district (38.77472°E, 8.105833°N) and Maeso district (40.58287°E, 9.17381°N) in Oromia.

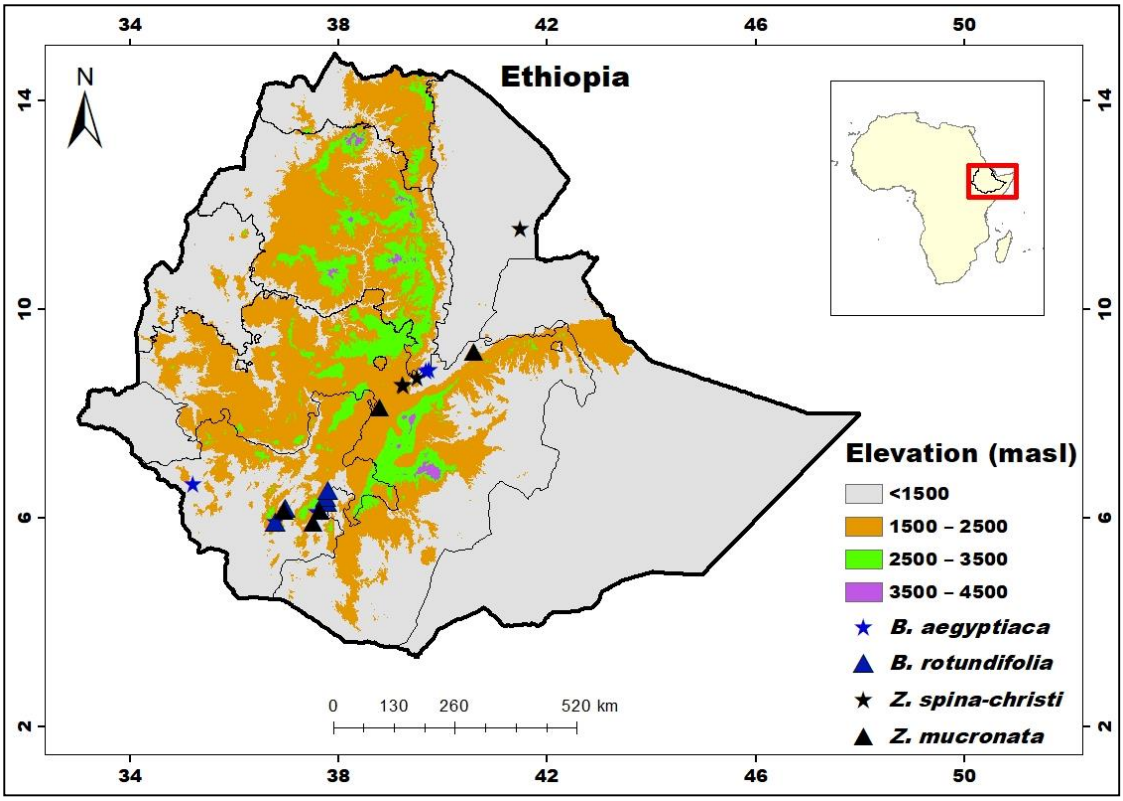

Figure 23. Map of Ethiopia showing collection areas for leaf samples of each plant species.

The plant specimens were processed and identified by the researchers (authors), and deposited in the National Herbarium of Ethiopia (ETH), Addis Ababa University (AAU), giving voucher specimen numbers: *Z. spina-christi* (EnTW-060, gsr/1767/13-081, gsr/1767/13-084, and EnTW-016), *Z. mucronata* (gsr/1767/13-104, EnTW-069, gsr/1767/13-127, EnTW-026, and gsr/1767/13-091), *B. aegyptiaca* (gsr/1767/13-017, EnTW-044, gsr/1767/13-124, EnTW-011, and EnTW-012), and *B. rotundifolia* (gsr/1767/13-123, gsr/1767/13-129, EnTW-050, EnTW-053, and EnTW-056).

Preparation of plant extracts

The collected leave samples were washed, rinsed with distilled water, and finally dried in the shade in neat condition. The dried plant material of each plant species was ground into a fine powder. The plant powders were initially diluted with a 5:1 (v/w) solvent-to-sample ratio (Chintalapani et al., 2018; Sachir et al., 2022). Accordingly, to prepare the alcoholic extracts, 50g of dried powdered leaves of each species were soaked in 250 ml of 96% ethanol absolute and 99% methanol absolute and placed on an orbital shaker for 48 h at 181 rpm (EDQM, 2016). To obtain a clear filtrate, the

macerated plant materials were first filtered through cotton wool and then through Whatman filters paper (No. 1).

The filtrates were evaporated and dried at 55^{o}C under reduced pressure using a rotatory vacuum evaporator (Wagtech-RE-300). In order to completely dry the extract, the remaining solvents were further dried in an oven at 40°C and obtained a semi-solid to solid yield. The purified extracts were capped airtight and preserved at -20°C in the refrigerator for further use (**Figure 24**).

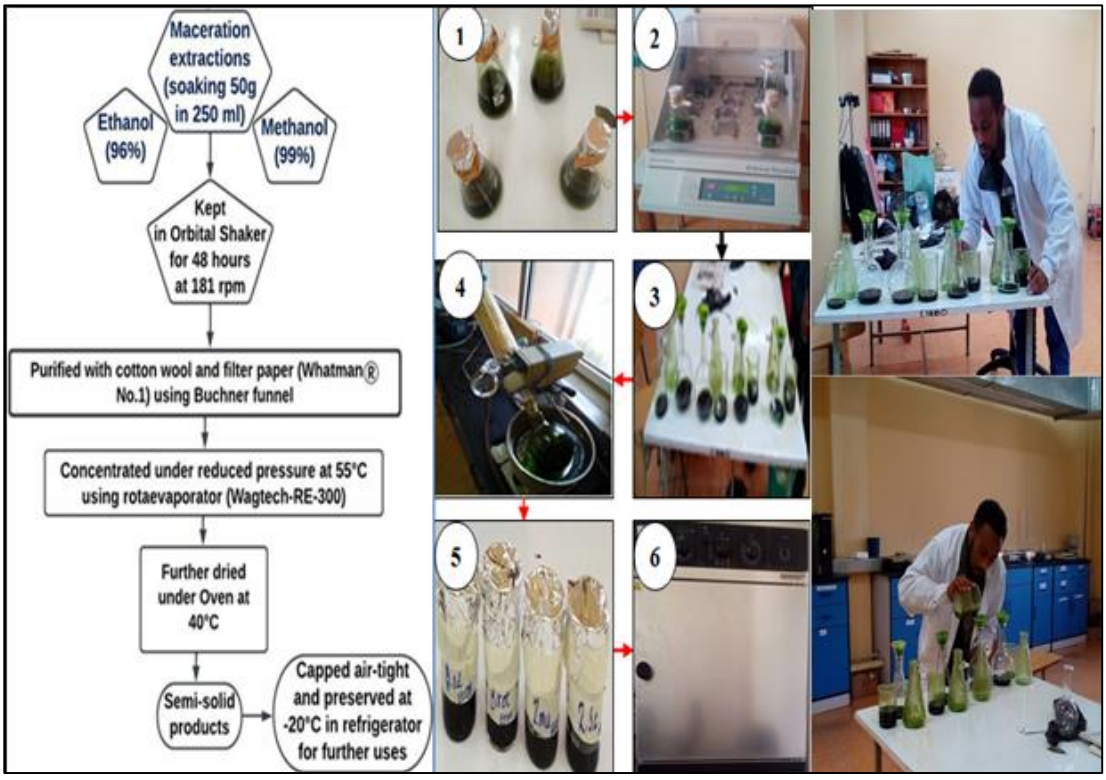

Figure 24. The general framework used to extract plant products using methanol and ethanol solvents.

In the figure 24 above, 1= Soaking leaf powder in alcoholic solvents, 2= Shaking on a digital shaker, 3 = Filtering using cotton wool and filter paper, 4 = Concentrating the extracts under rotaevaporator, 5 = Dispensing extracts in bakers, 6 = Evaporating remaining solvent in an oven).

Standard Culture of Test Bacteria

The selection of potential test bacteria was determined based on reports on ethnomedicinal uses of *Balanites* and *Ziziphus* species (Dafni et al., 2005; Kaleem et

al., 2014; Tilahun Teklehaymanot, 2017; Asmare Talie et al., 2018; Osman et al., 2020). Bacteria that are known to cause gastrointestinal diseases such as dysentery, hemorrhage, bloody diarrhea, stomach pain, and other ailments were considered in this study (Gonelimali et al., 2018; Manandhar et al., 2019). The Bacterial Glycerol Stocks (GBS) of six pathogenic bacteria (both gram-positive and gram-negative), namely *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 25212), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 13311), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumonia* (ATCC 700603), were obtained from the Ethiopian Public Health Institute (EPHI). The strains were immediately and continuously (3x) sub-cultured until clear colonies were obtained in agar media (*K. pneumoniae* and *E. coli* in Eosine Methylene Blue (EMB) agar; *S. aureus* in Mannitol Salt Agar (MSA); *E. faecalis* in Enterococcus-agar media; *P. aeruginosa* in *Cetrimide agar*; and *S. typhimurium* in Xylose Lysine Deoxycholate (XLD) agar (**Figure 25**). Then, stock cultures were placed in a refrigerator at 4°C for subsequent use. In the figure 25 below, 1 = S. aureus, 2 = *E. faecalis*, 3 = *E. coli*, 4 = *S. typhimurium*, 5 = *P. aeruginosa and 6 = K. pneumoniae*).

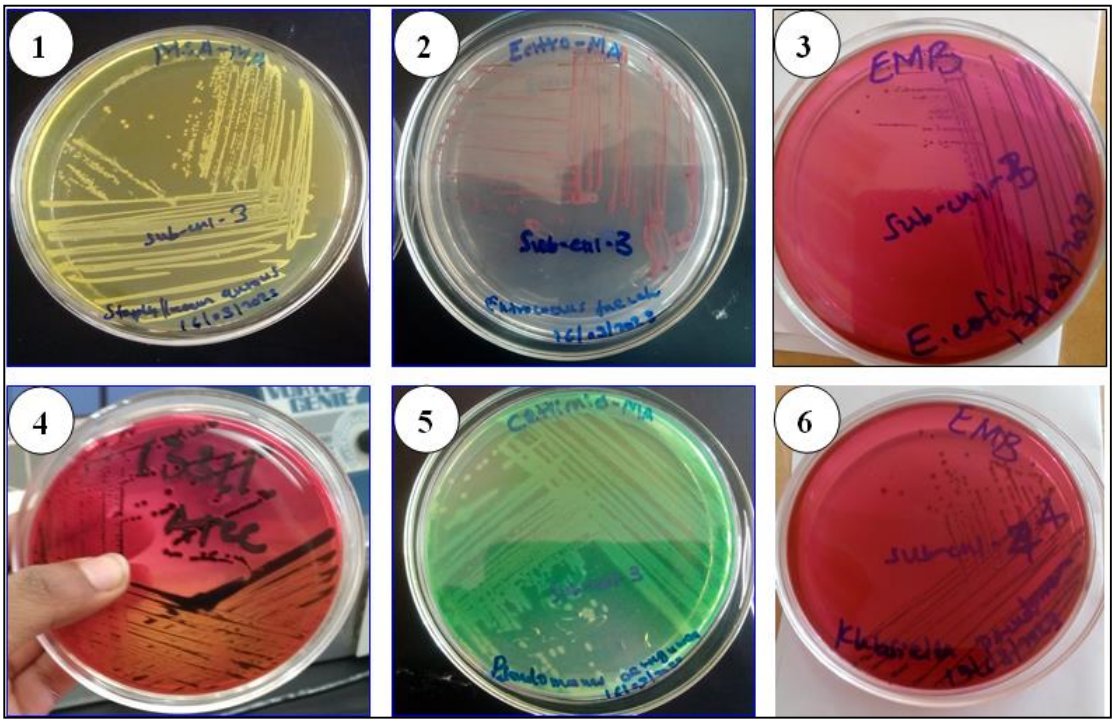

Figure 25. Plate of bacterial cultures grown from GBS with successive sub-cultures.

Inoculum preparation (broth culture)

Each bacterial strain was subcultured into 5 ml of Tryptic Soy Broth and incubated overnight at 37°C. For inoculum standardization, the turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard (1-2× 10^8 CFU/ml) using Tryptic Soy Broth. Separately, Mueller-Hinton (MH) agar was aseptically prepared, and an amount of 25 ml of MH media was poured into Petri plates of 100 mm size and allowed to solidify for 3–5 minutes. The MH medium was inoculated with bacterial inoculum suspension using cotton swabs and evenly spread over the entire MH surface.

Antimicrobial bioassay

Then, the purified and sterilized dry alcoholic plant extract residues were dissolved in 10% Dimethyl Sulfoxide-DMSO (Gurnani et al., 2016). Finally, three different concentrations of 500, 250, and 125 mg/ml plant extracts were formulated for the initial test of bacterial susceptibility against plant extracts (**Figure 26**).

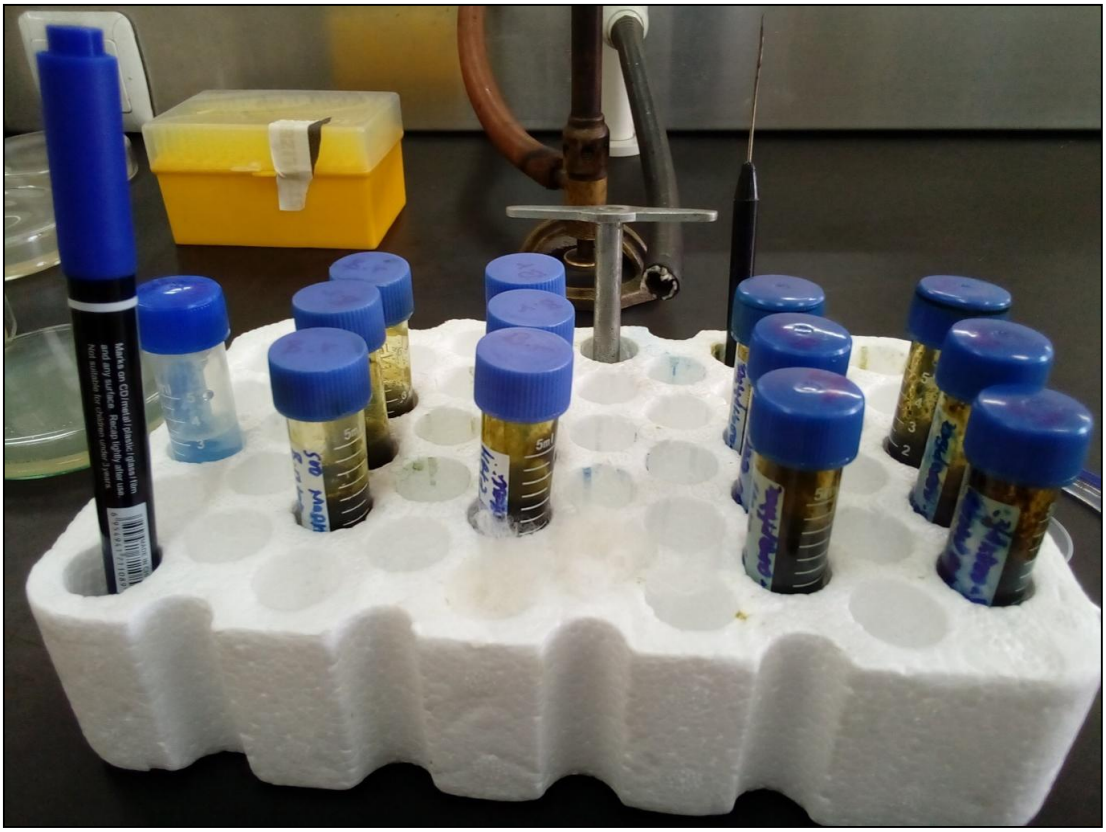

Figure 26. Plant extract concentrations of 500, 250 and 125 mg/ml formulated for initial test of bacterial susceptibility against plants extracts.

The agar-well diffusion method was used to evaluate the antimicrobial activity of plants extracts (Heatley, 1944; Magaldi et al., 2004; Valgas et al., 2007). Hence, for this study, seven wells of 6 mm diameter and 4 mm depth were perforated at an equal distance using a cork borer (**Figure 27**). Then, the volumes of 60 µL of the three extract concentrations (500, 250, and 125 mg/ml) were dispensed into the six marginal wells (1 for 500 mg/ml, 2 for 250 mg/ml, 3 for 125 mg/ml of ethanol extract; and 4 for 500 mg/ml, 5 for 250 mg/ml, and 6 for 125 mg/ml of methanol extract) with micropipettes. Gentamicin Sulfate (60 µL of 10 mg/ml) was dispensed into the 7th well at the center and used as a positive control. Each antibacterial activity test was performed in triplicate. So, a total of eighteen plates were used to test the antibacterial activity of the plant extracts against six bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *and Klebsiella pneumoniae*).

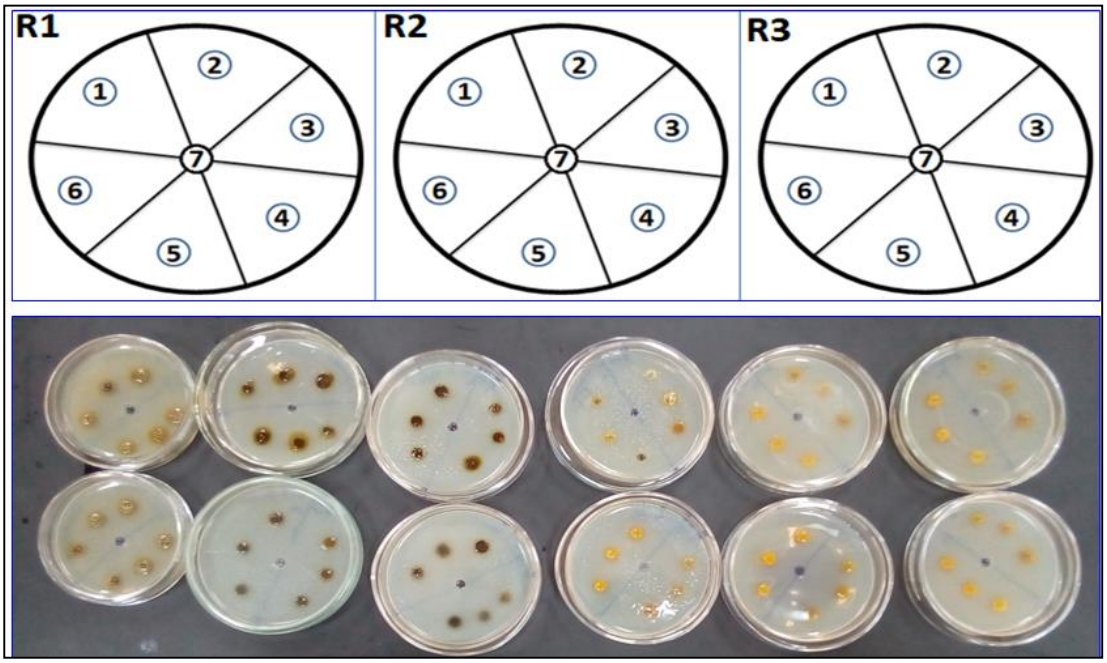

Figure 27. Illustration of the agar wells and antibacterial assay.

Figure 27 above shows the triplicated culture plates representing one bacterial strain tested against a given plant extract (Upper); actual plant extracts loaded into the plate wells inoculated with bacterial strains (Lower). Then, inoculated plates were left for 30 minutes to let the extracts diffuse well into the agar (Gonelimali et al., 2018). The plates were then properly labeled with the plant species, *viz.*, the bacterial strains, and

incubated at 37°C for 24 hours. Finally, the diameter of the zones of inhibition was measured in millimeters (mm) using a metric ruler and recorded.

Determination of minimum inhibitory and bactericidal concentrations

After preliminary screening of the plants for their antimicrobial activity, bacterial strains with positive susceptibility to the three extract concentrations (125, 250, and 500 mg/ml) were further tested to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC and MBC were determined following the broth serial dilution method as described by Eloff (1998). Accordingly, for a given plant species, six levels of broth macro-dilutions of plant extracts (i.e., 15.625, 31.25, 62.5, 125, 250, and 500 mg/ml) were prepared in duplicate (Singh & Kumar, 2013; Mogana et al., 2020), and 10µL broth culture suspension containing approximately 1–2 × 10^8 CFU/ml, adjusted with 0.5 McFarland standard turbidity, from an overnight broth culture was added in each dilution (**Figure 28**).

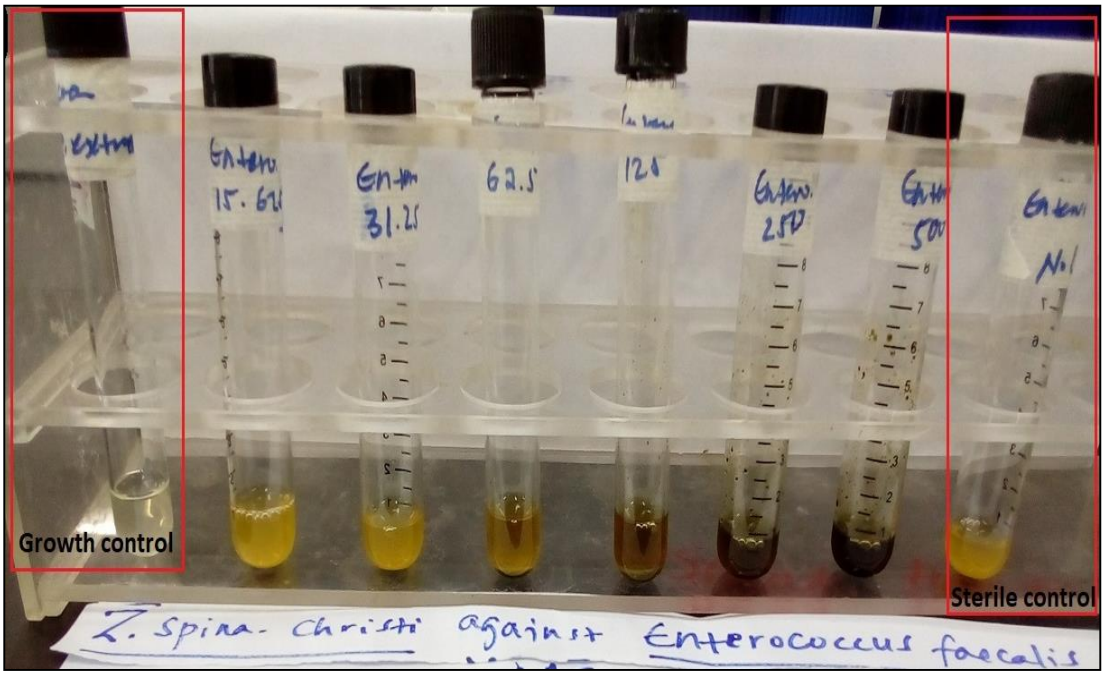

Figure 28. Example of broth serial dilutions of MeOH extract for determining the MIC and MBC of *Z. spina-christi* against *E. faecalis*.

Then, the inoculated dilutions were incubated for 24 hours and later subcultured in nutrient agar media to determine the MIC and MBC. MIC is the lowest concentration of antimicrobial agent that inhibits the growth of bacteria after overnight incubation (Levison, 2004), while MBC is the lowest concentration of antibacterial agent

required to completely kill a particular bacterium (Wiegand et al., 2008). When the MBC/MIC ratio is ≤4, the effect is considered bactericidal, but if the MBC/MIC ratio is >4, the effect is regarded as bacteriostatic (Levison, 2004; Benjamin et al., 2012).

Data analysis

The degree of susceptibility of each test organism was determined by measuring the zone of inhibition (i.e., a clear circular area where the growth of bacteria is inhibited). Following the classification of Morales et al. (2003) and Detha et al. (2018), the antimicrobial inhibition zone was evaluated as no or weak inhibition (<5 mm), moderate (5–10 mm), strong (>10–20 mm), and very strong (>20–30 mm). The degree of susceptibility was summarized as mean ± standard deviation. Moreover, one-way ANOVA was computed to see the level of significance difference among zone of inhibitions caused by the different treatment groups (i.e., control and 500, 250 and 125 mg/ml) at $p \leq 0.05$. Tukey's Honestly Significant Difference (THSD) was also computed to check the significance of difference between pairs of treatments at $p \leq 0.05$ (Steel et al., 1997), allowing all possible pairwise comparisons while keeping the family-wise error rate low.

4.3 Results

Susceptibility of bacterial strains to the plant extracts

The methanol extract of *Z. spina-christi* showed noticeable potency against *E. faecalis*, *P. aeruginosa*, and *S. aureus* under all three concentration gradients and was potent against the other tested bacteria at higher concentrations (**Table 14**, **Figure 29**). The ethanol extract of *Z. spina-christi* did not show potency against any of the tested bacteria strains. Moreover, both methanol and ethanol extracts of *B. aegyptiaca* showed noticeable potency against *K. pneumoniae* and *S. typhimurium*, while no detectable potency was observed against the other bacteria tested (**Table 14**, **Figure 30**). However, *Z. mucronata* and *B. rotundifolia* did not show any measurable potency against all the tested bacteria.

Table 14. Susceptibility of selected bacterial strains to 500, 250 and 125 mg/ml concentrations of methanol and ethanol leaf extracts of four plant species.

	Methanol extracts (mg/ml)											
	B. aegyptiaca			*B. rotundifolia*			*Z. spina-christi*			*Z. mucronata*		
	500	250	125	500	250	125	500	250	125	500	250	125
S. aureus	-	-	-	-	-	-	+	+	+	-	-	-
E. faecalis	-	-	-	-	-	-	+	+	+	-	-	-
E. coli	-	-	-	-	-	-	+	+	-	-	-	-
S. typhimurium	+	+	+	-	-	-	+	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	+	+	+	-	-	-
K. pneumoniae	+	+	+	-	-	-	+	-	-	-	-	-
	Ethanol extracts (mg/ml)											
	B. aegyptiaca			*B. rotundifolia*			*Z. spina-christi*			*Z. mucronata*		
	500	250	125	500	250	125	500	250	125	500	250	125
S. aureus	-	-	-	-	-	-	-	-	-	-	-	-
E. faecalis	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-
S. typhimurium	+	+	+	-	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-
K. pneumoniae	+	+	+	-	-	-	-	-	-	-	-	-

Note: "+" = positive, showed susceptibility), "-" = negative, doesn't show susceptibility).

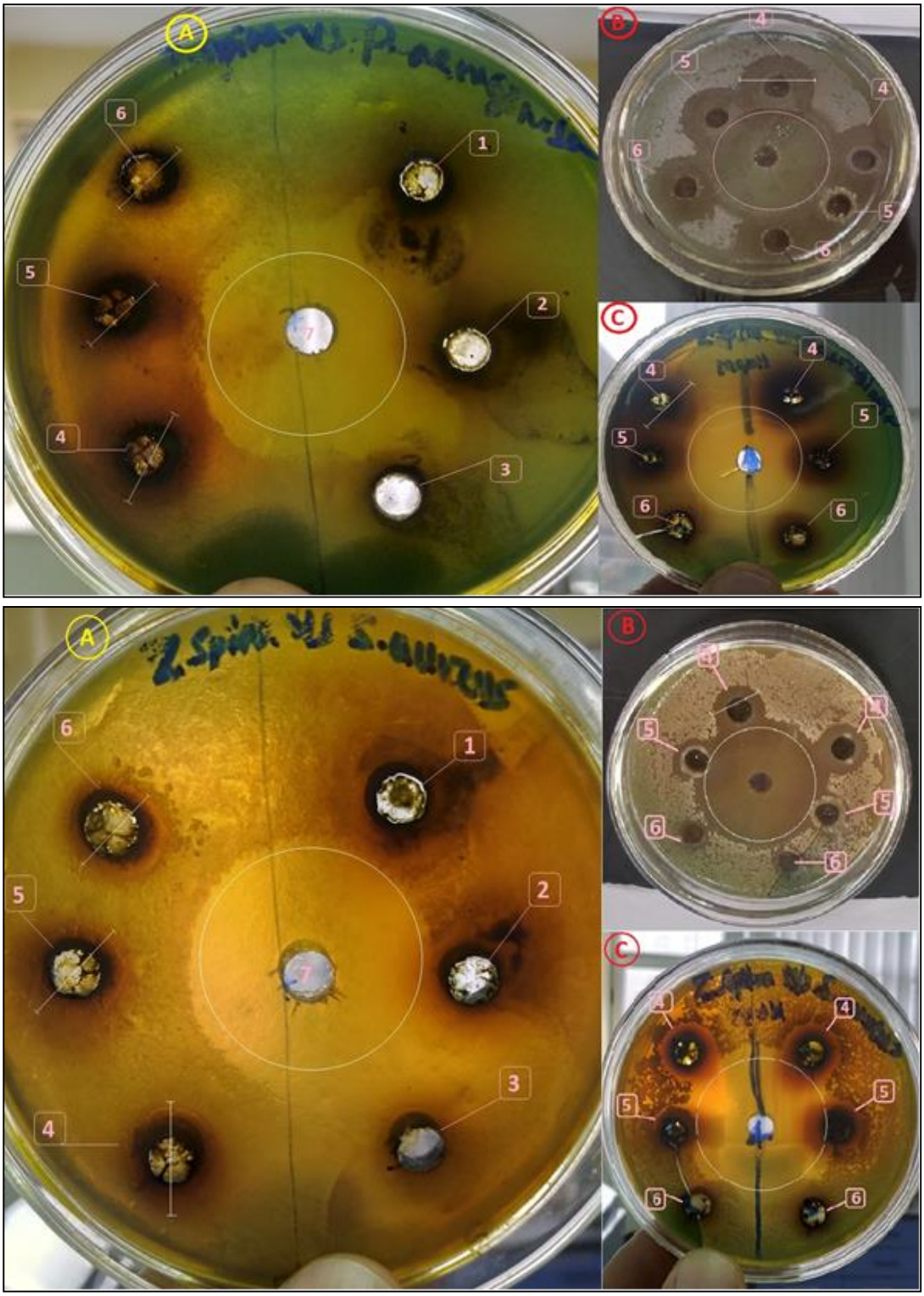

Figure 29. An initial susceptibility test of *P. aeruginosa* and *S. aureus* to *Z. spina-christ* with 500, 250 and 125 mg/ml concentrations of methanol (MeOH) and ethanol (EtOH) extracts.

In the figure 29 above, 1 = 500, 2 = 250 and 3 = 125 mg/ml of EtOH extracts; 4 = 500, 5 = 250 and 6 = 125 mg/ml of MeOH extracts. B (front views), and C (hind

views): susceptibility retest of *P. aeruginosa* and *S. aureus* to the MeOH extracts of *Z. spina-christi* rechecked in separate plates.

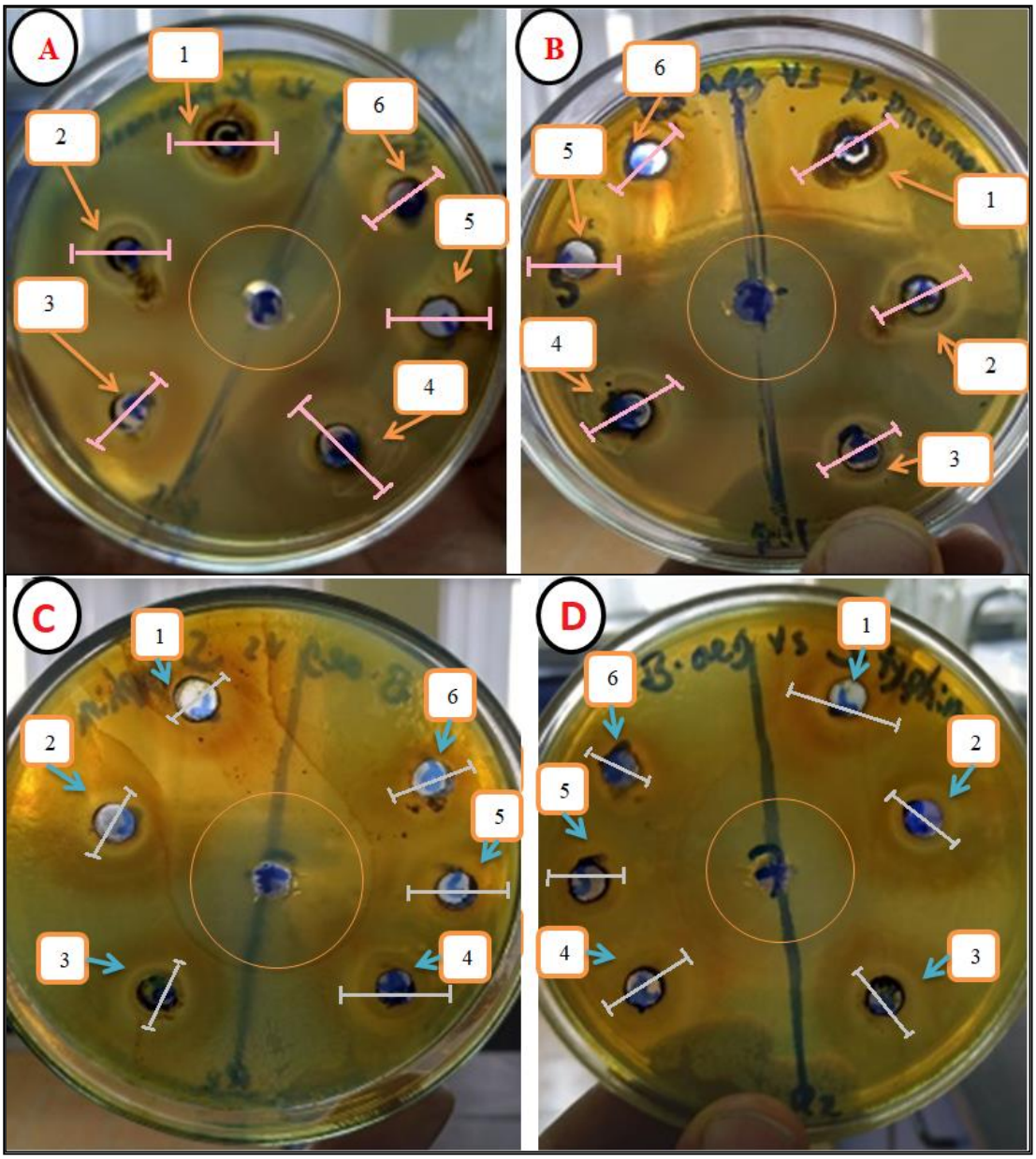

Figure 30. An initial susceptibility test of *K. pneumoniae* and *S. typhimurium* to *B. aegyptiaca* with 500, 250 and 125 mg/ml concentrations of methanol (MeOH) and ethanol (EtOH) extracts.

In the figure 30 above, A and C (front views), B and D (hind views); 1 = 500, 2 = 250 and 3 = 125 mg/ml of EtOH extracts; 4 = 500, 5 = 250 and 6 = 125 mg/ml of MeOH extracts. The rose and grey lines are the zone of inhibitions caused by methanol extracts of B. aegyptiaca against *K. pneumoniae* and *S. typhimurium*, respectively.

Methanol extract of *Z. spina-christi* showed activity against *E. faecalis*, *P. aeruginosa*, and *S. aureus*, which generally increases with the level of extract concentration used. The one-way ANOVA indicated that there exists significant mean variation among zone of inhibitions resulted by the different treatment groups (i.e., control and 500, 250, and 125 mg/ml extracts of *Z. spina-christi*) at $p \leq 0.05$ (**Table 15**).

Table 15. One-way ANOVA of the mean significance difference of zone of inhibitions among different leaf extracts of *Z. spina-christi* against the most susceptible tested bacteria.

	Df	***Sum Square***	***Mean Square***	***F-value***	***Pr(>F)***
			E. faecalis		
Treatments	3	680.7	226.9	56.72	9.82e-06***
Residuals	8	32.0	4.0		
			S. aureus		
Treatments	3	775.6	258.53	193.9	8.29e-08***
Residuals	8	10.7	1.33		
			P. aeruginosa		
Treatments	3	288.25	96.08	16.47	0.000874***
Residuals	8	46.67	5.83		

However, THSD test showed that except for the control (i.e., Gentamicin) there exists no mean significant difference among the zone of inhibitions resulted by 500, 250 and 125 mg/ml extract concentrations of *Z. spina-christi* against *E. faecalis* and *P. aeruginosa* at $p \leq 0.05$. However, significant mean variations are observed between the effects of 500 mg/ml (14.3±2.1) and 125 mg/ml (6.7±0.6) as well between 250 mg/ml (11.7±0.6) and 125 mg/ml extract concentrations of *Z. spina-christi* against *S. aureus* at $p < 0.05$ (**Table 16**).

Table 16. Summary of the mean ($\bar{x}$) and standard deviation (SD) of zone of inhibition calculated for the different concentrations of methanol (MeOH) extract of *Z. spina-christi* against *E. faecalis*, *P. aeruginosa*, and *S. aureus*.

Concentrations (mg/ml)	***E. faecalis***		***P. aeruginosa***		***S. aureus***	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
500	12.3[a]	2.5	18.0[a]	2.6	14.3[a]	2.1
250	10.7[a]	1.5	15.3[a]	1.5	11.7[a]	0.6
125	7.3[a]	0.6	13.0[a]	1.0	6.7[b]	0.6
Gentamicin	27.0[b]	2.6	26.0[b]	3.6	28.3[c]	0.6

Overall, the control has significantly higher inhibition effects against all tested bacterial strains compared to the plant extracts of different concentrations (500, 250 and 125 mg/ml) of *Z. spina-christi*. Furthermore, *Z. spina-christi* showed a moderate-to-strong inhibition against *E. faecalis*, a strong inhibition against *P. aeruginosa*, and a moderate-to-strong inhibition against *S. aureus*. Similarly, the one-way ANOVA also indicated that there exists significant mean variation among zone of inhibitions resulted by the different treatment groups (i.e., control and 500, 250 and 125 mg/ml extracts of *B. aegyptiaca*) at $p \leq 0.05$ (**Table 17**).

Table 17. One-way ANOVA of the mean significance difference of zone of inhibitions among different leaf extracts of *B. aegyptiaca* against the most susceptible tested bacteria.

	Df	***Sum Square***	***Mean Square***	***F-value***	***Pr(>F)***
			K. pneumoniae		
Treatments (EtOH)	8	686.3	228.75	144.5	2.64e-07***
Residuals	3	12.7	1.58		
			K. pneumoniae		
Treatments (MeOH)	8	764.9	254.97	611.9	8.71e-10***
Residuals	3	3.3	0.42		
			S. typhimurium		
Treatments (EtOH)	8	733.7	244.6	122.3	5.06e-07***
Residuals	3	16.0	2.0		
			S. typhimurium		
Treatments (MeOH)	8	652.9	217.64	58.04	9e-06***
Residuals	3	30.0	3.75		

However, except with the control (i.e., Gentamicin) and among the methanol extracts of *B. aegyptiaca* against *K. pneumoniae*, there exist no mean significant difference among the zone of inhibitions resulted by the 500, 250 and 125 mg/ml extract at $p \leq 0.05$ for ethanol extracts of *B. aegyptiaca* against *K. pneumoniae* as well both methanol and ethanol extracts of *B. aegyptiaca* against *S. typhimurium* (**Table 18**). However, significant mean variations are observed between the effects of 500 mg/ml (12.7±0.6) and 125 mg/ml (10.3±0.6) as well between 250 mg/ml (12.0±1.0) and 125 mg/ml of methanol extract of *B. aegyptiaca* against *K. pneumoniae* at $p<0.05$ (**Table 18**). Generally, however, *B. aegyptiaca* showed strong inhibition against *K. pneumoniae* and *S. typhimurium*.

Table 18. Summary of the mean ($\bar{x}$) and standard deviation (SD) of zone of inhibition calculated for the different concentrations of methanol (MeOH) and ethanol (EtOH) extracts of *B. aegyptiaca* against *K. pneumoniae* and *S. typhimurium*.

	K. pneumoniae				***S. typhimurium***			
	MeOH extract		EtOH extract		MeOH extract		EtOH extract	
Concentrations (mg/ml)	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
500	12.7^{a}	0.6	13.0^{a}	0.0	12.3^{a}	2.3	11.7^{a}	0.6
250	12.0^{ab}	1.0	12.7^{a}	1.5	11.7^{a}	2.1	10.3^{a}	1.5
125	10.3^{b}	0.6	12.0^{a}	2.0	11.0^{a}	2.0	10.0^{a}	2.0
Gentamicin	30.0^{c}	0.0	30.0^{b}	0.0	28.7^{b}	1.2	28.7^{b}	1.2

Minimum inhibitory and bactericidal concentrations

The MIC and MBC for the methanol extract of *Z. spina-christi* showed antibacterial activity at all three concentration gradients tested. However, the ethanol extract of *Z. spina-christi* did not show any potency against the tested bacteria. In this regard, *E. faecalis* is found to be the most susceptible bacterial strain to *Z. spina-christi* (MIC = 15.625 mg/ml, MBC = 31.25 mg/ml), while *S. aureus* and *P. aeruginosa* are less susceptible to *Z. spina-christi* (MIC = 250 mg/ml, MBC = 500 mg/ml each) (**Table 19**, **Figure 31**). However, for all tested bacteria, the MIC/MBC ratio is found to be ≤4 indicating that the effect of *Z. spina-christi* is bactericidal against the tested bacteria.

Similarly, the MIC and MBC for *B. aegyptiaca* calculated against tested bacteria showed that *S. typhimurium* is a relatively susceptible bacterium to *B. aegyptiaca* (MIC = 125 mg/ml, MBC = 500 mg/ml) compared to *K. pneumoniae* (MIC =

250mg/ml, MBC = 500 mg/ml) under both ethanol and methanol extracts (**Table 19**, **Figure 32**). However, for both bacteria, the MIC/MBC ratio is ≤ 4. This indicates that *B. aegyptiaca* has a bactericidal effect against the tested bacteria, in both the ethanol and methanol extracts.

Table 19. MIC and MBC for methanol extract of *Z. spina-christi* and *B. aegyptiaca* against most susceptible bacterial strains.

	Z. spina-christi		
Test bacteria	**MIC (mg/ml)**	**MBC (mg/ml)**	**MBC/MIC ratio**
E. faecalis	15.625	31.25	2.0
S. aureus	250	500	2.0
P. aeruginosa	250	500	2.0

	B. aegyptiaca				
Test bacteria	**MIC (mg/ml)**		**MBC (mg/ml)**		**MBC/MIC ratio**
	EtOH extract	MeOH extract	EtOH extract	MeOH extract	
K pneumoniae	250	250	500	500	2.0
S. typhimurium	125	125	500	500	4.0

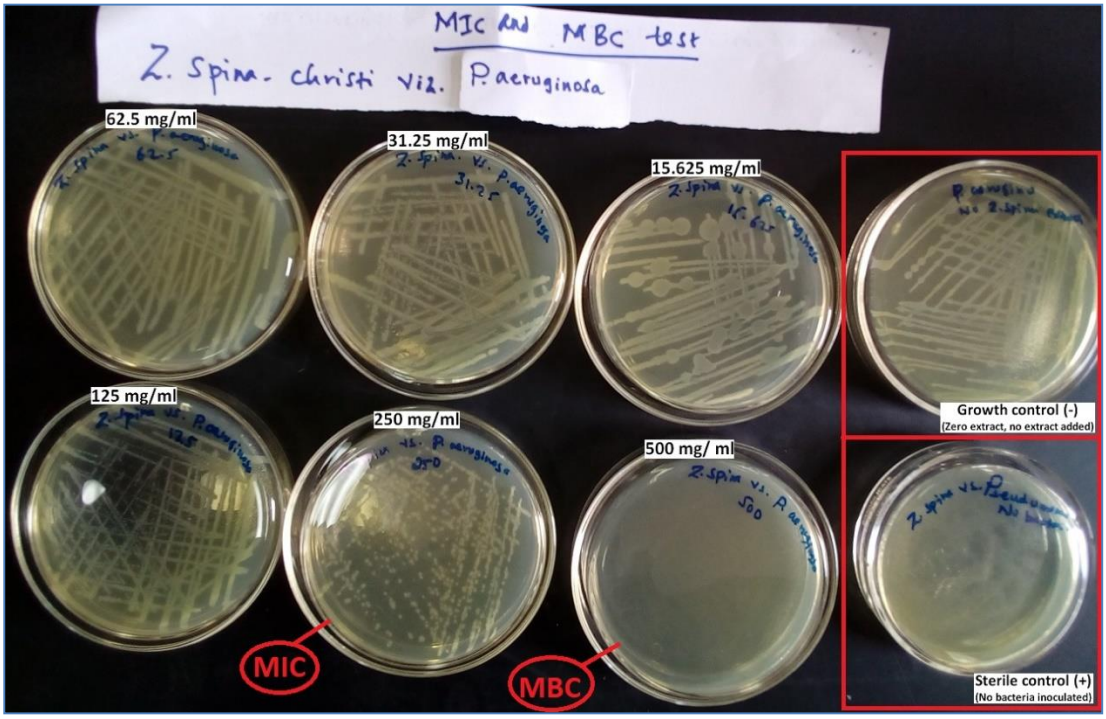

Figure 31. MIC and MBC tests results for MeOH extract of *Z. spina-christi* against *P. aeruginosa*.

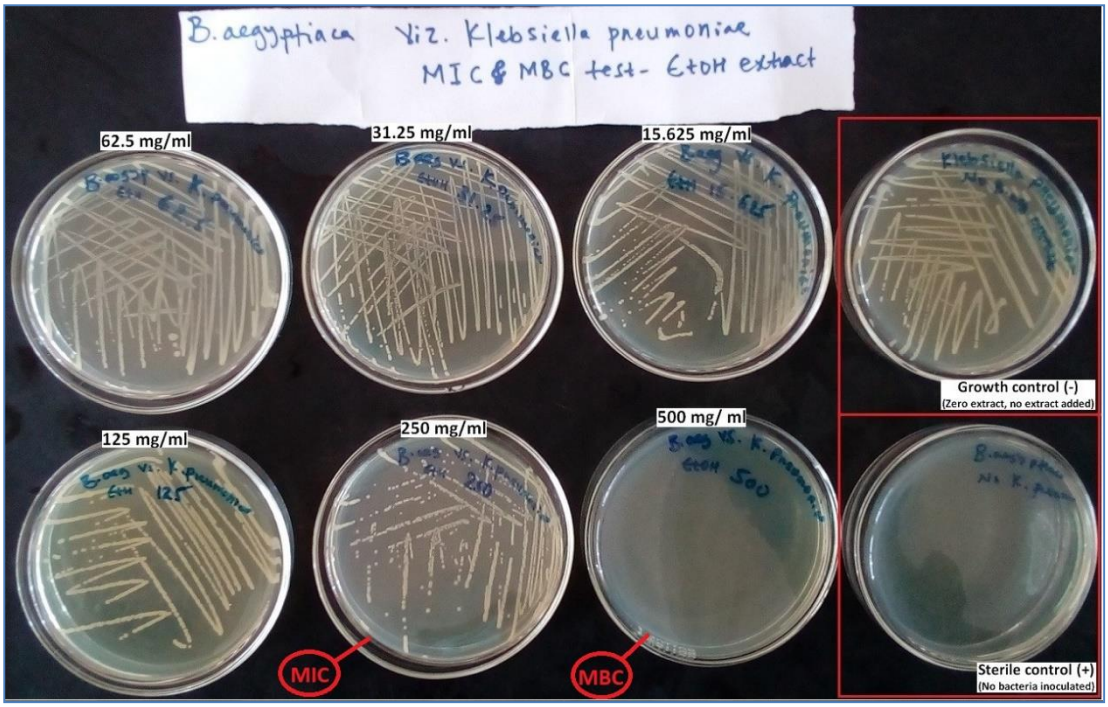

Figure 32. MIC and MBC tests results for ethanol (EtOH) extract of *B. aegyptiaca* against *K. pneumoniae*.

4.4 Discussions

This study showed that *Z. spina-christi* has strong antimicrobial properties against a wide range of bacterial pathogens (*E. faecalis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *S. typhimurium*, and *K. pneumoniae*) compared to other tested medicinal plants (*B. aegyptiaca*, *B. rotundifolia*, and *Z. mucronata*). Overall, *Z. spina-christi* showed potency against the tested bacteria with the leave extracts of 500 mg/ml and below. Previous studies also reported a high degree of susceptibility of *Klebsiella sp.*, *S. aureus*, *E. coli*, and *P. aeruginosa* against alcoholic extracts of leaves of *Z. spina-christi* using agar-well and disc diffusion methods (Al-Kaabi et al., 2021; Elaloui et al., 2022; Al-Azawi et al., 2022). Thus, it can be suggested that further fractionation of the crude extract of leaves from *Z. spina-christi* could result in a higher level of potency at lower concentrations of fractionates. Generally, this study verified the medicinal use of *Z. spina-christi* by different communities in many parts of Ethiopia (Mirutse Giday & Tilahun Teklehaymanot, 2013; Asmare Talie et al., 2018). The antibacterial activity of *Z. spina-christi* is supposed to be emanated the numerous bioactive compounds it possesses including flavonoids, triterpenoids, alkaloids, sapogenins, saponins, and β-sitosterol (Hussein, 2019; Taghipour et al., 2020; Abdulrahman et al., 2022).

Similarly, *B. aegyptiaca* was proven to have inhibitory effects against two pathogenic bacteria, namely *S. typhimurium* and *K. pneumoniae*, at an exact concentration of 500 mg/ml and below. However, it is expected that *B. aegyptiaca* may show inhibitory effects on the remaining bacterial strains at higher extract concentrations (e.g., ≥ 750 mg/ml). A study by Koko et al. (2017) also showed that the leave extracts of *B. aegyptiaca* showed inhibitory effects against *S. aureus, Bacillus subtilis, E. coli,* and *P. aeruginosa*. Nevertheless, this study verified the traditional medicinal values of *B. aegyptiaca* claimed by different communities in many parts of Ethiopia (Mirutse Giday & Tilahun Teklehaymanot, 2013; Tilahun Teklehaymanot, 2017; Ali Zeynu et al., 2021). Though the current study did not identify phytochemicals from *B. aegyptiaca*, the antibacterial property of *B. aegyptiaca* is probably associated with the presence of secondary metabolites such as alkaloids, flavonoids, saponins, coumarins and phenolic acids in the leaves (Murthy et al., 2021).

In this study, the leave extracts of *Z. mucronata* and *B. rotundifolia* did not show potency against the test bacterial strains up to 500 mg/ml concentration. However, this does not necessarily mean that plant species have no antimicrobial properties. It is expected that the antimicrobial properties of *Z. mucronata* and *B. rotundifolia* could be detected above the 500 mg/ml concentration of the leave extracts (e.g., ≥750 mg/ml). In this regard, however, Nemudzivhadi and Masoko (2015) reported that the leave extract of *Z. mucronata* showed inhibitory effects against *S. aureus, E. faecalis, E. coli*, and *P. aeruginosa* using the agar-well diffusion method. The difference in the findings between the current and the aforementioned reports might be due to the effects of environment on plants' ability to synthesize bioactive compounds. The biosynthesis and accumulations of bioactive properties are greatly reliant on environmental aspects such as temperature, light, soil properties and moisture availability (Jan et al., 2021). Moreover, the degree of potency of plant extracts against pathogenic bacteria is greatly reliant on the kind of solvent used for extraction (Masoko et al., 2008). For instance, according to Nemudzivhadi and Masoko (2015), the leave extracts of *Z. mucronata* using hexane and chloroform showed greater inhibitory effects against *S. aureus, E. faecalis, E. coli*, and *P. aeruginosa* compared to the leave extracts of *Z. mucronata* using ethanol and methanol solvents. In Ethiopia, the traditional medicinal use of *B. rotundifolia* was reported by Mirutse Giday & Tilahun Teklehaymanot (2013). However, it becomes the least studied

Balanites species for its antimicrobial properties in-vitro where no reports were found or examined for its inhibitory effects against microbial pathogens. In the current study, however, the leave extracts of *B. rotundifolia* did not show inhibitory effects on the tested bacteria at 125, 250 and 500 mg/ml. However, it is expected that it could show inhibitory effects above 500 mg/ml of leave extracts.

The dermal and oral administrations of the products of *Z. spina-christi* and *B. aegyptiaca* by the local communities in Ethiopia indicate the potential antimicrobial effects of the species against many pathogens that cause both topical and internal infections. Therefore, this study revealed that *Z. spina-christi* and *B. aegyptiaca* can heal boils (abscesses), bloodstream infections, dysentery (or *bloody diarrhea)*, pneumonia, urinary tract infections, abdominal and pelvic infections, wounds, and enteric infections which are caused by *S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, *S. typhimurium*, and *K. pneumoniae*. The effectiveness of methanol extracts of both *Z. spina-christi* and *B. aegyptiaca* against most bacteria could be related to the strong polarity of methanol compared to ethanol, which enables it to extract many polar compounds that have antibacterial activities.

This study did not include a phytochemical characterization or molecular elucidation of bioactive compounds from *Ziziphus* and *Balanites*. This could be considered one of the limitations of this study. Therefore, it is recommended to further investigate the molecular constituents of leaf extracts of *Z. spina-christi* and *B. aegyptiaca* using techniques such as Nuclear Magnetic Resonance (NMR) and mass spectroscopy. Other available techniques should also be considered

4.5 Conclusions

The study verified the potential inhibitory properties of leaves of *Z. spina-christi* and *B. aegyptiaca* extracted by methano and ethanol against both gram-positive and gram-negative bacteria collected form EPHI. Particularly, it was observed that the methanol extracts of leaves through macerationn technique for both *Z. spina-christi* and *B. aegyptiaca* are generally more effective in inhibiting the growth of some bacterial strains. Therefore, it is also important to further investigate and elucidate the phytochemical composition of the different provenances of both *Z. spina-christi* and *B. aegyptiaca* in Ethiopia. Overall, the findings of the current study provide the basics for prospecting for antibiotic agents from *Z. spina-christi* and *B. aegyptiaca* against

drug-resistant bacterial strains. The findings of this study suggest that plants products for pharmaceutical purposes should undergo bioprocessing. Furthermore, based on the results of this study, we recommend conducting further investigations into the medicinal properties of *Balanites* and *Ziziphus* using various plant parts such as roots, fruits, and barks. These investigations should be based on traditional claims and the analysis of the phytochemical compositions of extracts.

Chapter 5

5. Modelling and Mapping Habitat Suitability for *Ziziphus spina-christi* (L.) Willd., *Ziziphus mucronata* Willd., *B. aegyptiaca* (L.) Del. and *B. rotundifolia* (Tiegh.) Blatt. under Climate Change in Ethiopia

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Abstract

In Ethiopia, *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia* are known multipurpose shrub or small trees. However, their species distributions are not well documented and understood in Ethiopia. Therefore, this study aimed to model the habitat suitability of these species under climate change in Ethiopia. The occurrence points of *Z. spina-christi* (n = 159) and *Z. mucronata* (n = 101), *B. aegyptiaca* (n = 224) and *B. rotundifolia* (n = 80) were collected from field surveys and herbarium. Bioclimatic, soil, and landscape variables were also used to model habitat suitability using GLM, GAM, BRT, RF, MARS, and SVM. The ensemble SDM under the current climate predicted that the Ethiopian Rift Valley and the Eastern Amhara are suitable habitats for *Z. spina-christi*, where its distribution is largely affected by pH, Bio17, Bio13, and elevation. Similarly, the ensemble SDM showed that the Central Ethiopian Rift Valley, a few pocket areas in Northern Wollo and Southern Tigray, and areas in eastern Ethiopia are suitable habitats for *Z. mucronata*, where its distribution is largely affected by clay, sand, silt, and BD. The ensemble SDM under the current climate predicted that about 8.6% of the areas of Ethiopia are suitable for *Z. spina-christi*, while 8.2% (ΔA = 4%↓) and 9.4% (ΔA = 9.7%↑) will be suitable under SSP2-45 and SSP5-85, respectively, in the years 2061–2080. Similarly, the ensemble SDM under the current climate predicts that about 6.4% of the areas of Ethiopia are suitable for *Z. mucronata*, while 5.5% (ΔA = 13.8%↓) and 5.7% (ΔA = 11%↓) will be suitable under SSP2-45 and SSP5-85, respectively. The ensemble SDMs under the current climate showed that the Central Ethiopian Rift Valley (8.6% area of Ethiopia for *B. aegyptiaca*) and Southern Ethiopian Rift Valley (3.1% area of Ethiopia for *B. rotundifolia*) are highly suitable habitats. Bio07 and Bio15 for *B. aegyptiaca;* and Bio17 and Bio12 for *B. rotundifolia* are the most key bioclimatic variables that affect their distributions. The ensemble SDM under SSP2-45 and SSP5-85 (HadGEM3-GC31-LL) climates showed that the highly suitable areas will remain suitable for both species (*B. aegyptiaca* with 8.8% (ΔA = 2.1%↑) and 9.5% (ΔA = 7.5%↑) and *B. rotundifolia* with 2.2% (ΔA = 28.6%↓) and 3.8% (ΔA = 23%↑), respectively). The findings of this study provide insight to decision-makers for the sustainable conservation of these valuable species in Ethiopia.

Keywords: *Balanites* Del.; Climate Change; Main Ethiopian Rift Valley; Precipitation; Species Distribution Models; Temperature; *Ziziphus* Mill.

5.1 Introduction

The key drivers of biodiversity loss are habitat loss and fragmentation, over-harvesting, invasive alien species and climate change (Hill & Curran, 2001; Santos et al., 2007; Alemayehu Wassie et al., 2010). Such prolonged habitat reduction has remarkably changed the structure and species composition of different ecosystems (Echeverria et al., 2006). Similarly, the current vicious impact of climate change and the rising human population growth with increasing demand for natural resources have put severe pressure on these resources. Presently, climate change instigates the expansion of global drylands (Cervigni & Morris, 2016; Yao et al., 2020; Peng et al., 2021). In this regard, climate predictions indicate that the warming temperature and drought frequency will increase in drylands, where *Ziziphus* species are widely distributed, at a faster rate than the global average (Osborne et al., 2022). As a result, the effect of climate change on the drylands is posing greater risks to biodiversity, food security, and livelihood in Africa (Hirwa et al., 2022; Ahmed et al., 2022). Climate change has many impacts on biodiversity, including shifting the timing of life-cycle events and habitat range, changing abundance and population sizes, morphology and reproduction physiology, etc. (Post & Forchhammer, 2008).

Several studies indicated that most extinction involved a synergy of these factors, with individual causes being difficult or impossible to isolate (Brook et al., 2008). Habitat loss and fragmentation are currently the major drivers of global biodiversity loss (Pereira et al., 2012). Climate change is projected to become equally or more important drivers of global biodiversity loss in the coming decades (Pereira et al., 2012; Dawson et al., 2011). Previous studies have indicated that prolonged habitat reduction resulting from climate change has significantly altered the structure and species composition of various ecosystems (Echeverria et al., 2006). In this regard, endemic species with limited habitat ranges are prone and vulnerable to the changing climate (Malcolm et al., 2006). For that reason, it is predicted that over 5,000 plant species in Africa will lose their ideal habitat ranges by 2085 (McClean et al., 2005).

The majority of the Ethiopian landmass is arid and semi-arid with erratic and low rainfall patterns (Wondimagegn Amanuel et al., 2019) and enormous biodiversity resources supporting the large numbers of communities (Kidane Georgis, 2014). It

occurs in three vegetation types, i.e., *Vachellia -Commiphora* woodland and bushland proper, *Vachellia* wooded grassland of the Ethiopian Rift Valley, and *Combretum-Terminalia* woodland and wooded grassland. In recent years, a dramatic climate change has been witnessed in Ethiopia, manifested by increasing temperatures and decreasing rainfall (Haileab Zegeye, 2018; Zenebe Mekonnen, 2022). Since the 1960s, the mean annual temperature of Ethiopia has risen by about 1.3°C with increasing spatial and temporal rainfall variability (Haileab Zegeye, 2018).

Ziziphus (Rhamnaceae) includes trees and shrubs with multi-purpose roles that provide food, income, and traditional medicine for rural communities in dryland ecosystems (Panghal et al., 2010). The genus consists of 170 species, mainly distributed in tropical and subtropical regions (Liu & Cheng, 1995). Six species of Ziziphus, namely *Ziziphus spina-christi, Z. pubescens*, *Z. mucronata*, *Z. hamur, Z. abyssinica*, and *Z. mauritiana*, occur in Ethiopia (**Figure 33**), where *Z. spina-christi* and *Z. mucronata* are the most widely distributed species (Hedberg & Edwards, 1989).

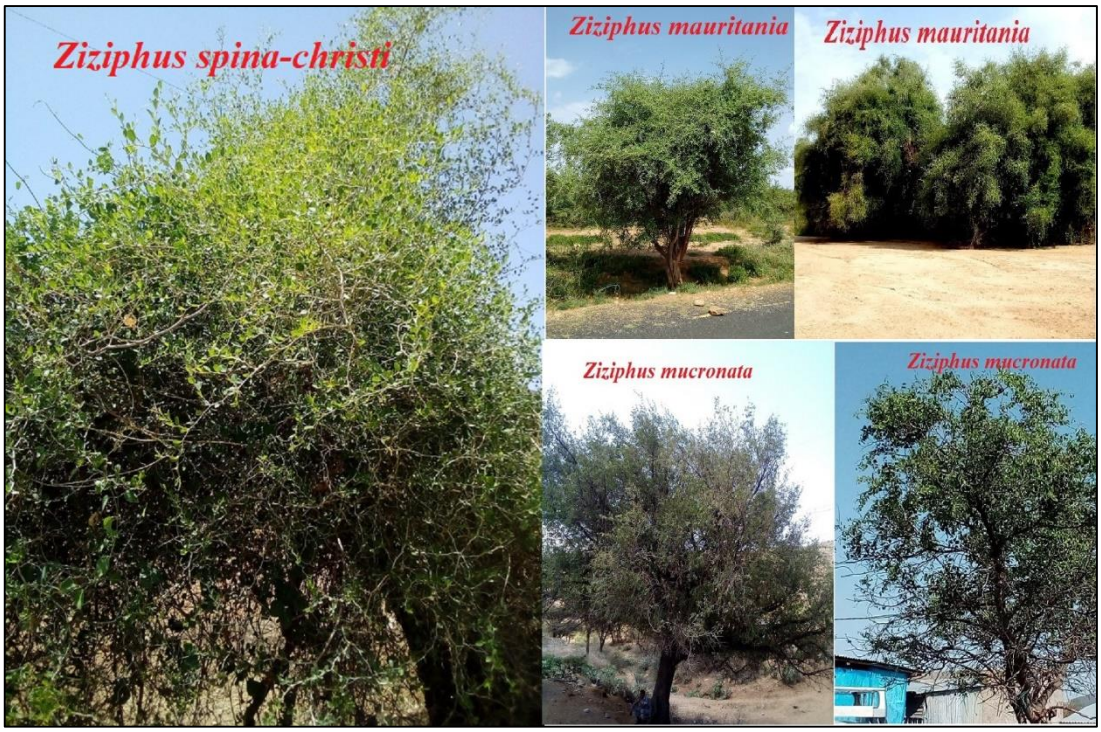

Figure 33. Field samples of some species of *Ziziphus* (Source: Mohammed Adefa).

The last two listed species (*Z. spina-christi* and *Z. mucronata*) are multipurpose species and underutilized plants in Africa (Saied et al., 2008; Orwa et al., 2009;

Mokgolodi et al., 2011). They are also highly valuable plants used by the rural and pastoral communities in Ethiopia for food, medicine, fodder, cosmetics, etc. *(*Mokgolodi et al., 2011; Abraha Teklay et al., 2013; Ali Zeynu et al., 2021). *Z. spina-christi* is a shrub or small tree growing up to 20 meters high, distributed between 550 and 2,000 meters above sea level, with a mean annual rainfall of 100 to 500 mm. It is commonly distributed in Africa, extending from Mauritania through the Sahara and Sahelian zones of West Africa to the Red Sea (Orwa et al., 2009). In Ethiopia, it occurs from 500 to 1,900 (-2,400) meters above sea level in semi-arid and arid ecosystems (Hedberg & Edwards, 1989; Azene Bekele-Tesemma, 2007). It is commonly used as a medicinal plant in the Ada'ar District of the Afar region (Mirutse Giday & Tilahun Teklehaymanot, 2013) and heals dandruff *(*Ali Zeynu et al., 2021).

Ziziphus mucronata is a shrub or medium-sized tree up to 9 meters high that occurs up to 2,000 meters above sea level with a mean annual rainfall of 446 to 1,200 mm (Orwa et al., 2009). In Ethiopia, *Z. mucronata* grows from 100 to 1,950 (-2,100) meters above sea level in *Vachellia-Terminalia, Vachellia-Balanites, Anogeissus-Boswellia* woodland and bushlands, and *Vachellia* woodland (Hedberg & Edwards, 1989; Azene Bekele-Tesemma, 2007). It is also reported to treat chronic cough, boils, toothache, rheumatism, and swellings (Mokgolodi et al., 2011).

Balanites Del. is a genus in the family Balanitaceae that includes multipurpose species distributed in arid and semiarid areas in Ethiopia. The varied species in this genus were reported for their uses as sources of medicinal, wild edible fruit and fuel woods (Panghal et al., 2010). *Balanites aegyptiaca* is a multi-branched, spiny shrub or tree up to l0 m high, and distributed in a wide ecological range (**Figure 34**). It occurs in altitudinal range from below 500 up to 2,000 meter above sea level (Orwa et al., 2009). *Balanites rotundifolia* is a multi-stemmed evergreen thorny bushy shrub or tree 2-5, and drought resistant and grows on different soil types.

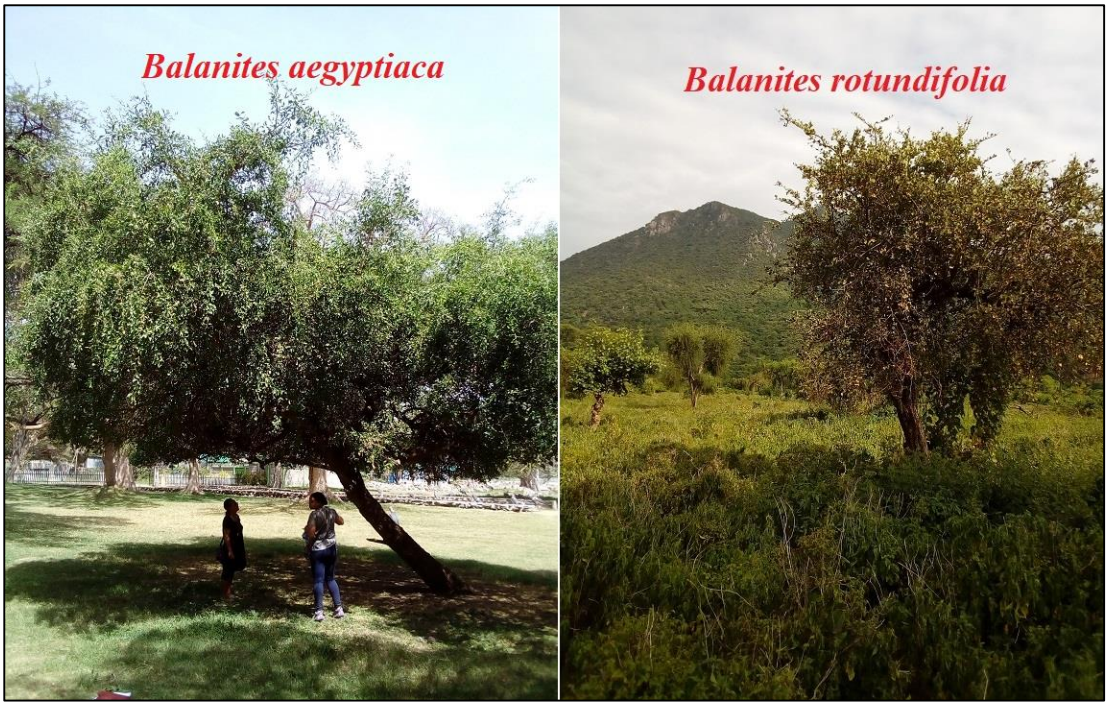

Figure 34. Field samples of *B. aegyptiaca* and *B. rotundifolia* (Source: Mohammed Adefa).

However, despite having a wider distribution and much ecological and economic importance, the habitat suitability of *Z spina-christi, Z. mucronata, B. aegyptiaca* and *B. rotundifolia* under climate change in Ethiopia is not well known. Hence, understanding these potential distributions fosters their future conservation and sustainable uses. Species distribution models are used as tool to better understand the occurrences and vulnerabilities of the species and environmental factors that linked to the species distributions (Guisan & Thuiller, 2005; Guillera-Arroita et al., 2015; Soultan & Safi, 2017); and are crucial to effectively monitor the distribution, ecology and evolution of species across vast ecological zones (Phillips et al., 2006). Therefore, the objectives of this study were to model the habitat suitability of *Z spina-christi, Z. mucronata, B. aegyptiaca* and *B. rotundifolia* under the current climate; predict future distributions of *Z spina-christi, Z. mucronata, B. aegyptiaca* and *B. rotundifolia* using SSP2-45 and SSP5-85 scenarios for the years 2061–2080; and identify the main environmental variables influencing species distributions under the changing climate in Ethiopia. Therefore, it was hypothesized that the *Z spina-christi, Z. mucronata, B. aegyptiaca* and *B. rotundifolia* would change their species distributions (i.e., either increase or decrease their areas of suitable habitats) under the changing climate.

5.2 Material and Methods

Study area

This study area is based on the semi-arid (-arid) ecosystems of Ethiopia, i.e., the Ethiopian Rift Valley, the drylands of Northeastern, Northwestern, Northern, Southern, and Eastern Ethiopia (**Figure 35**). In other words, the study focuses on the arid and semi-arid ecosystems (up to 2,300 meters above sea level) that include the *Vachellia-Commiphora* woodland and bushland proper, *Vachellia* wooded grassland of the Ethiopian Rift Valley, and *Combretum-Terminalia* woodland wooded grassland ecosystems of Ethiopia (Friis et al., 2010). However, this study ultimately aimed to produce model outputs for the whole country (i.e., Ethiopia) located between 3.3º N to 14.9º N (*Lat.*) and 32.99º E to 47.99º E (*Long.*).

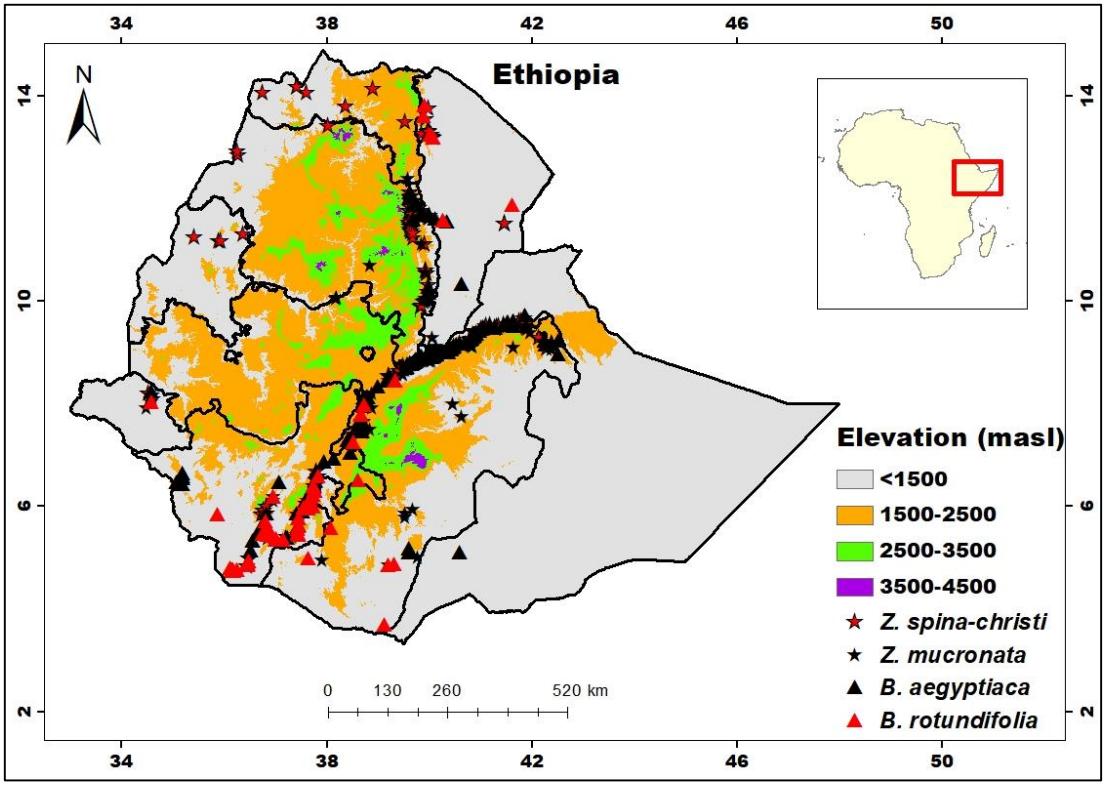

Figure 35. Map of the study area and the 564 (i.e., 159 + 101 + 224 +80) occurrence points (sampling areas).

Species occurrence data

Occurrence points of *Z. spina-christi* (*n* = 159), *Z. mucronata* (*n* = 101), *B. aegyptiaca* (*n* = 224) and *B. rotundifolia* (*n* = 80) were collected mainly from field data and specimen records of the National Herbarium of Ethiopia (ETH), Addis

Ababa University (**Figure 35**). Occurrence points from fields were gathered randomly at a distance of 5–10 km, 50–100 m off the roadside, across the Ethiopian Rift Valley region and other dryland areas. The work of gathering occurrence points from the field was done in two phases: (i) between January 2019 and October 2020 (collection during this period was made by Dr. Tigist Wondimu for the purpose of phylogeography study by herself), and (ii) between January and June 2022. Moreover, background data for *Z. spina-christi* (*n* = 159), *Z. mucronata* (*n* = 101), *B. aegyptiaca* (*n* = 224) and *B. rotundifolia* (*n* = 80) were generated at random points using occurrence data as domains in R4.1.3.

Environmental predictor variables

The 30s resolution raster data of bioclimatic variables (i.e., Bio01–Bio19), elevation, Srad, and Tpi (all averaged for 1970–2000), and CMIP6's future climate was downloaded from www.worldclim.com/version2, the Worldclim database (Fick & Hijmans, 2017), and downscaled to the study area (i.e., Ethiopia) using ArcGis 10.8. Moreover, the data for soil variables at 30–60 cm rootable soil depth (e.g., clay, silt, and sand in g/100g (%), and soil pH, Cation exchange capacity (CEC) in cmol(c)/kg, soil organic carbon content (SOC) in g/kg, and bulk density (BD) in kg/dm^3) from https://files.isric.org/soilgrids/latest/data_aggregated/1000m/ **(**Poggio et al., 2021), as well as landscape variables (e.g., aspects and slopes) from https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/harmonized-world-soil-database-v12/en/ (Fischer et al., 2008) were downloaded and downscaled using ArcGis 10.8. All predictor variables were adjusted to have a similar spatial scale (i.e., 30s resolution) and projection (i.e., GCS/WGS/1984).

The Intergovernmental Panel on Climate Change (IPCC) equally treats all global climate models for their accuracy (IPCC, 2001, 2007). However, for this study, three future global climate models (GCMs) of CMIP6 [WCRP (World Climate Research Programme) Coupled Model Intercomparison Project (CMIP)] (Eyring et al., 2016) namely the second generation Euro-Mediterranean Centre on Climate Change Earth System Model (CMCC-ESM2) (Peano & Lovato, 2019; Lovato et al., 2022), the Goddard Institute for Space Studies Model version E2.1-H (GISS-E2-1-H) (NASA-GISS, 2018; Kelley et al., 2020), and the third Hadley Centre Global Environmental Model run in the Global Coupled configuration 3.1 (HadGEM3-GC31-LL) (Webb,

2020) were downloaded owing to their better performance in Ethiopian environments (Jury, 2015; Solomon Addisu, 2016; Koch et al., 2022) and preliminarily tested to identify the best performing GCM amongst them. As a result, HadGEM3-GC31-LL relatively performs better as to the metrics of AUC, correlation, TSS, and deviation. Hence, HadGEM3-GC31-LL was finally selected for SDMs of *Z. spina-christi* and *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia* in the years 2061–2080 under SSP2-45 and SPP5-85. The SSPs are the new set of CO_2 emissions scenarios driven by different socioeconomic assumptions. The SSP2-45 and SSP5-85 projections represent global temperature anomalies of 2.4ºC (or around 3ºC) and 4.9ºC above pre-industrial levels by 2100 with atmospheric CO_2 equivalents of 650 and 1370 ppm, respectively (Rogelj et al., 2012; Platts et al., 2015).

Collinearity analysis for environmental variables

Collinearity among predictors decreases the efficiency and increases the uncertainty of species distribution models (De Marco & Nóbrega, 2018). Hence, it is very essential to partially exclude collinear and less important variables to increase efficiency and decrease the uncertainty of models' projections. Hence, following Manly (1994) and Cruz-Cárdenas et al. (2014), principal component analysis was computed for 42 environmental variables based on their correlation matrix. Then, using the first four principal components (PCA1–4) that explained the largest proportions (>80%) of the total variance and less collinearity ($r \leq 0.6$) most important variables influencing species distribution were finally selected and used in SDMs (De Marco & Nóbrega, 2018). Accordingly, a total of 16, 15, 14, 13 environmental predictor variables were selected to compute the SDMs of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia*, respectively (**Table 20**, **Table 21**). In this regard, Wisz et al. (2008) and Anand et al. (2021) proposed that small occurrence points (~30) are generally sufficient in exploratory species distribution modeling for small- to medium-scale areas. Moreover, according to van Smeden et al. (2016), no evidence has validated the assumption of ten events per variable (EPV). Hence, the occurrence points used for *Z. spina-christi* (n = 159), *Z. mucronata* (n = 101), *B. aegyptiaca* (n = 224) and *B. rotundifolia* (n = 80) in this study were sufficient for SDMs, provided that background points for *Z. spina-christi* (n = 159), *Z. mucronata* (n = 101), *B. aegyptiaca* (n = 224) and *B. rotundifolia* (n = 80) were also included and used.

Table 20. Lists of environmental variables used to compute SDMs of *Z. spina-christi* and *Z. mucronata*.

Z. spina-christi		*Z. mucronata*	
Environmental variables	**Descriptions**	**Environmental variables**	**Descriptions**
Bio02	Mean Diurnal Range [Mean of monthly (max temp - min temp)]	Bio02	Mean Diurnal Range [Mean of monthly (max temp - min temp)]
Bio06	Min Temperature of Coldest Month	Bio03	Isothermality (Bio2/Bio7)
Bio13	Precipitation of Wettest Month	Bio16	Precipitation of Wettest Quarter
Bio17	Precipitation of Driest Quarter	Bio17	Precipitation of Driest Quarter
Bio18	Precipitation of Warmest Quarter	Bio18	Precipitation of Warmest Quarter
Bio19	Precipitation of Coldest Quarter	Elev	Elevation
Elev	Elevation	AspectClW	Aspect: West: 225˚ < aspect ≤ 315˚
Srad	Solar radiation	CEC	Cation Exchange Capacity of the soil
Tpi	Topographic index	Clay	
AspectClE	Aspect: East: 45˚ < aspect ≤ 135˚	Sand	Proportion of clay particles (< 0.002 mm)
AspectClU	Aspect: Undefined: Slope aspect undefined	BD	Bulk density of the fine earth fraction
CEC	Cation Exchange Capacity of the soil	Silt	Proportion of silt particles (≥ 0.002 mm and ≤ 0.05 mm)
pH	pH	SlopesCl1	0 % ≤ slope ≤ 0.5 %
SOC	Soil organic carbon content in the fine earth fraction	SlopesCl3	2 % ≤ slope ≤ 5 %
Silt	Proportion of silt particles (≥ 0.002 mm and ≤ 0.05 mm)	SlopesCl4	5 % ≤ slope ≤ 10 %
SlopesCl5	10 % ≤ slope ≤ 15 %		

Table 21. Lists of environmental variables used to compute SDMs for *B. aegyptiaca* and *B. rotundifolia*.

B. aegyptiaca		*B. rotundifolia*	
Variables	**Descriptions**	**Variables**	**Descriptions**
Bio07	Temperature Annual Range (Bio5-Bio6)	Bio02	Mean Diurnal Range (Mean of monthly (max temp - min temp))
Bio08	Mean Temperature of	Bio12	Annual Precipitation

	Wettest Quarter		
Bio14	Precipitation of Driest Month	Bio17	Precipitation of Driest Quarter
Bio15	Precipitation Seasonality (Coefficient of Variation)	AspectClS	Aspect: South: 135˚ < aspect ≤ 225˚
Bio16	Precipitation of Wettest Quarter	AspectClW	Aspect: West: 225˚ < aspect ≤ 315˚
Bio18	Precipitation of Warmest Quarter	CEC	Cation Exchange Capacity of the soil
Tpi	Topographic index	pH	pH
AspectClE	Aspect: East: 45˚ < aspect ≤ 135˚	Silt	Proportion of silt particles (≥ 0.002 mm and ≤ 0.05 mm)
AspectClS	Aspect: South: 135˚ < aspect ≤ 225˚	SlopesCl2	0.5 % ≤ slope ≤ 2 %)
AspectClU	Aspect: Undefined: Slope aspect undefined	SlopesCl3	2 % ≤ slope ≤ 5 %
AspectClW	Aspect: West: 225˚ < aspect ≤ 315˚	SlopesCl4	5 % ≤ slope ≤ 10 %
Clay	Proportion of clay particles (< 0.002 mm) in the fine earth fraction)	SlopesCl6	15 % ≤ slope ≤ 30 %
Silt	Proportion of silt particles (≥ 0.002 mm and ≤ 0.05 mm)	SlopesCl8	Slope > 45 %
SlopesCl4	5 % ≤ slope ≤ 10 %		

Extracting values from rasters, model fitting and prediction

The presence-background data records for the selected environmental predictor variables corresponding to the present occurrence and background points for *Z. spina-christi* (*n* = 159), *Z. mucronata* (*n* = 101), *B. aegyptiaca* (*n* = 224) and *B. rotundifolia* (*n* = 80) were extracted and generated using the SDM package (Naimi & Araújo, 2016) with the support of other required packages such as Dismo (Hijmans et al., 2015), Raster (Hijmans & van Etten, 2012; Hijmans, 2015). Hence, data models with 1157, 1086, 1210 and 1077 environmental records were generated to predict the habitat suitability of *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia* respectively, under the current and future climates. The data model was partitioned into the training dataset (75%) and testing dataset (25%) for model fitting and validation, respectively. Hence, the training data was used to fit (or train) the models, and the testing data was used to evaluate (or test) the models (Phillips et al., 2006). The models were fitted to the data model using SDM and SP packages in R 4.1.3 (Pebesma & Bivand, 2005; Bivand et al., 2013; Naimi & Araújo, 2016) using the

cross-validation partitioning method with 10 replication runs. The models were optimized with the same feature classes, regularization multiplier (*reg = 1*), and iterations (*iter = 500*). So, two regression-based models, namely the generalized linear model (GLM) and generalized additive model (GAM), and four machine learning models, namely the boosted regression tree (BRT), random forest (RF), multivariate adaptive regression spline (MARS) and support vector machine (SVM) were computed for both the current and future climates. Then, predictions using models for habitat suitability were done using the predictor variables and the models' objects as domains (a combination of independent variables). Accordingly, the ensemble SDMs were computed from the weighted means of individual models under both the current and future climates based on their performances. Future projections of species distribution were based on the premise that only climate variables would change while soil and topographical variables would remain unchanged in the future. The SDMs were then converted to raster tiff files using the Raster packages in R 4.1.3 (Hijmans, 2015) for further analysis using ArcGis 10.8. The relative variable importance of predictors in the ensemble models' performance was also computed in R 4.1.3 based on Pearson correlation and AUC metrics for test data (averaged for 6 models after 10 permutations of each model). Relative variable importance measures either the percentage (not probability) that the prediction error increases when the variable is removed or the change in the purity of each node when the variable is removed.

Model performance evaluation

Four performance evaluation metrics, namely Area under the Receiver Operator Curve (AUC), Correlation (COR), True Skill Statistic (TSS), and Deviance were used to evaluate the performance and validity of individual and ensemble SDMs. The AUC (ranges from 0 to 1) is mainly used to determine the performance of the models (Allouche et al., 2006; Phillips et al., 2006). It is simply the proportion of the true presence rate (sensitivity related to presence data) and the true absence rate (specificity related to absence data). AUC values ranging between 0.5 and 0.7 are considered weak models; values between 0.7 and 0.9 show good performance, and values greater than 0.9 indicate high model performance (Allouche et al., 2006; Phillips et al., 2006). Overall, models with high AUC scores are assumed to effectively differentiate between suitable and unsuitable habitats. Swets (1988) also

classified model performance using AUC as failing (0.5 to 0.6), bad (0.6 to 0.7), reasonable (0.7 to 0.8), good (0.8 to 0.9), or great (0.9 to 1). TSS gives more weight to model sensitivity than specificity and has values ranging between -1 and 1, where values below 0.4 and above 0.8 are considered "poor" and "excellent", respectively (Allouche et al., 2006; Ahmed et al., 2022). A correlation (COR) also has a value ranging from -1 and 1. Deviance is also one of a goodness-of-fit used, and so the lower the deviance the better the goodness-of-fit of the model. Therefore, as a rule of thumb, a comparison of the accuracy of individual models was done by taking into consideration the overall performance of each model under each evaluation metric. The performances of ensemble SDMs were also evaluated based on their weighted mean scores (and standard deviations) under each evaluation metric. Additionally, the spatial autocorrelations among individual SDMs were also computed in R4.1.3.

ArcGIS analysis of habitat suitability classes

Based on probability occurrence, the ensemble SDMs were reclassified into four suitability classes using ArcGIS 10.8 following Hamid et al. (2019): (i) unsuitable (0.0–0.25), (ii) low-suitable (0.25–0.50), (iii) moderately suitable (0.50–0.75), and (iv) highly suitable (0.75–1.00). Hence, the change in habitat area (ΔA) between current and CMIP6's climate conditions was predicted using two indicators (Duan et al., 2016) using the formula: ΔA= ((Af-Ac)/Ac) *100; Where ΔA is the percentage change of suitable habitat area; Af is the predicted area of suitable habitat for species of *Ziziphus* in the future, and Ac is the predicted area of suitable habitat under current conditions. Here, highly suitable, moderately suitable, low-suitable, and unsuitable areas were combined while calculating habitat area changes along the temporal scales of current and future climatic change.

5.3 Results

Model performance and spatial autocorrelation under the current climate

Analysis of the performance of SDMs indicated that the RF model better predicted the habitat suitability of *Z. spina-christi* with 0.98 AUC, 0.88 TSS, 0.83 COR and 0.29 Deviance; followed by SVM (AUC = 0.93, TSS = 0.77, COR = 0.66, Deviance = 0.46), and BRT (AUC = 092, TSS = 0.73, COR = 0.65, Deviance = 0.56) (**Table 22**). Similarly, under the current climate, the RF model showed higher performance in predicting the habitat suitability of *Z. mucronata* with 0.97 AUC, 0.87 TSS, 0.78

COR and 0.24 Deviance; followed by BRT (AUC = 0.95, TSS = 0.81, COR = 0.63, Deviance = 0.37), and SVM (AUC = 0.94, TSS = 0.8, COR = 0.6, Deviance = 0.38) (**Table 22**). Similarly, RF is the most robust model under both *B. aegyptiaca* (0.99 AUC, 0.91 TSS, 0.9 COR, 0.23 Deviance) and *B. rotundifolia* (0.97 AUC, 0.87 TSS, 0.84 COR, 0.17 Deviance), followed by SVM for *B. aegyptiaca* (0.97 AUC, 0.85 TSS, 0.82 COR, 0.33 Deviance) and *B. rotundifolia* (0.96 AUC, 0.85 TSS, COR = 0.77, 0.21 Deviance), and BRT for *B. rotundifolia* (0.96 AUC, 0.85 TSS, 0.79 COR, 0.25 Deviance) (**Table 22**). The receiver operator characteristics (ROC) curves of ensemble SDMs of each species under the current climate showed that the AUC for the training and testing data generally ranges from 0.85, indicating the good performance of ensemble models (**Figure 36**, **Figure 37, Figure 38, Figure 39**).

Table 22. Model performance of *Z. spina-christi* and *Z. mucronata* under the current climate ($\bar{x}$ = mean, SD = standard deviation).

Species	**presence points (#)**	**Model**	**AUC**	**TSS**	**COR**	**Deviance**
Z. spina-christi	159	GLM	0.87	0.61	0.49	0.59
		GAM	0.89	0.77	0.7	3.7
		BRT	0.92	0.73	0.65	0.56
		RF	0.98	0.88	0.83	0.29
		MARS	0.91	0.72	0.65	0.58
		SVM	0.93	0.77	0.66	0.46
		$\bar{x}$±SD	***0.92±0.04***	***0.75±0.09***	***0.66±0.09***	***1.03±1.2***
Z. mucronata	101	GLM	0.89	0.69	0.43	0.41
		GAM	0.84	0.7	0.69	2.81
		BRT	0.95	0.81	0.63	0.37
		RF	0.97	0.87	0.78	0.24
		MARS	0.91	0.74	0.61	0.38
		SVM	0.94	0.8	0.6	0.38
		$\bar{x}$±SD	***0.92±0.03***	***0.77±0.07***	***0.62±0.1***	***0.76±0.9***
B. aegyptiaca	GLM	0.93	0.70	0.68	0.53	
	GAM	0.92	0.83	0.8	3.14	
	BRT	0.95	0.8	0.77	0.55	
	RF	0.99	0.91	0.9	0.23	
	MARS	0.96	0.82	0.8	0.42	
	SVM	0.97	0.85	0.82	0.33	
	$\bar{x}$±SD	***0.95±0.02***	***0.8±0.06***	***0.79±0.06***	***0.87±1.02***	
B. rotundifolia	GLM	0.93	0.78	0.67	0.28	
	GAM	0.9	0.77	0.72	1.97	
	BRT	0.96	0.85	0.79	0.25	
	RF	0.97	0.87	0.84	0.17	
	MARS	0.93	0.81	0.73	0.39	
	SVM	0.96	0.85	0.77	0.21	
	$\bar{x}$±SD	***0.9±0.2***	***0.8±0.4***	***0.8±0.6***	***0.5±0.7***	

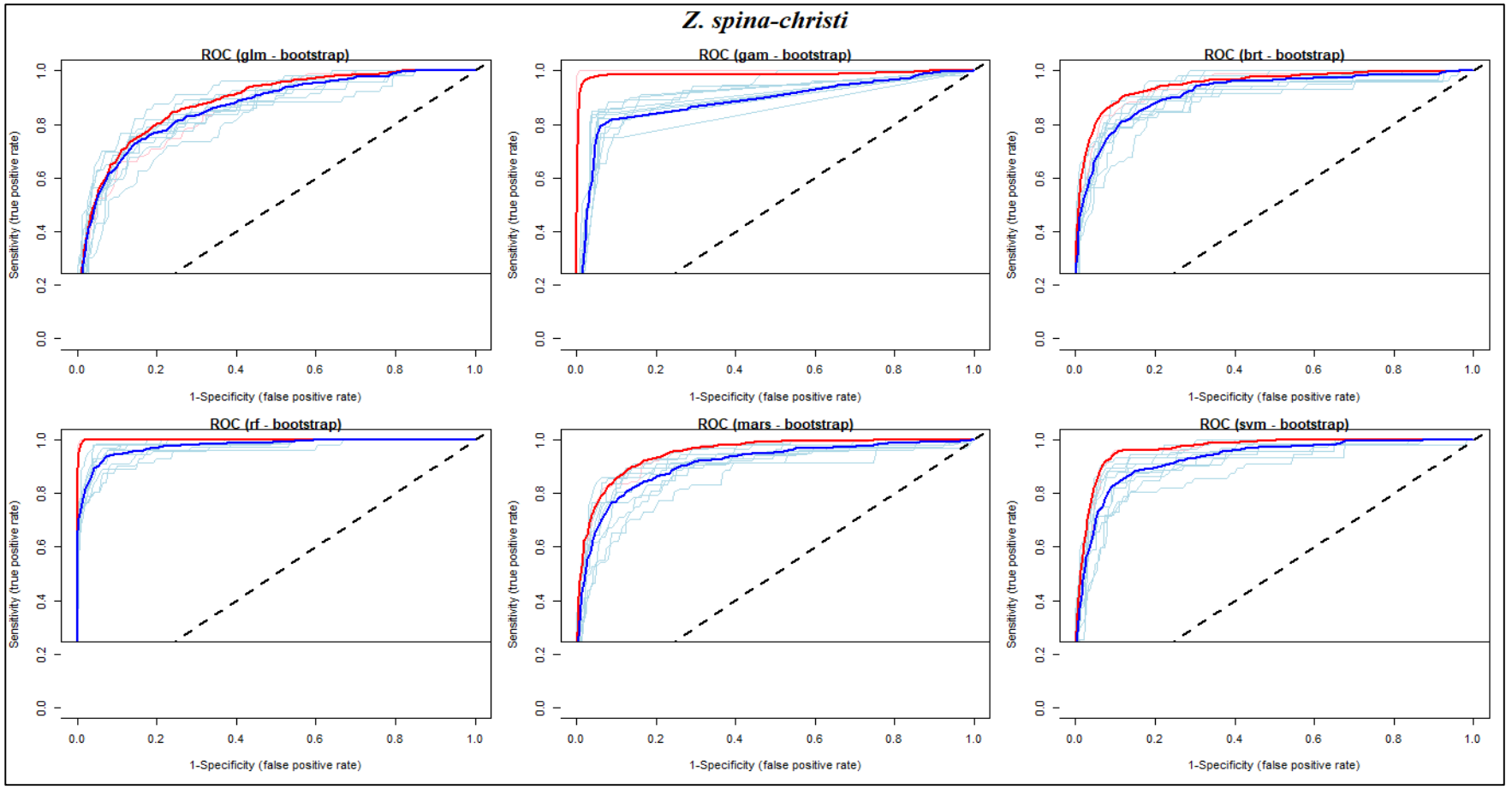

Figure 36. The receiver operator characteristics (ROC) curves for SDMs of *Z. spina-christi* under the current climate.

In the figure 36 above, Sensitivity (true positive rate for presence data) of the vertical line and 1-specificity (false positive rate for absence data) of the horizontal line describe the proportion of correctly and incorrectly classified samples. The red and blue curves represent the mean of AUC using training and testing data, respectively.

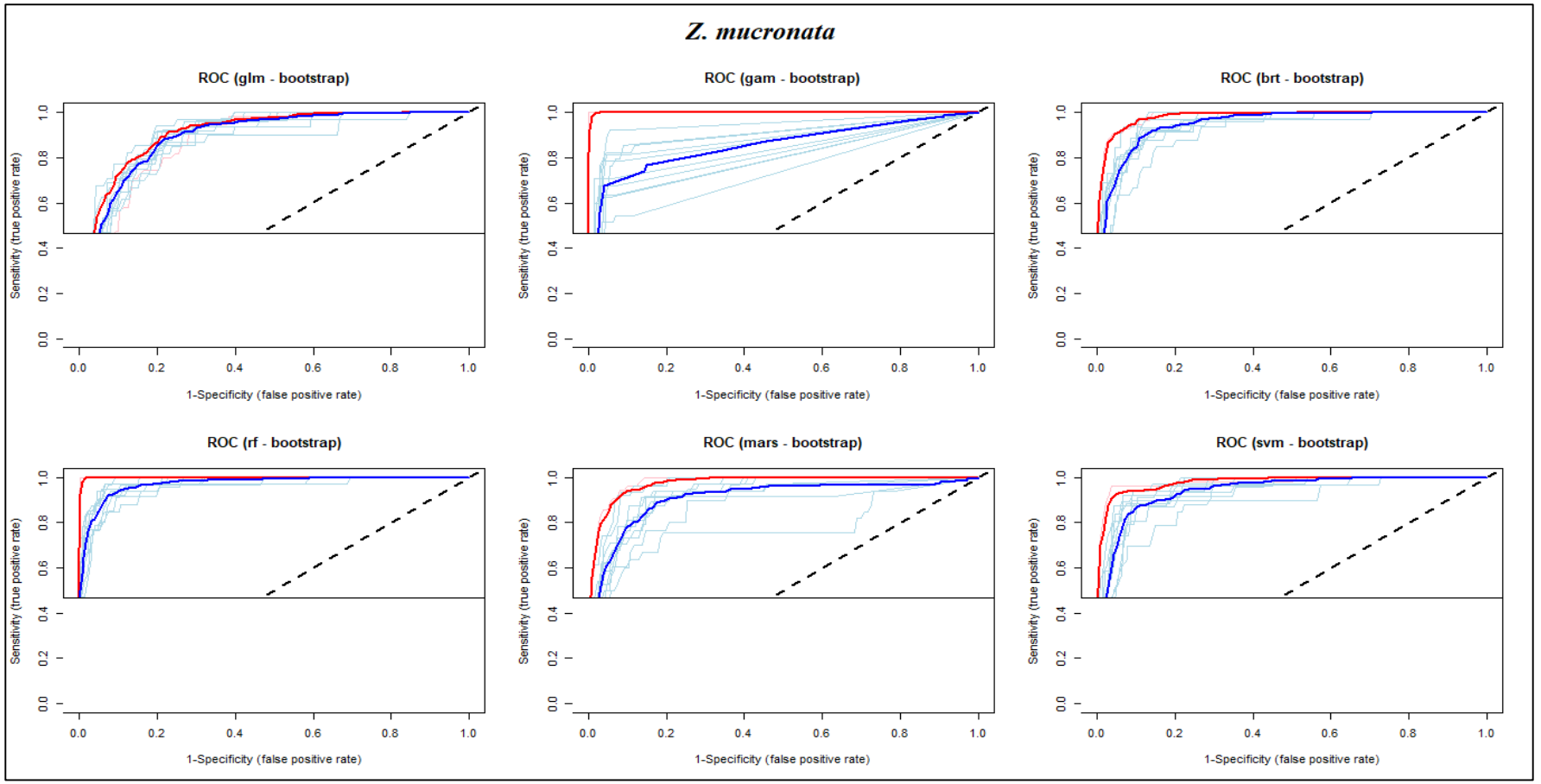

Figure 37. The receiver operator characteristics (ROC) curves for SDMs of *Z. mucronata* under the current climate.

In the figure 37 above, Sensitivity (true positive rate for presence data) of the vertical line and 1-specificity (false positive rate for absence data) of the horizontal line describe the proportion of correctly and incorrectly classified samples. The red and blue curves represent the mean of AUC using training and testing data, respectively.

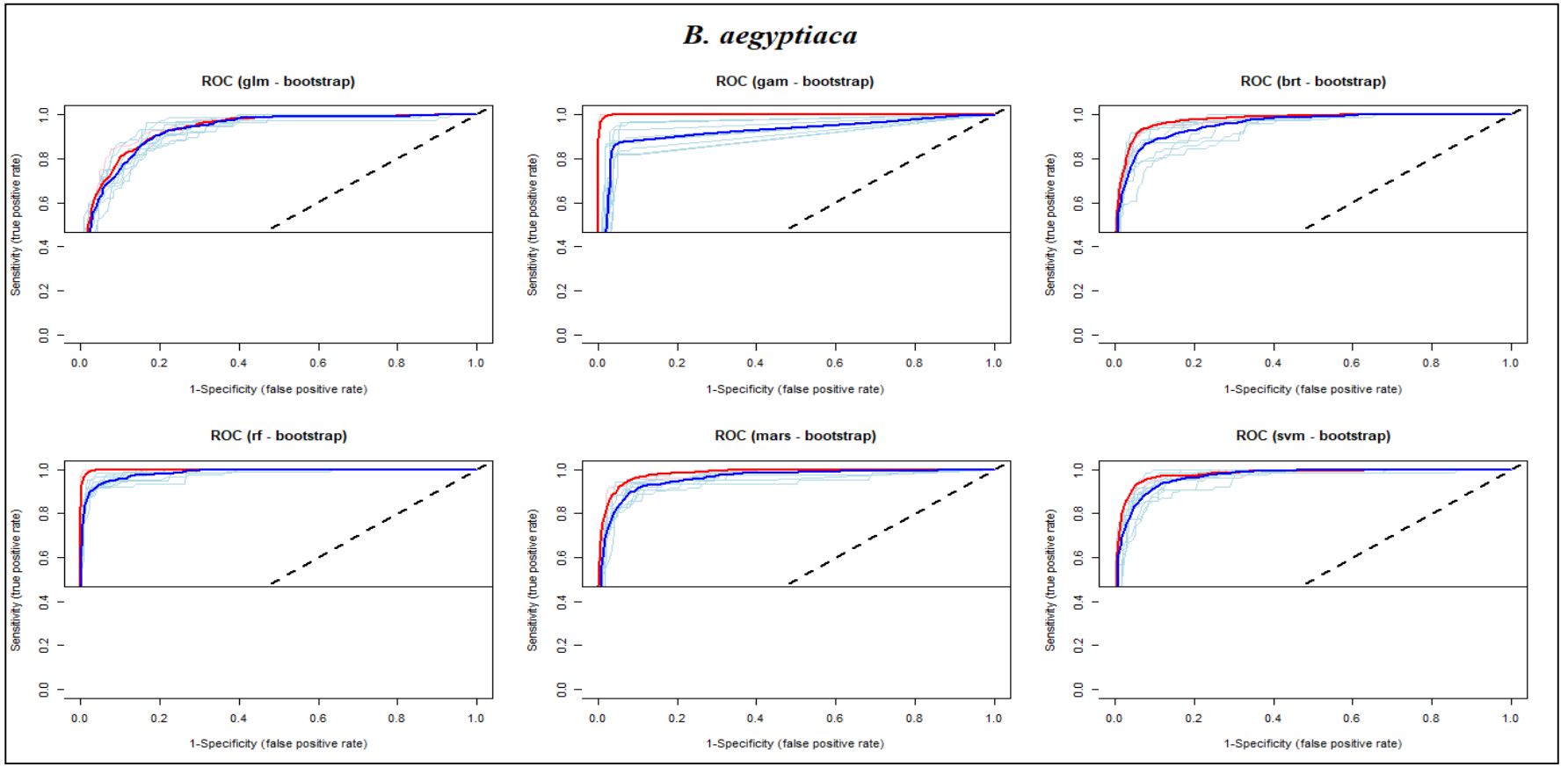

Figure 38. The receiver operator characteristics (ROC) curves of SDMs for B. aegyptiaca under the current climate.

In the figure 38 above, Sensitivity (true positive rate for presence data) of the vertical line and 1-specificity (false positive rate for absence data) of the horizontal line describe the proportion of correctly and incorrectly classified samples. The red and blue curves represent the mean of AUC using training and testing data, respectively.

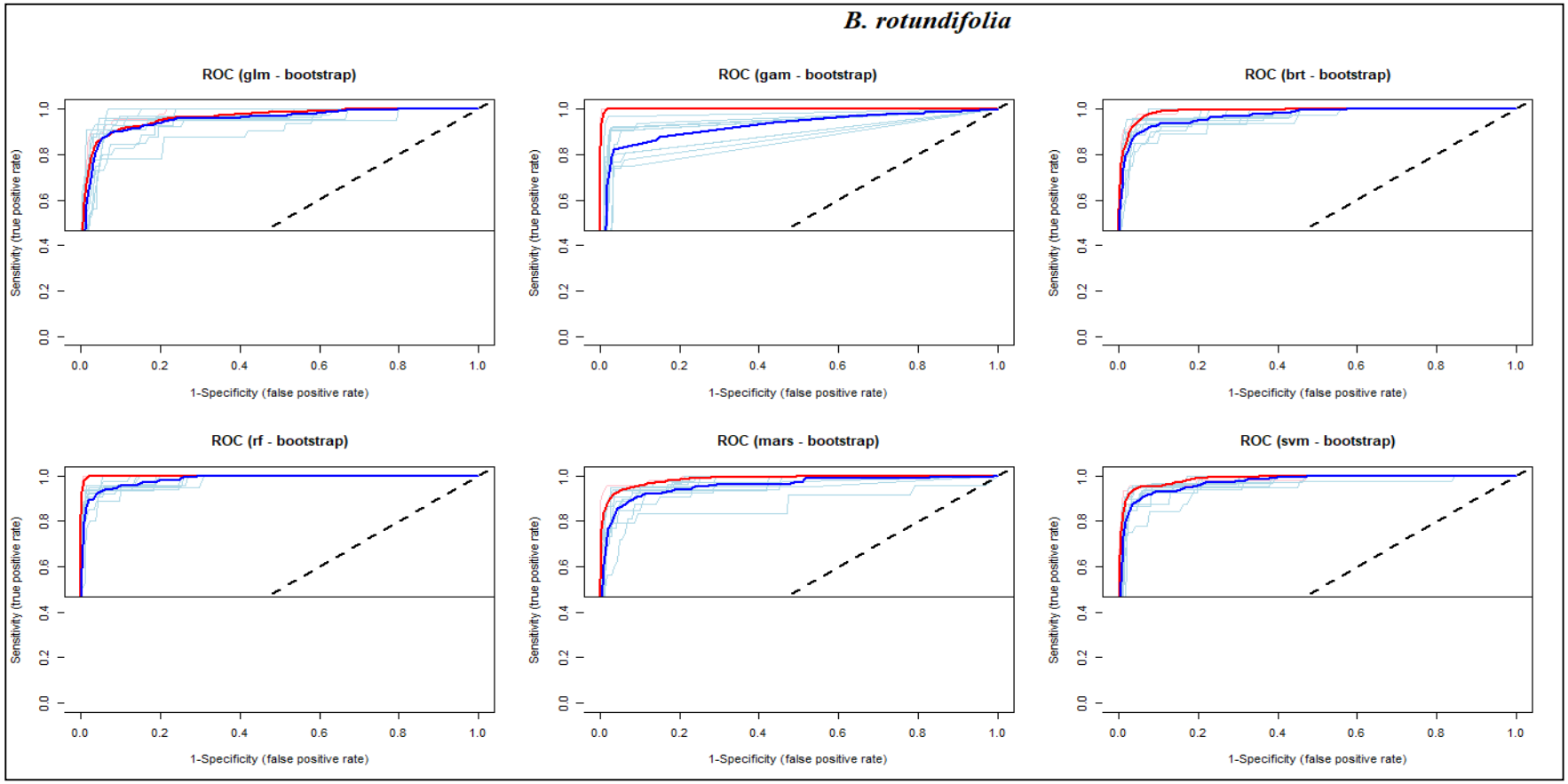

Figure 39. The receiver operator characteristics (ROC) curves of SDMs for *B. rotundifolia* under the current climate.

In the figure 39 above, Sensitivity (true positive rate for presence data) of the vertical line and 1-specificity (false positive rate for absence data) of the horizontal line describe the proportion of correctly and incorrectly classified samples. The red and blue curves represent the mean of AUC using training and testing data, respectively.

The spatial autocorrelations of models produced for *Z. spina-christi* under the current climate showed that the highest correlation was observed between BRT and RF (74%), followed by BRT vs. SVM (73%), RF vs. SVM (67%), GLM vs. BRT (67%), GLM vs. RF (61%), and GLM vs. SVM (61%), while the least correlation was observed between GAM and SVM (28%), GAM vs. MARS (28%), followed by GAM vs. BRT (29%) (**Figure 40**).

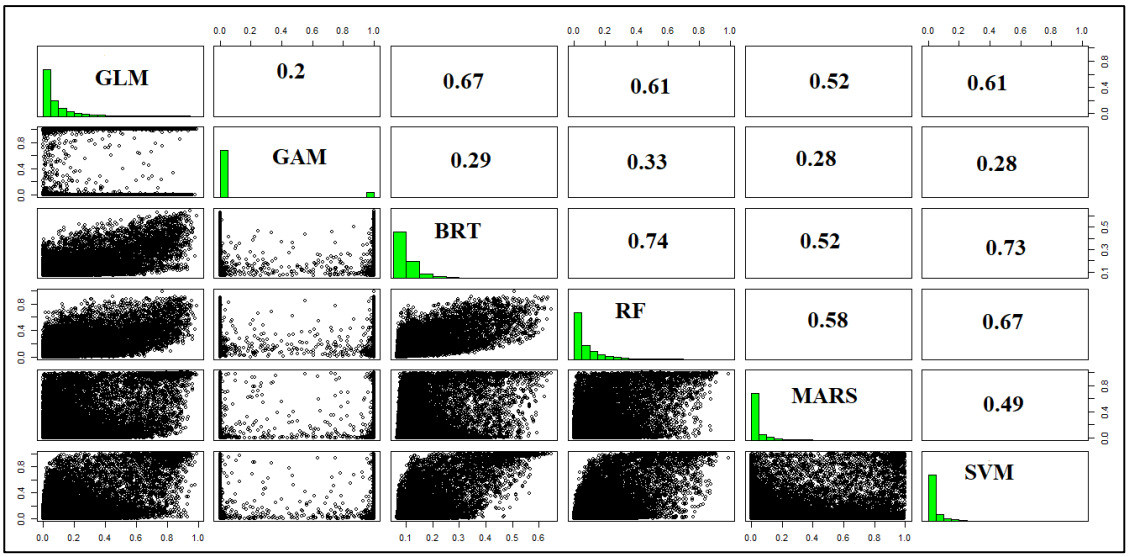

Figure 40. Spatial autocorrelation coefficients among different SDMs of *Z. spina-christi* under the current climate.

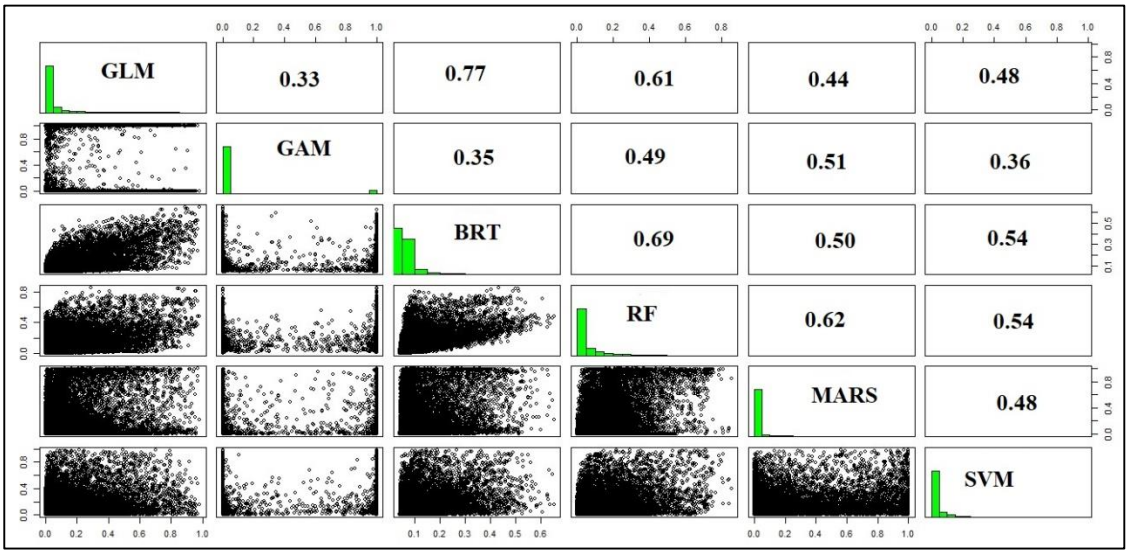

Figure 41. Spatial autocorrelation coefficients among different SDMs of *Z. mucronata* under the current climate.

Similarly, for *Z. mucronata* the highest spatial autocorrelation among SDMs under the current climate was observed between GLM and BRT (77%), followed by BRT vs. RF (69%), RF vs. MARS (62%), and GLM vs. RF (61%), while the least correlations were observed for GAM vs. BRT (35%), followed by GAM vs. GAM vs. SVM (36%). In other words, the GAM was noted to have lower correlations with other models (**Figure 41**).

The spatial autocorrelations of models produced for *B. aegyptiaca* under the current climate (**Figure 42**) showed that the highest correlation was observed between RF and SVM (77%), followed by between BRT and RF (76%), BRT and SVM (73%), GLM and BRT (68%), RF and MARS (66%), BRT and MARS (66%), and GLM and SVM (63%). However, the lowest correlations were observed between GLM and GAM (25%).

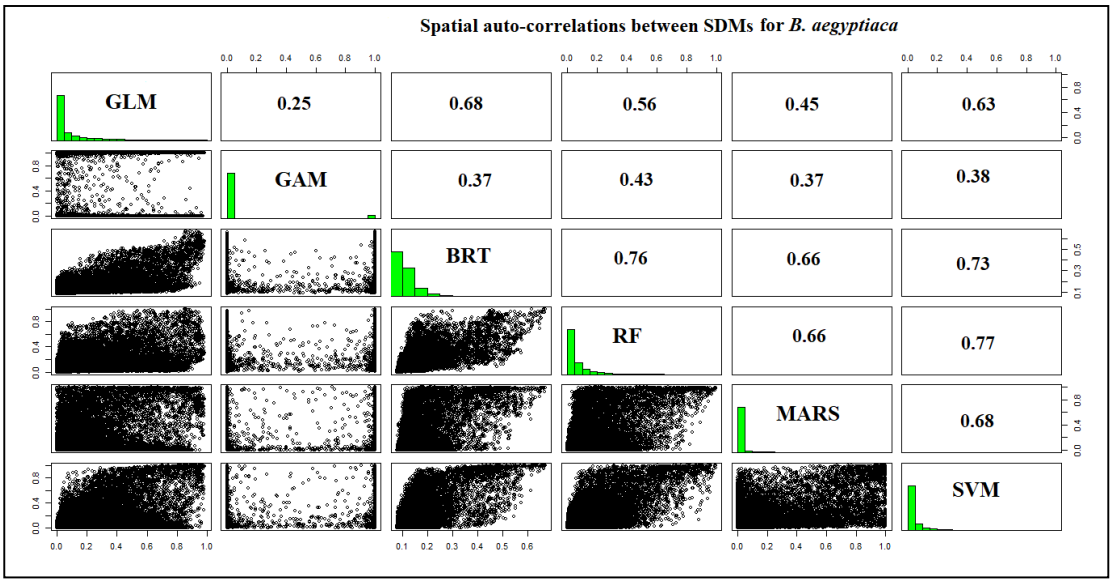

Figure 42. Spatial autocorrelation coefficients among different SDMs for *B. aegyptiaca* under the current climate.

Similarly, for *B. rotundifolia,* the highest spatial autocorrelation among SDMs under the current climate (**Figure 43**) was observed between RF and BRT (75%), followed by between BRT and GLM (67%), BRT and SVM (64%), and BRT and MARS (59%). However, the lowest correlations were observed between MARS and GAM (34%), followed by GLM and GAM (36%).

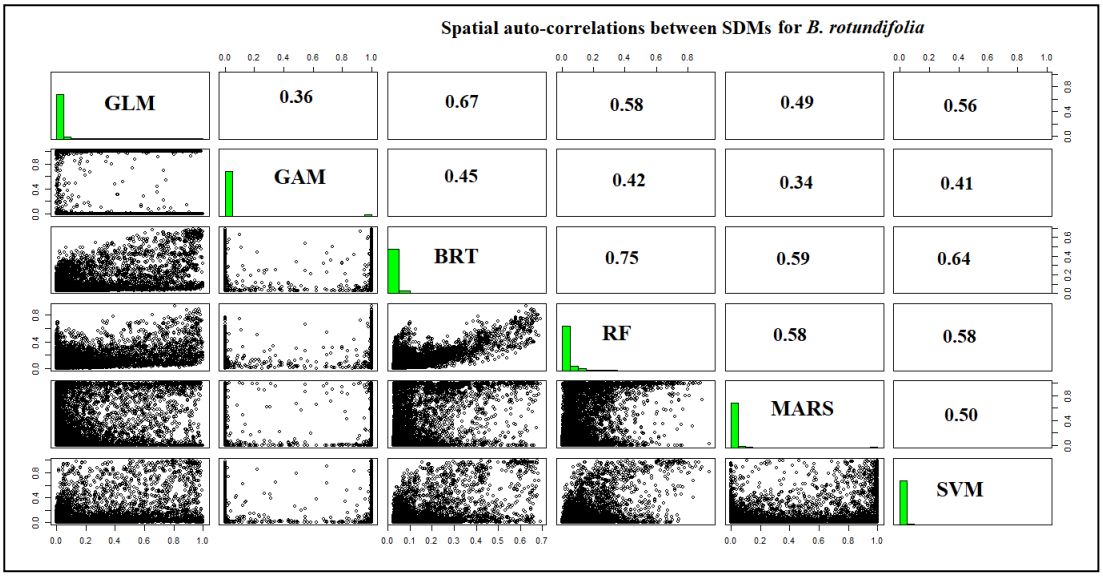

Figure 43. Spatial autocorrelation coefficients among different SDMs for *B. rotundifolia* under the current climate.

Potential distribution of Ziziphus spina-christi

Under the current climate in Ethiopia, SDMs of *Z. spina-christi* indicated that the Ethiopian Rift Valley regions, dryland areas of Ethiopia in East Amhara, and a few areas in West Amhara, Northeast Tigray, and Southern Central Oromia are identified as suitable habitats for *Z. spina-christi* (**Annex 10**).

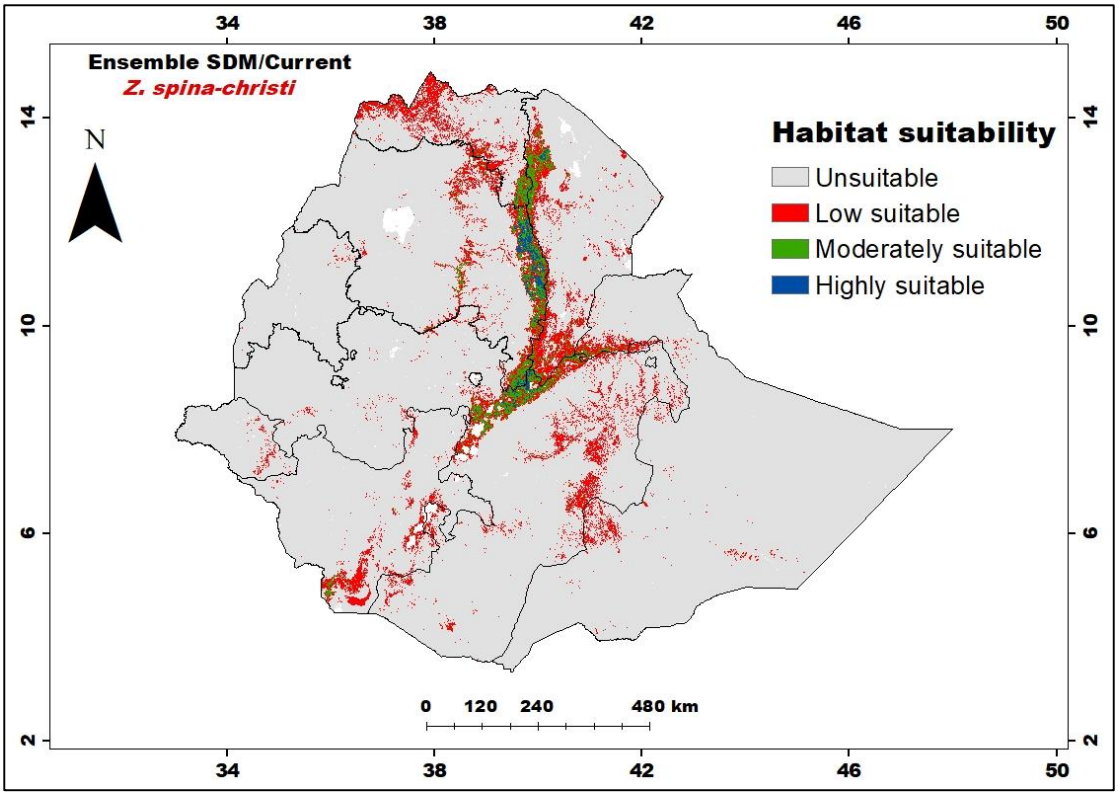

Figure 44. An ensemble SDM of *Z. spina-christi* reclassified using ArcGIS10.8 under the current climate in Ethiopia.

The ensemble SDM computed under the current showed that the Ethiopian Rift Valley regions and the Eastern Amhara regions, along with the Afar region, are highly suitable habitats for *Z. spina-christi* (**Figure 44**). Most notably, the Central Ethiopian Rift Valley and areas extending to the east and north along its ridges are identified as highly suitable habitats for *Z. spina-christi*. In this regard, under the current climate, the average SDM showed that areas designated as suitable habitat for *Z. spina-christi* cover about 8.6% of the area of the country, of which 6.6% are low-suitable, 1.7% are moderately suitable, and 0.3% are highly suitable (**Table 23**).

Moreover, the ensemble SDMs under both SSP2-45 and SSP5-85 of HadGEM3-GC31-LL's future climate also predicted that the Ethiopian Rift Valley regions, the Eastern Amhara region, and part of Southern Tigray will remain suitable habitats for *Z. spina-christi* (**Figure 45**, **Annex 11**, **Annex 12**).

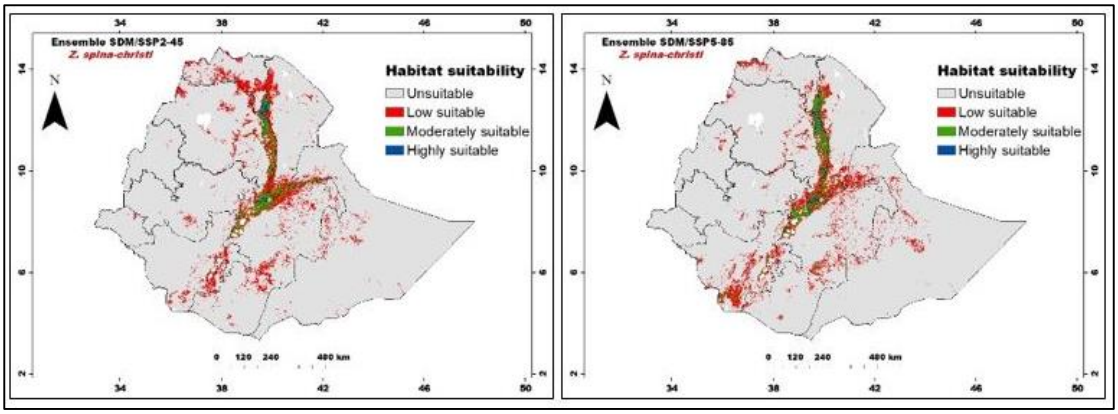

Figure 45. Ensemble SDMs of *Z. spina-christi* under SSP2-45 (left) and SSP5-85 (right) in Ethiopia.

Furthermore, the ensemble SDM under the SSP2-45 climate predicted that 8.2% of the total area of the country will be suitable for *Z. spina-christi*, while 9.4% of the area of the country is projected to be suitable for *Z. spina-christi* under the SSP5-85 future climate (**Table 23**).

Table 23. Analysis of habitat area changes of *Z. spina-christi* between the current and projections under SSP2-45 and SSP5-85 of HadGEM3-GC31-LL.

SSP	***Not suitable* (km^2)**	**Estimated area of suitability (km^2) = LS + MS + HS**	**Area changes (ΔA = ((Af-Ac)/Ac) *100)**
SSP2-45	1,216,757 (91.8%)	86,466 (6.5%) + 20,027 (1.5%) + 2,665 (0.2%) = **Af**	–4 %↓, decreasing
SSP5-85	1,201,231 (90.6%)	80,605 (6.1%) + 20,027 (1.8%) + 4,225 (1.5%) = **Af**	+9.7%↑, increasing
Current average	1,212,208 (91.4%)	87,408 (6.6%) + 21918 (1.7%) + 4381 (0.3%) = **Ac**	

Furthermore, under the current climate, analysis of the relative importance of predictor variables, based on the Pearson correlation metric for "test" data, revealed that pH highly affects the distribution of *Z. spina-christi* with more than 43% relative importance, followed by Bio17 (Precipitation of Driest Quarter) with more than 23%, Bio13 (Precipitation of Wettest Month) with more than 20% contributions, and elevation (>15%) (**Figure 46**).

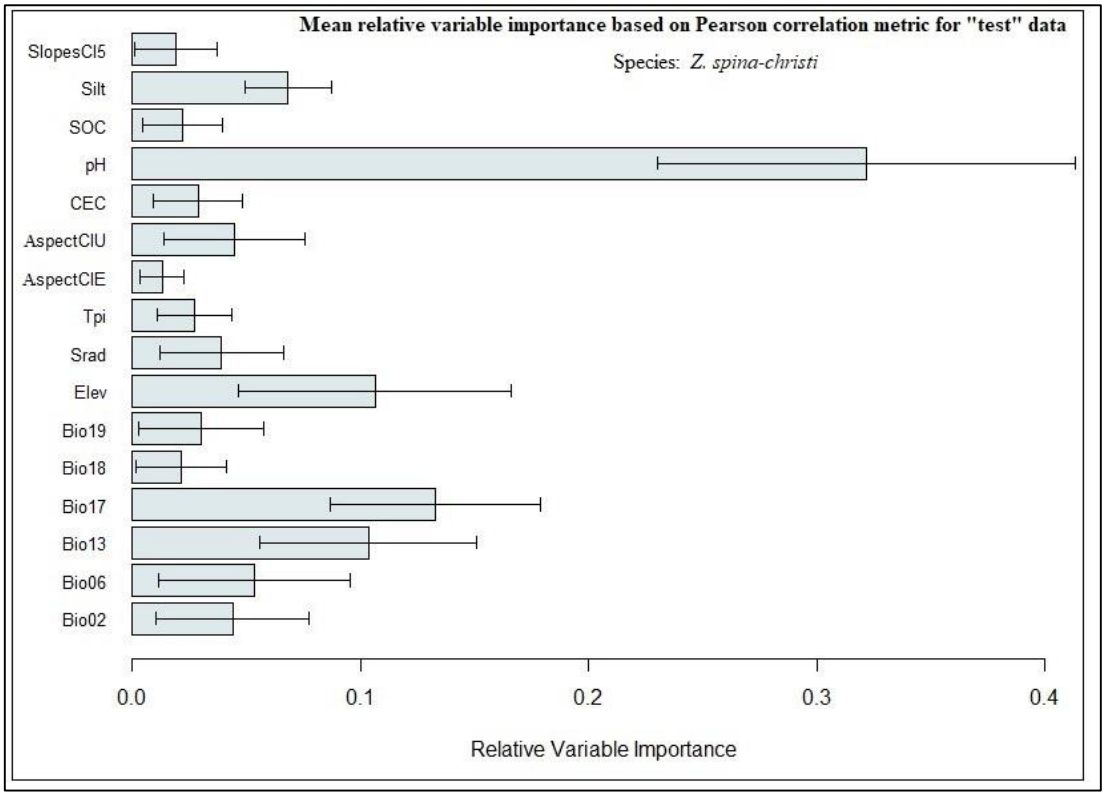

Figure 46. Relative variable importance to the distribution of *Z. spina-christi* based on Pearson correlation for "test" data (permutations = 10).

Based on the AUC metric, pH still shares the largest relative importance (>32%), followed by Bio17 (>13%), and elevation and Bio13 (>10% each) variables (**Figure 47**). The habitat suitability of *Z. spina-christi* generally increases nonlinearly with pH, Bio17, and Bio13, where the predictor variables contribute positive mean variances. However, habitat suitability for *Z. spina-christi* decreases nonlinearly with elevation (**Figure 48**). The result also showed that *Z. spina-christi* has a unimodal response to pH and elevation and a nearly bimodal response to Bio17 and Bio13.

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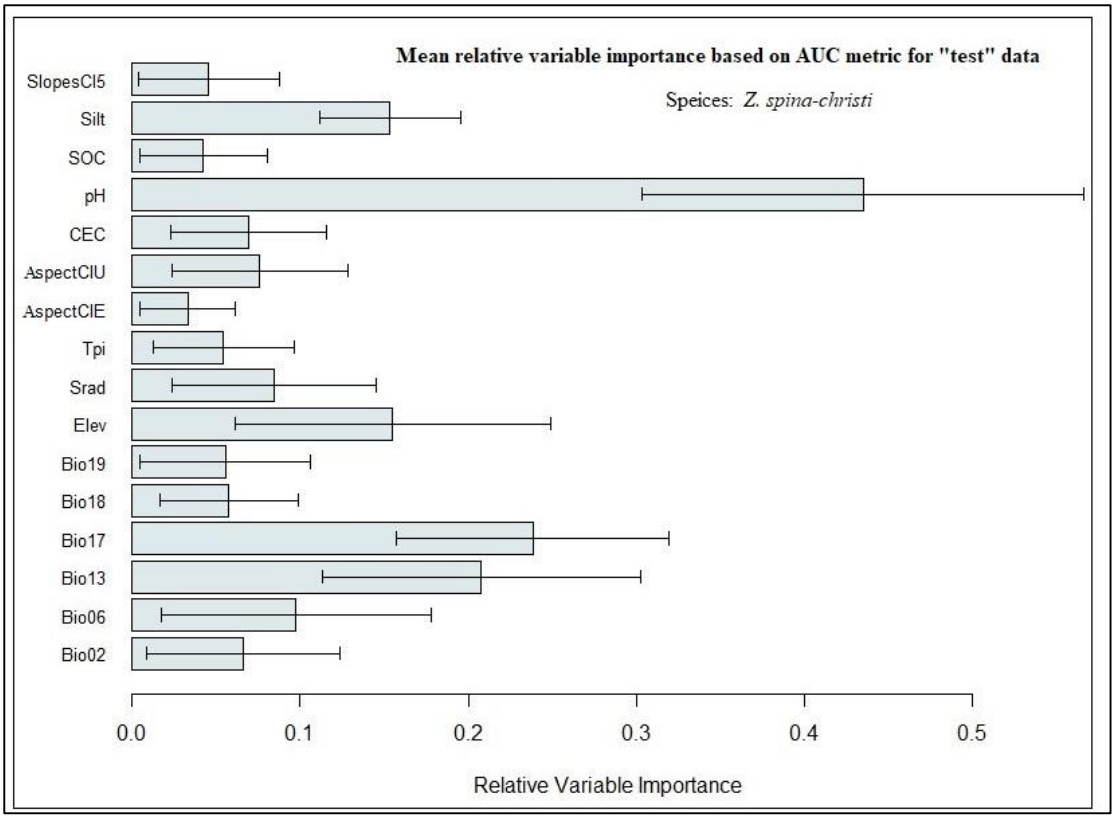

Figure 47. Relative variable importance to the distribution of *Z. spina-christi* based on AUC metrics for "test" data (permutations = 10).

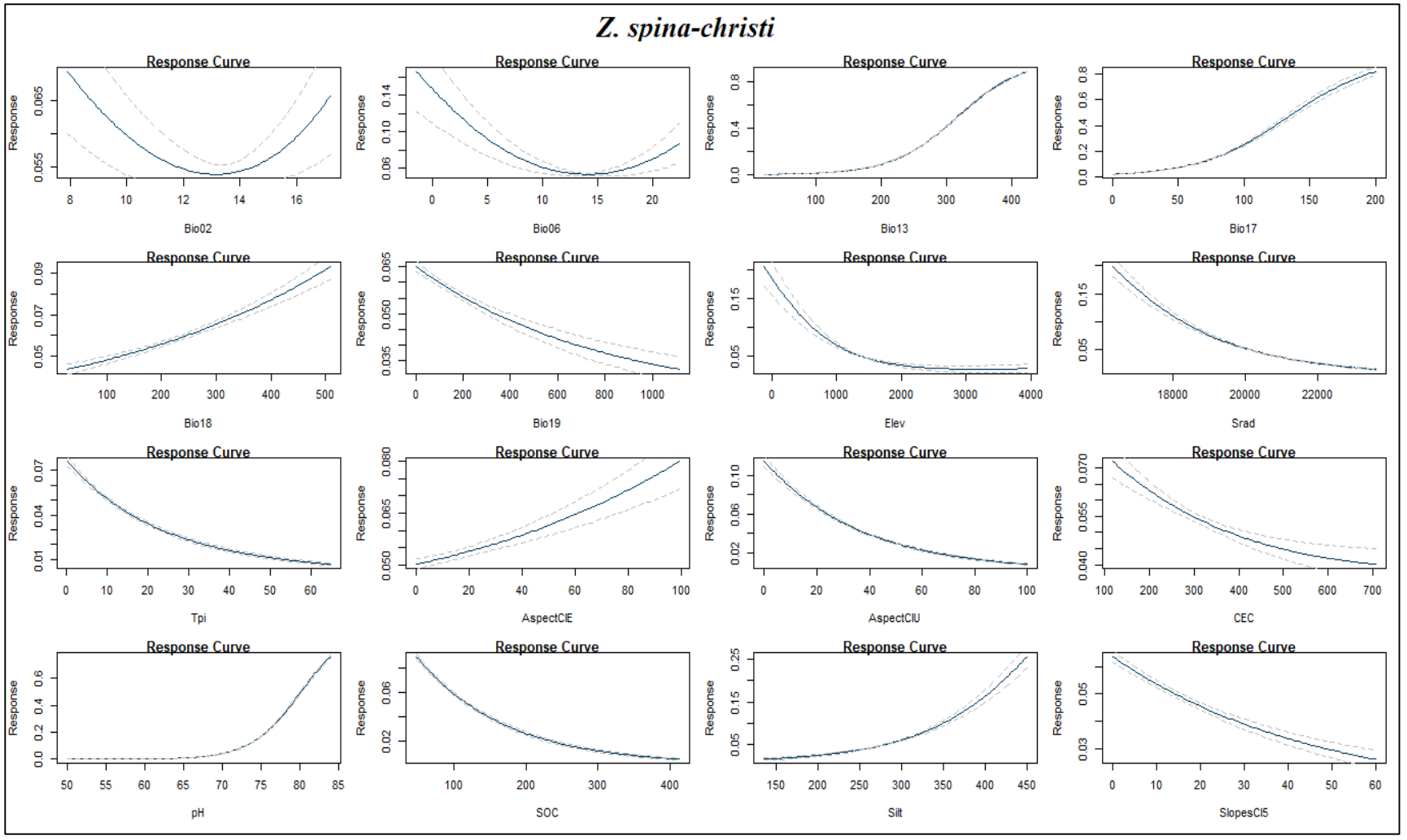

Figure 48. The variability of responses (i.e., habitat suitability) of *Z. spina-christi* to environmental predictor variables.

Potential distribution* of *Ziziphus mucronata

The SDMs under the current climate generally revealed that mainly the Ethiopian Rift Valley regions, Southern Oromia and North Shewa, a few areas of East Hararge and Bale, a few areas of Somali, South Omo areas, a few areas of Gambella, and a few areas of Southern Tigray and North Wollo are identified as moderately to highly suitable habitats for *Z. mucronata* (**Annex 13**). Under the current climate, the ensemble SDM indicated that the Central Ethiopian Rift Valley is the most suitable habitat area for *Z. mucronata* (**Figure 49**). Furthermore, a few spots are also identified across Northern Wollo and Southern Tigray, and between Eastern Hararge and Somali regions with potential suitability for *Z. mucronata* (**Figure 49**). Overall, the ensemble SDM under the current climate predicted that 6.4% of the area of Ethiopia is suitable to *Z. mucronata* of which 5.33% is low-suitable, 1.05% is moderately suitable, and 0.01% is highly suitable (**Table 24**).

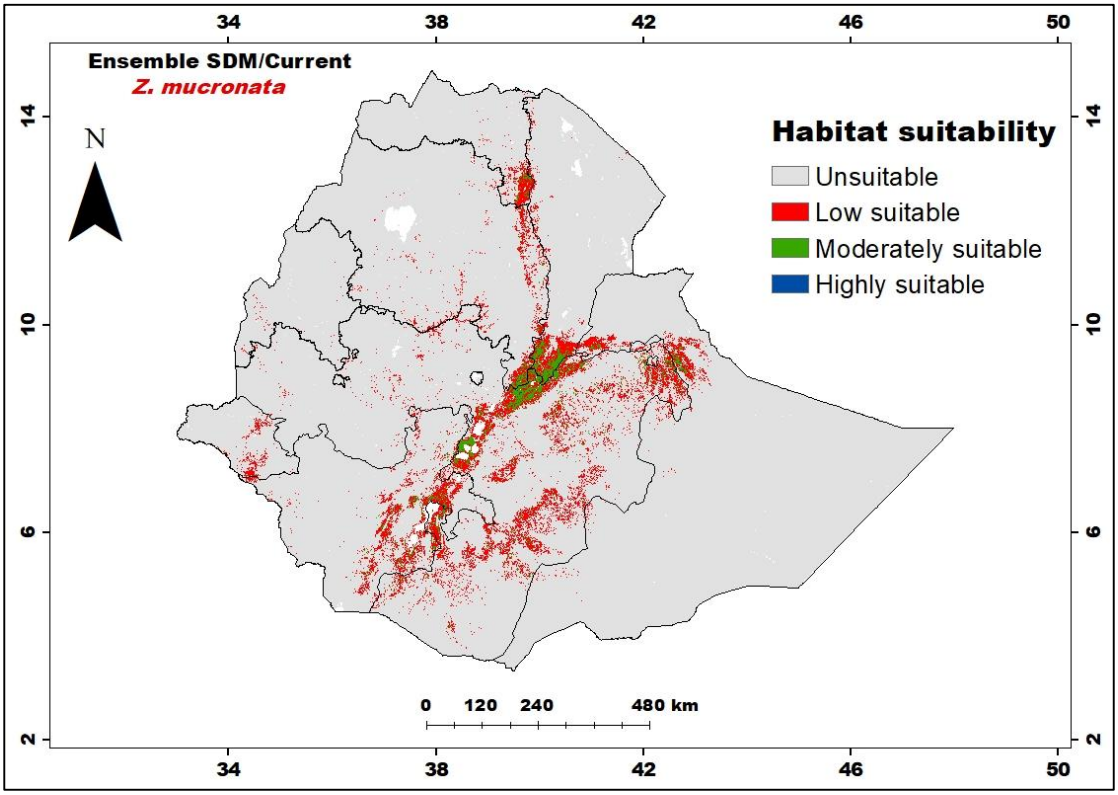

Figure 49. An ensemble SDM of *Z. mucronata* reclassified using ArcGIS10.8 under the current climate in Ethiopia.

Moreover, the ensemble SDMs under both SSP2-45 and SSP5-85 of the HadGEM3-GC31-LL climate model also revealed that mainly the Central Ethiopian Rift Valley, the area bordering North Wollo and South Tigray, and areas between east Hararge and

Somali will remain highly suitable habitats for *Z. mucronata* (**Figure 50**, **Annex 14**, **Annex 15**). The remaining parts of the Ethiopian Rift Valley regions, such as the Southern Ethiopian Rift Valley, and a few areas along East Hararge and Somali region, and the eastern drylands of Wollo and South Tigray, will remain low and moderately suitable habitats for *Z. mucronata* (**Figure 50**).

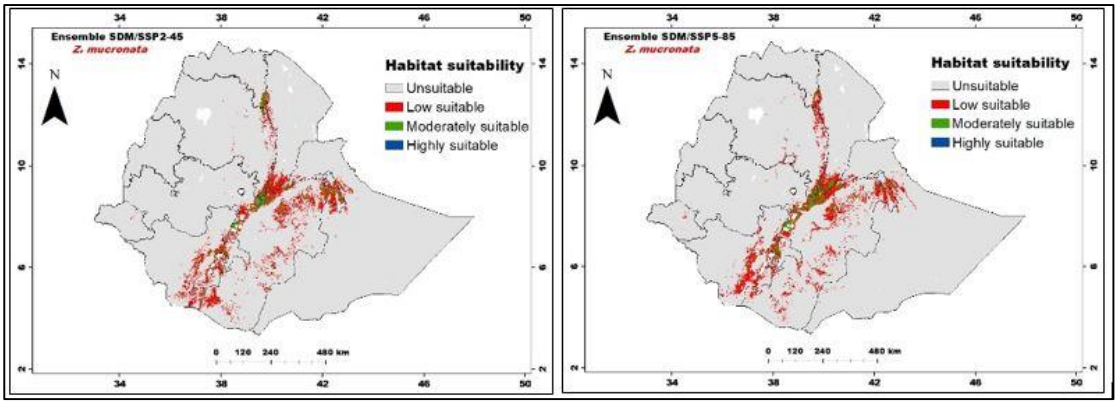

Figure 50. Ensemble SDMs of *Z. mucronata* under SSP2-45 (left) and SSP5-85 (right) in Ethiopia.

Furthermore, the ensemble SDM under the SSP2-45 climate predicted that 5.5% of the total area of the country will be suitable to *Z. mucronata*, while 5.7% of the area of the country is projected to be suitable to *Z. mucronata* under the SSP5-85 future climate (**Table 24**).

Table 24. Analysis of habitat area changes of *Z. mucronata* between the current and projections under SSP2-45 and SSP5-85 of HadGEM3-GC31-LL.

SSP	***Not suitable*** **(km^2)**	**Estimated area of suitability (km^2) = LS + MS + HS**	**Area changes (ΔA = ((Af-Ac)/Ac) *100)**
SSP2-45	1,252,870 (94.5%)	60,322 (4.54%) + 12,204 (0.92%) + 511 (0.04%) = **Af**	–13.8%↓, decreasing
SSP5-85	1,250,508 (94.31%)	61,965 (4.67%) + 13,135 (0.99%) + 299 (0.03%) = **Af**	–11%, decreasing
Current average	1,241,155 (93.61%)	70,731 (5.33%) + 13912 (1.05%) + 109 (0.01%) = **Ac**	

Furthermore, analysis of the variable contribution to the model's performance under the current climate based on the Pearson correlation metric for "test" data showed that

clay highly affects the distribution of *Z. mucronata*, accounting for more than 35% of the relative importance, followed by sand (>33%), silt (>31%), and BD (>30%) variables (**Figure 51**). Based on the AUC metric, clay still shares the largest relative importance (> 25%), followed by BD (21%), and sand and silt (>20% each) (**Figure 52**). The habitat suitability of *Z. mucronata* tends to have variable (nonlinear and multimodal) responses (mostly decreasing) to clay, sand, and silt at the different proportions of the soil particles (**Figure 53**).

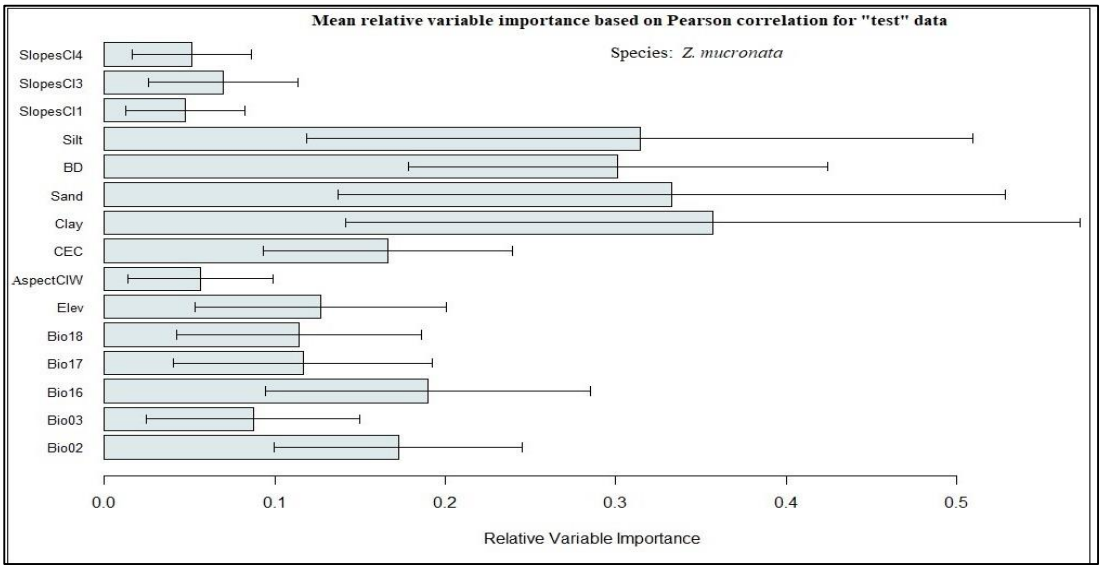

Figure 51. Relative variable importance to the distribution of *Z. mucronata* based on Pearson correlation for "test" data (permutations = 10).

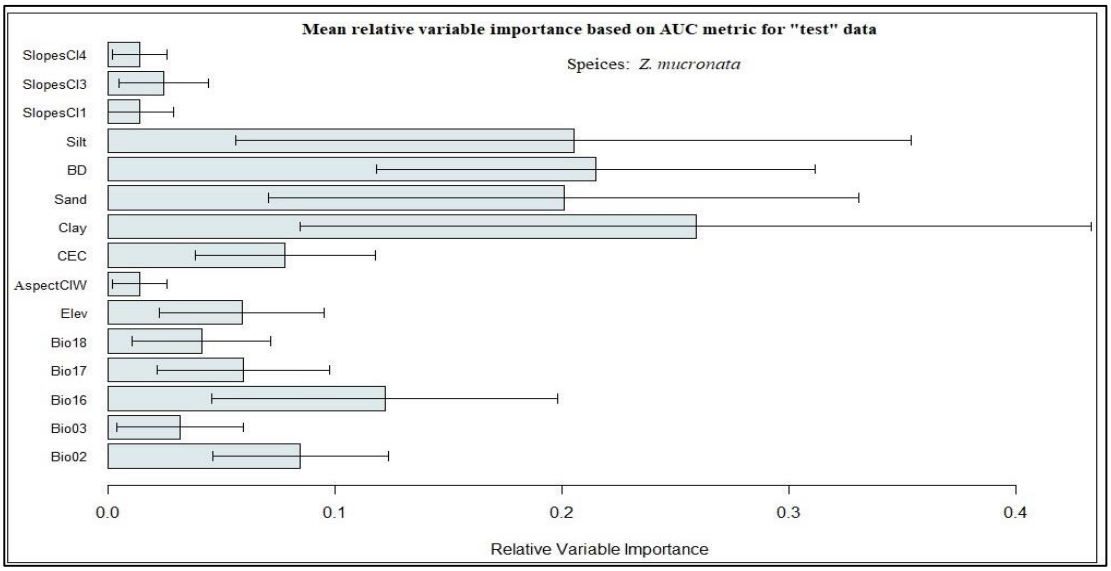

Figure 52. Relative variable importance to the distribution of *Z. mucronata* based on Pearson correlation for "test" data (permutations = 10).

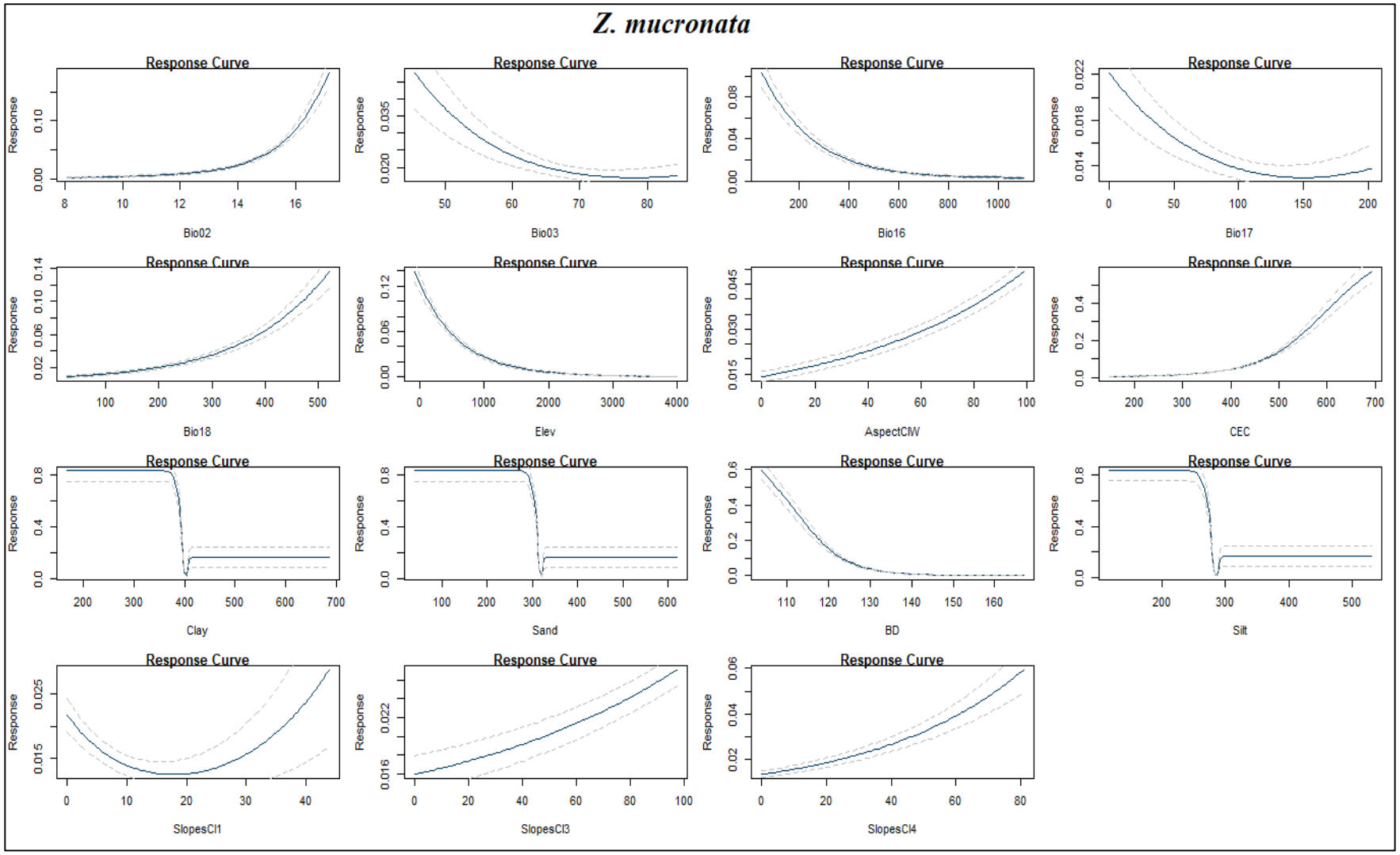

Figure 53. The variability of responses (i.e., habitat suitability) of *Z. mucronata* to environmental predictor variables.

The habitat suitability of *Z. mucronata* tends to have variable (nonlinear) responses to clay, sand, and silt at different proportions of the soil particles. For instance, the habitat suitably suitable for *Z. mucronata* remains stable (unchanging) with a clay proportion of 390 g/100g (%), making a sharp decrease until the clay proportion reaches 400 g/100g (%), while making a sharp increase until the clay proportion reaches 410 g/100g (%), finally remaining stable after 400 g/100g (%) of clay proportion. However, the habitat suitability of *Z. mucronata* decreases with BD but appears to be nonlinear (**Figure 53**). The result also revealed that *Z. mucronata* has a multimodal response to clay, sand, and silt while having a unimodal response to BD.

Potential distribution of Balanites aegyptiaca

The varied species distribution models (SDMs) computed under the current climate predicted that mainly the Ethiopian Rift Valley and a few dryland ecosystems of Ethiopia are the most suitable habitats for *B. aegyptiaca* (**Annex 16**). The ensemble SDM showed that the Central Ethiopian Rift Valley is the most suitable habitat area for *B. aegyptiaca* (**Figure 54**). Moreover, parts of Gambella, parts of Hararge, Bale, and Borena in Oromia, and parts of the Somali region are identified as low- to moderately-suitable habitats for the species.

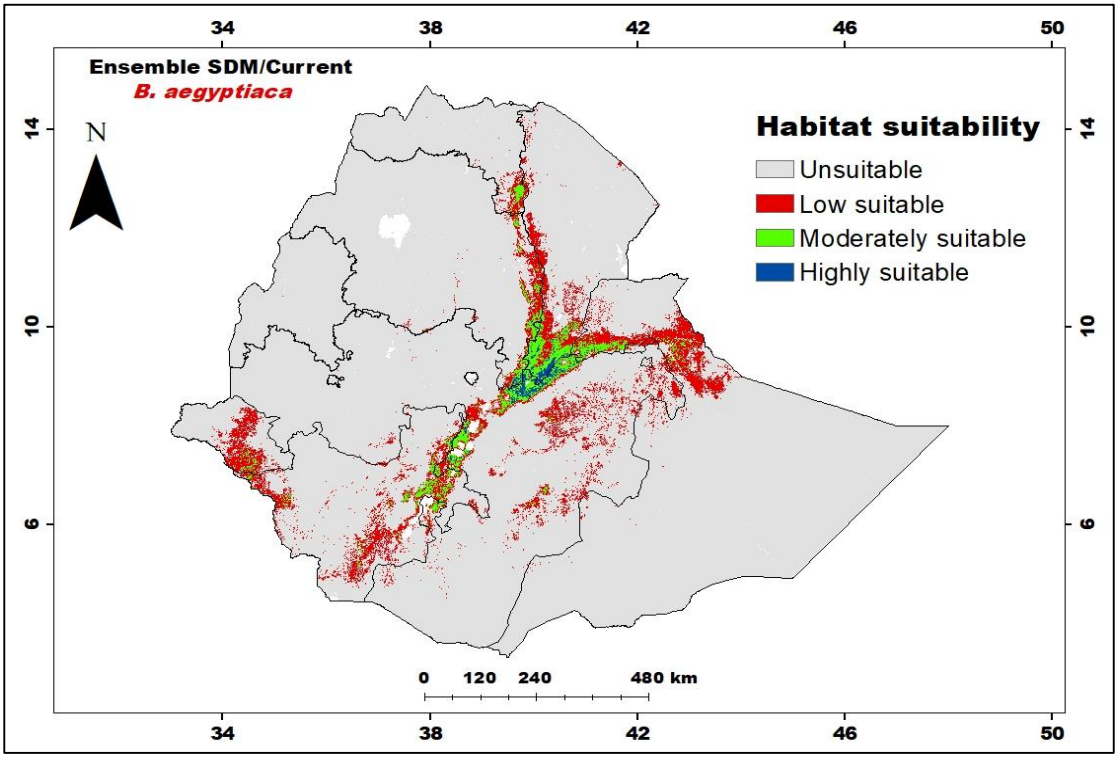

Figure 54. Current ensemble SDM for *B. aegyptiaca* in Ethiopia.

Under the current climate, the areas identified as unsuitable habitats for *B. aegyptiaca* account for 91.4% of the area of Ethiopia, while 6.4% are low-suitable habitats, 1.8% are moderately suitable habitats, and 0.4% are highly suitable habitats (**Table 25**).

Table 25. Average aerial extent of habitat classes of *B. aegyptiaca* under current and future climate scenarios.

Habitat suitability class	**Areas in km^2**		
	Current condition	**SSP45**	**SSP85**
Highly suitable	4,838 (0.4%)	4,854 (0.4%)	7,799 (0.6%)
Moderately suitable	24,424 (1.8%)	24,200 (1.8%)	22,246 (1.7%)
Low suitable	85,255 (6.4%)	87,880 (6.6%)	95,712 (7.2%)
Unsuitable	1,213,366 (91.4%)	1,210,949 (91.2%)	1,202,126 (90.5%)

The relative importance of the predictor variables averaged for all SDMs of *B. aegyptiaca* indicated that Bio07 (Temperature Annual Range) is the most important variable with 53.1% contribution based on the correlation matrix and 29.4% contribution based on the AUC matrix for the "training data"; and 53.8% contribution based on the correlation matrix and 29.1% contribution based on the AUC matrix for the "testing data"; followed by Bio15 (Precipitation Seasonality) with 29.5% contribution based on the correlation matrix and 22.4% contribution based on the AUC matrix for the "training data"; and 34.3% contribution based on the correlation matrix and 22.6% contribution based on the AUC matrix for the "testing data". In other words, the results showed that temperature and precipitation-related environmental variables were the ones that highly influenced the distribution of *B. aegyptiaca* (**Table 26**). However, it was observed that Bio07 is positively correlated (nonlinear, unimodal) with the habitat suitability of *B. aegyptiaca*, while Bio15 is negatively correlated (nonlinear, unimodal) with the habitat suitability of *B. aegyptiaca* (**Figure 55**).

Table 26. Relative variable importance (%) for the distribution of *B. aegyptiaca* based on two metrics.

Variable	Relative variable importance (RVI)			
	Training data		Test data	
	Based on correlation matrix	Based on AUC matrix	Based on correlation matrix	Based on AUC matrix
Bio07	53.1	29.4	53.8	29.1
Bio08	1.8	0.8	1.7	0.7
Bio14	0.3	0.1	0.3	0.1
Bio15	29.5	22.4	34.3	22.6
Bio16	0.6	0.4	0.7	0.1
Bio18	3.8	1.9	3.6	3.7
Tpi	17.8	15	19.9	14.4
AspectClE	5	2.1	6.2	3
AspectClS	3.5	1.5	3.6	0.4
AspectClU	0.3	0.2	0.3	0.1
AspectClW	3.3	2	4	2.8
Clay	0.01	0.01	0.01	0.01
Silt	5.3	2.5	6.5	4.4
SlopesCl4	1.8	0.6	2.1	0.7

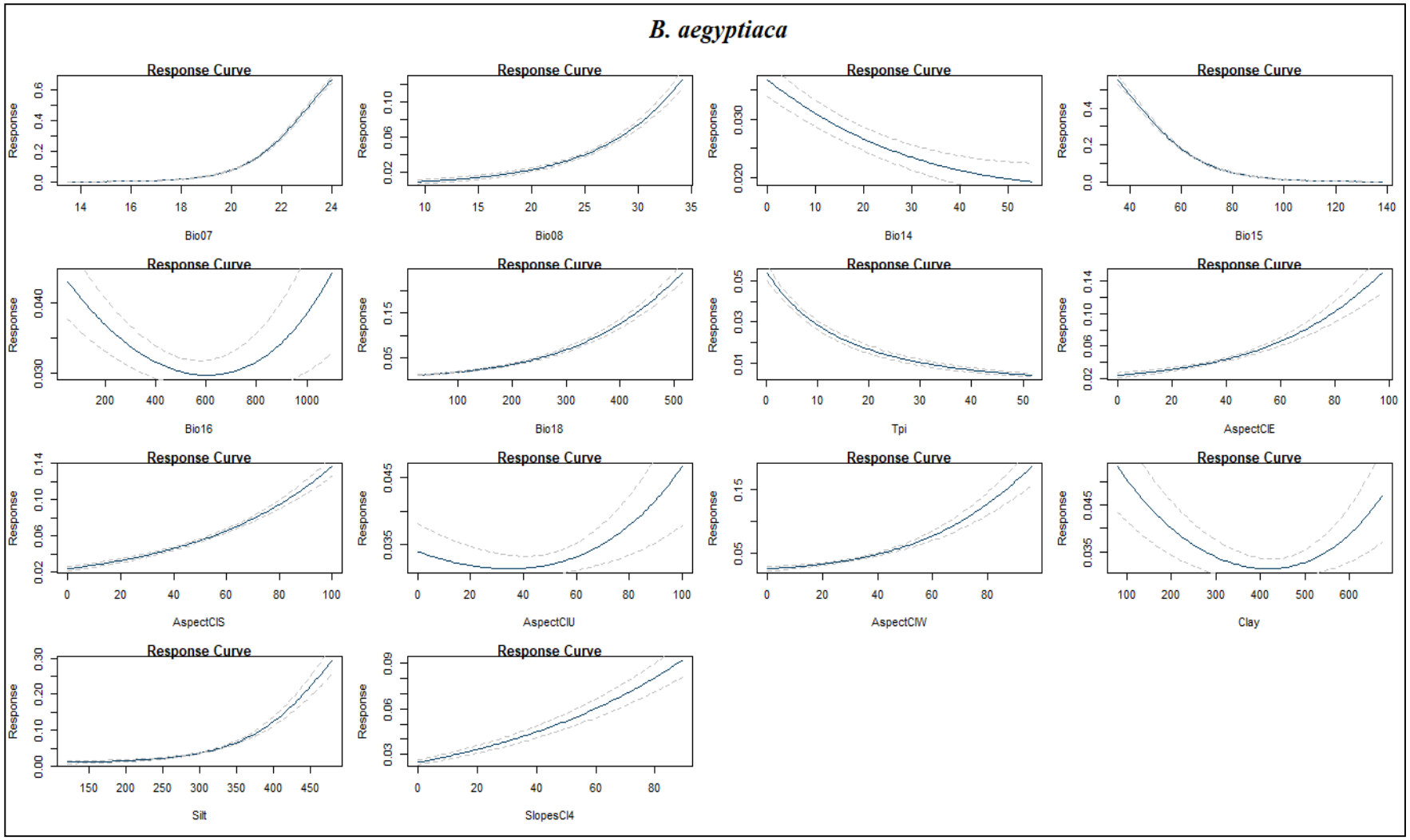

Figure 55. The variability of responses (i.e., habitat suitability) of *B. aegyptiaca* to environmental predictor variables.

Furthermore, analysis of the habitat suitability of *B. aegyptiaca* under both SSP2-45 and SSP5-85 of the HadGEM3-GC31-LL climate showed that the Ethiopian Rift Valley region will remain the ideal habitat for *B. aegyptiaca*. Moreover, the GLM, GAM, MARS, and SVM also predicted that considerable areas of the drylands of Eastern and Southeastern Ethiopia will be suitable for *B. aegyptiaca* (**Annex 17**, **Annex 18**). The ensemble SDM for *B. aegyptiaca* under SSP2-45 indicated that the Ethiopian Rift Valley region and parts of the Somali and Gambella regions will remain suitable habitats for *B. aegyptiaca* (**Figure 56**).

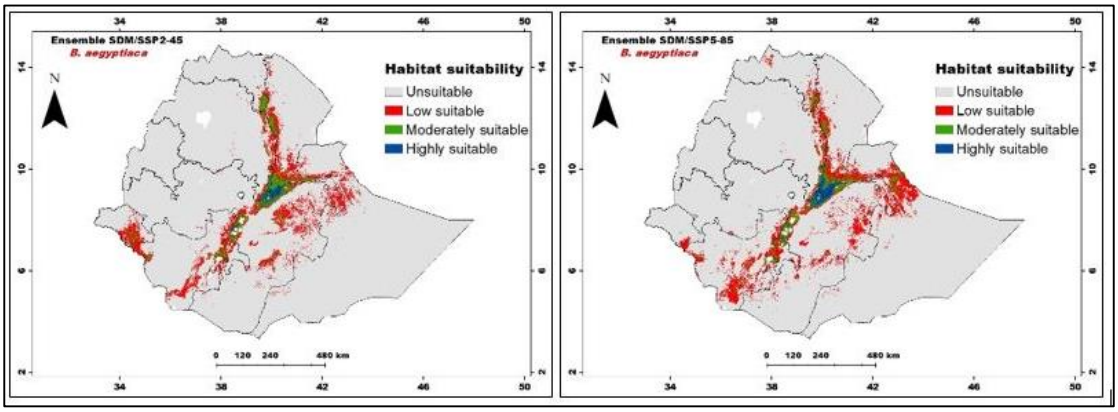

Figure 56. Future ensemble SDMs for *B. aegyptiaca* under SSP2-45 (left) and SSP5-85 (right) of HadGEM3-GC31-LL climate in Ethiopia.

Table 27. Analysis of habitat area changes for *B. aegyptiaca* between the current and projected under SSP2-45 and SSP5-85 (HadGEM3-GC31-LL) in 2061–2080.

SSP	***Not suitable* (km^2)**	**Estimated area of suitability (km^2) = LS + MS + HS**	**Area changes (ΔA = ((Af-Ac)/Ac)*100)**
SSP2-45	1,210,949 (91.2%)	116,934 (8.8%), **Af**	+2.1%↑, increasing
SSP5-85	1,202,126 (90.5%)	125,757 (9.5%), **Af**	+7.5%↑, increasing
Current average	1,213,366 (91.4%)	114,517 (8.6%), **Ac**	

*Note that "ΔA" is the percentage changes of suitable habitat area; "Af" is the predicted area of suitable habitat for HadGEM3-GC31-LL using SSP2-45 and SSP5-85, and "Ac" is the predicted area of suitable habitat under the current climate.

Therefore, analysis of the habitat area changes in habitat suitability indicated that areas of 8.8% and 9.5% of the area of Ethiopia are predicted to be suitable for *B.*

aegyptiaca under SSP2-45 and SSP5-85 in 2061–2080, respectively (**Table 27**). Analysis of the habitat change between the current and future climate of HadGEM3-GC31-LL for the years 2061-2080 showed that *B. aegyptiaca* will likely expand its habitat ranges by 2.1% and 7.5% of its current area under SSP2-45 and SSP5-85, respectively.

Potential distribution of Balanites rotundifolia

The projected SDMs for *B. rotundifolia* under the current climate indicated that the Southern Ethiopian Rift Valley region, mainly the South Omo, and areas of the Omo River watershed system are identified as the most important habitat areas for *B. rotundifolia* (**Annex 19**). Moreover, analysis of the ensemble SDM under the current climate showed that the Southern Ethiopian Rift Valley regions, particularly the South Omo, are the most suitable habitat for *B. rotundifolia* (**Figure 57**).

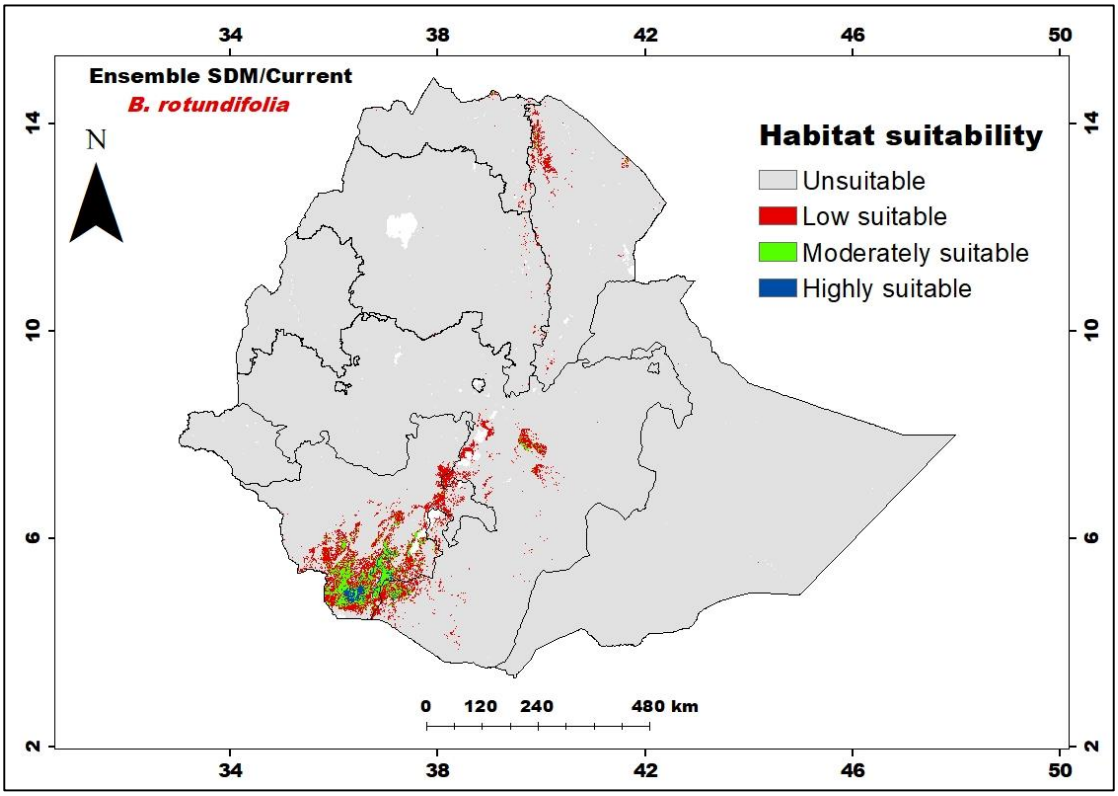

Figure 57. Current ensemble SDM for *B. rotundifolia* in Ethiopia.

Moreover, some arid and semi-arid areas of Gamo and Wolayta, Sidama and Guji areas, and a few areas of the Northern Ethiopian Rift Valley (Northern Afar region) are identified as low-suitable habitat areas for *B. rotundifolia* (**Figure 57**). Moreover, under the current climate, the ensemble SDM estimated that unsuitable habitat for *B.*

rotundifolia account for 96.9% of the area of Ethiopia, while 2.2% is low-suitable habitat, 0.7% is moderately suitable habitat, and 0.2% is highly suitable habitat (**Table 28**).

Table 28. Average aerial extent of habitat classes of *B. rotundifolia* under current future climate change scenarios.

Habitat suitability class	**Areas in km^2**		
	Current condition	**SSP45**	**SSP85**
Highly suitable	2,001 (0.2%)	2,574 (0.2%)	4,181 (0.3 %)
Moderately suitable	9,683 (0.7%)	5,120 (0.4%)	13,126 (1.0 %)
Low suitable	29,689 (2.2%)	21,853 (1.6%)	33,587 (2.5 %)
Unsuitable	1,286,510 (96.9%)	1,298,336 (97.8%)	1,276,989 (96.2 %)

Analysis of the relative contributions of predictor variables averaged for all SDMs for *B. rotundifolia* showed that Bio17 (Precipitation of the Driest Quarter) is highly contributing to the models' performance of *B. rotundifolia*, accounting for 79.6% and 40.6% based on correlation and AUC matrices for the "training data," respectively, and 82.3% and 42.5% based on correlation and AUC matrices for the "testing data," respectively; followed by Bio12 (Annual Precipitation) with 64.8% and 36.2% contribution based on correlation and AUC matrices for the "training data", respectively; and 61.4% and 29.3% contribution based on correlation and AUC matrices for the "testing data", respectively (**Table 29**).

Table 29. Relative variable importance (%) for the distribution of *B. rotundifolia* based on two metrics.

Variable	Relative variable importance (RVI)			
	Training data		Test data	
	Based on correlation matrix	Based on AUC matrix	Based on correlation matrix	Based on AUC matrix
Bio02	15.7	6	11.8	0.01
Bio12	64.8	36.2	61.4	29.3
Bio17	79.6	40.6	82.3	42.5
AspectClS	0.01	0.01	0.01	0.01
AspectClW	0.1	0.1	0.1	0.01
CEC	27.9	8.4	20	2.4
pH	8.6	2.7	6.7	2.4
Silt	0.01	0.01	0.01	0.2
SlopesCl2	1.5	0.4	1.2	0.3
SlopesCl3	15.3	4.3	10.9	0.6
SlopesCl4	2.1	0.7	0.2	0.01
SlopesCl6	2.1	0.6	1.6	0.7
SlopesCl8	5.1	6.6	4.3	0.7

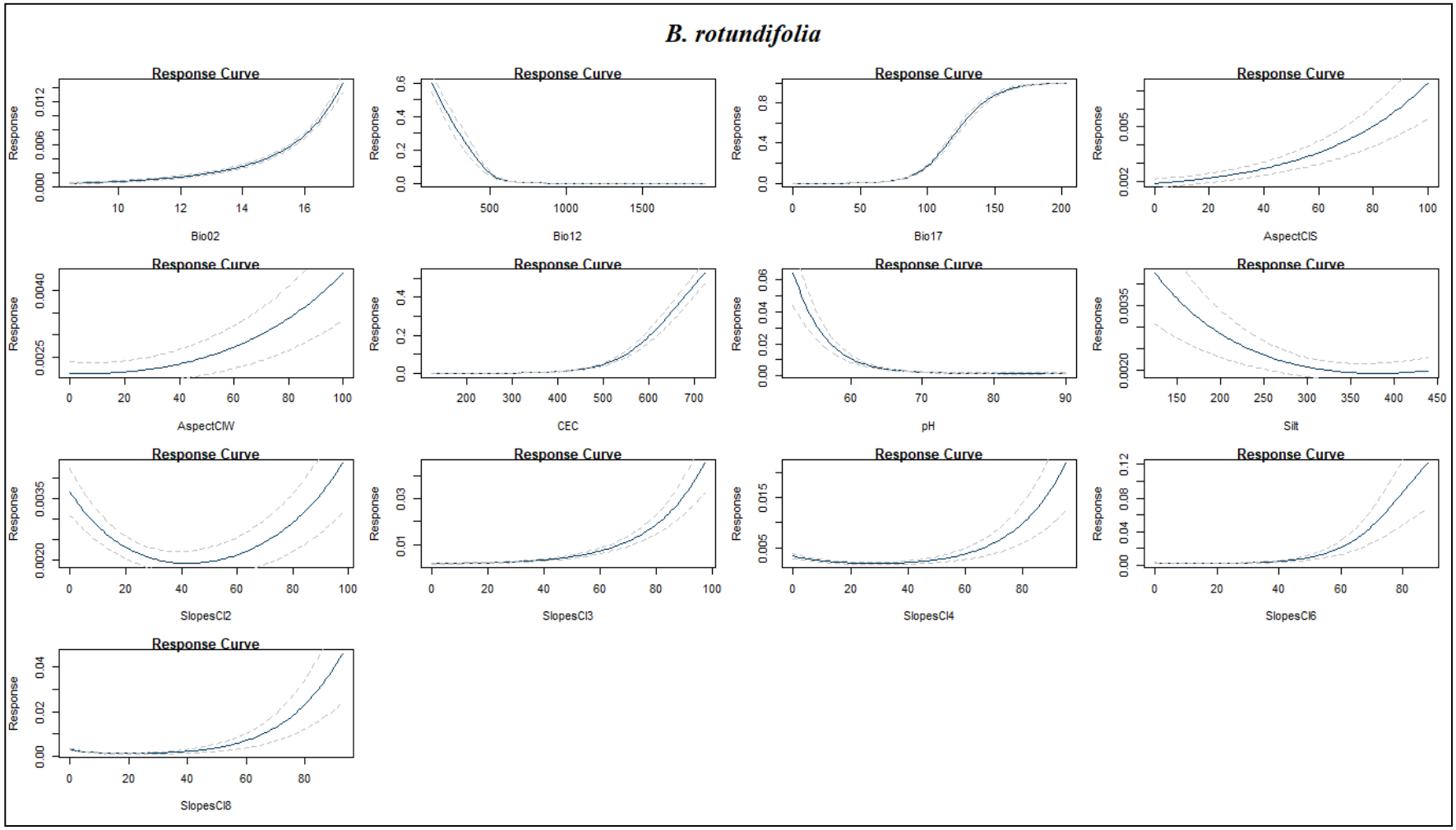

Figure 58. The variability of responses (i.e., habitat suitability) of *B. rotundifolia* to environmental predictor variables.

However, it was observed that Bio17 is positively correlated (nonlinear, bimodal) with the habitat suitability of *B. rotundifolia*, while Bio12 is negatively correlated (nonlinear, unimodal) with the habitat suitability of *B. rotundifolia* (**Figure 58**).

The projected SDMs for *B. rotundifolia* under SSP2-45 of the HadGEM3-GC31-LL climate model indicated that the Southern Ethiopian Rift Valley regions will remain suitable for the species in the years between 2061 and 2080 (**Annex 20**, **Annex 21**). Under both SSP2-45 and SSP5-85, the ensemble SDMs showed that the Southern Ethiopian Rift Valley, mainly the South Omo region, will remain the most suitable habitat for *B. rotundifolia* in the years 2061–2080 (**Figure 59**).

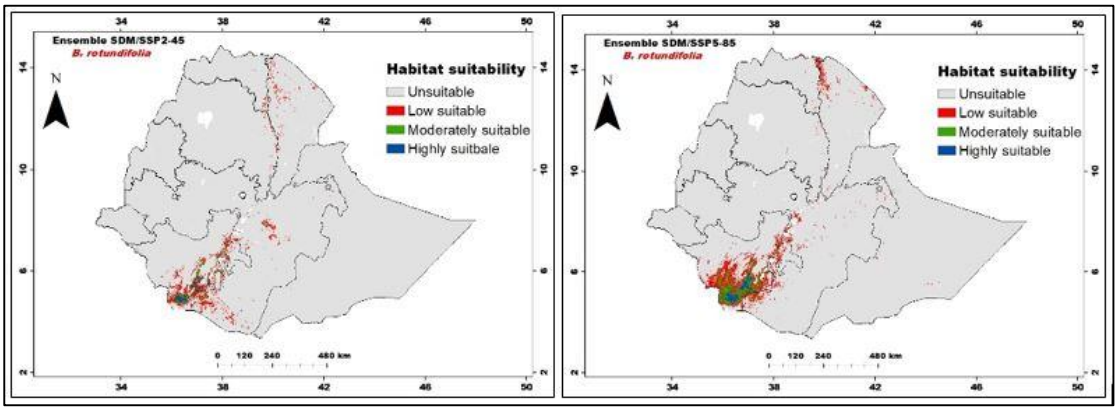

Figure 59. Future ensemble SDMs for *B. rotundifolia* under SSP2-45 (left) and SSP5-85 (right) of HadGEM3-GC31-LL climate in Ethiopia.

Furthermore, analysis of the area changes in habitat suitability indicated that areas of 2.2% and 3.8% of the area of Ethiopia are predicted to be suitable for *B. rotundifolia* under SSP2-45 and SSP5-85 in 2061–2080, respectively (**Table 30**).

Table 30. Analysis of habitat area changes for *B. rotundifolia* between the current and projected under SSP2-45 and SSP5-85 (HadGEM3-GC31-LL) in 2061–2080.

SSP	*Not suitable* (km^2)	Estimated area of suitability (km^2) = LS + MS + HS	Area changes (ΔA = ((Af-Ac)/Ac)*100)
SSP2-45	1,298,336 (97.8 %)	29,547 (2.2 %), **Af**	–28.6%↓, decreasing
SSP5-85	1,276,989 (96.2 %)	50,894 (3.8 %), **Af**	+23.0%↑, increasing
Current average	1,286,510 (96.9 %)	41,373 (3.1 %), **Ac**	

*Note that "ΔA" is the percentage changes of suitable habitat area; "Af" is the predicted area of suitable habitat for HadGEM3-GC31-LL using SSP2-45 and SSP5-85, and "Ac" is the predicted area of suitable habitat under the current climate.

Analysis of the habitat change between the current and future climate of HadGEM3-GC31-LL for the years 2061-2080 showed that *B. rotundifolia* will likely decrease its habitat range by 28.6% (↓) under SSP2-45 and increase by 23% (↑) of its current area under SSP5-85 (**Table 30**).

5.4 Discussions

Habitat suitability of Ziziphus spina-christi under climate change in Ethiopia

In the current climate in Ethiopia, the SDMs predicted that *Z. spina-christi* occurs in large-scale habitat areas across the country. The suitable habitat areas identified under the current and future climates can be used as inputs for conservation strategies and dryland afforestation programs in Ethiopia. Previously, no SDMs of Z. *spina-christi* were produced and reported under climate change in Ethiopia, which could be referred to for checking the compatibility of the current study. Nevertheless, this study generally indicated that Z. *spina-christi* will expand its distribution ranges with the increasing climate changes in Ethiopia. Hence, *Z. spina-christi* can be taken as a key species for the enhancement of dryland agro-biodiversity in Ethiopia, thereby supplementing human nutrition and other multiple uses (Debela Hunde et al., 2014). Similarly, the study of the Maximum Entropy (MaxEnt) of *Z. spina-christi* in the United Arab Emirate (UAE) by Ksiksi et al. (2019) also revealed that the habitat suitability of *Z. spina-christi* will potentially increase with climate change under both CMIP5's RCP4.5 scenarios in the 2050s and 2070s. Most importantly, the SSP5-85 climate scenario will likely favor *Z. spina-christi* in expanding its habitat ranges. Both SSP2-45 and SSP5-85 scenarios project the possibility of a warmer climate compared to the current average. However, the two models show different projected temperatures (SSP5-85 has a projected temperature of 1.9 ºC higher than SSP2-45). Hence, this might be the reason why the area extent of habitat suitability projected under SSP2-45 is lower compared to SSP5-85.

The temperature-loving nature of *Z. spina-christi*, tolerating heat and drought, may provide windows of opportunity and ecological advantages for the species to increase its habitat ranges with the increasing temperature. Moreover, the deep taproot system it possesses can provide peculiar regenerative potential and survival for *Z. spina-christi* under the changing climate (Ksiksi et al., 2019). This finding agrees with the report by Zait and Schwartz (2018), who confirmed *Z. spina-christi* to has adaptive

strategies for photosynthesis and drought under the changing climate. The ensemble models of habitat suitability produced for *Z. spina-christi* under the current and future climates are ecologically sound considering their present occurrences in Ethiopia. In this regard, Azene Bekele-Tesemma (2007) also documented that *Z. spina-christi* normally occurs up to 1,900 m altitude in arid and semi-arid agro-climatic zones in the Afar plains, Dire-Dawa, Hararge, Bale, Gamo, Gofa, Shewa, Wollo, and Tigray of Ethiopia. Overall, our study identified potentially suitable habitat areas that should be delineated for the conservation, management, and sustainable utilization of *Z. spina-christi in Ethiopia.* The findings in this study also strengthen the idea of applying high-precision machine learning algorithms to better conceptualize the actual habitat ranges of the species. Hence, the selection and implementation of models require greater care to avoid misleading policymakers (Elith & Leathwick, 2009). Nevertheless, it is most recommendable to ensemble the different models, thereby avoiding model uncertainty.

The pH and precipitation-related environmental variables (i.e., Bio17 and Bio13) and elevation largely determine the distribution of *Z. spina-christi* in Ethiopia. The habitat suitability of *Z. spina-christi* increases with the former three variables while decreasing with elevation. However, according to the study by Ksiksi et al. (2019), the distribution of *Z. spina-christi* is mainly influenced by Precipitation of the Wettest Month (Bio13), followed by Precipitation of the Coldest Quarter (Bio19), and Annual Precipitation (Bio12) in the hyper-arid landscape of the UAE. Model outputs resulting from using one algorithm (e.g., only MaxEnt) cannot be reliable unless multiple models are run in ensemble to significantly reduce potential biases in model performance and so increase the robustness of SDM models (Zhang et al., 2015). In fact, different provenances of the same species from diverse ecological ranges may show variable responses to environmental variables due to the adaptation evolution of the different ecotypes. Furthermore, a report by Orwa et al. (2009) also indicates that *Z. spina-christi* prefers alluvial plains with deep soils, but also clay where water is available and saline soils, and has a physical limit of 0–2,000 m altitude, 19–28 °C mean annual temperature, and 100–500 mm mean annual rainfall.

Habitat suitability of Ziziphus mucronata under climate change in Ethiopia

The current study identified potential habitat areas that should be prioritized for the conservation, management, and sustainable use of the species under current and future climates. However, the SDMs of *Z. mucronata* computed under the current climate in Ethiopia showed that the species has narrower habitat ranges compared to *Z. spina-christi*. This might be related to the fact that *Z. mucronata* is highly influenced by the edaphic nature of soil textures, namely clay, BD, sand, and silt particles. Hence, it is important to improve the management of arid and semi-arid ecosystems and biodiversity resources to secure their long-term ecological functioning. The current study revealed that the habitat suitability of *Z. mucronata* is negatively correlated with silt, while positively correlated with the other environmental predictors. As per Orwa et al. (2009), the ecosystem of *Z. mucronata* is predominantly characterized by fluvisols and a variety of fine-textured soils with a physical limit of up to 2,000 m altitude, a mean annual temperature of 12–30 °C, and a mean annual rainfall of 446–1,200 mm. The species was also reported to be tolerant of shallow soils, seasonal waterlogging, salt spray, and soil salinity (Orwa et al., 2009). A report by Azene Bekele-Tesemma (2007) also indicated that *Z. mucronata* occurs up to 2,000 m altitude in areas with a mean annual rainfall of 446 to 1,200 mm, predominantly in *Vachellia-Terminalia*, *Vachellia-Balanites*, and *Boswellia* woodlands and bushlands, and arid riverine forests in all regions of Ethiopia.

Hence, the increasing CO_2 and atmospheric temperature, under both emission scenarios, will very likely affect the distribution of *Z. mucronata* in the future. The SDMs indicated the higher sensitivity and low adaptability of *Z. mucronata* to the changing climate. Hence, species with high sensitivity and low adaptive capacity are most likely at risk of extinction (Dawson et al., 2011). In this regard, a global drought prediction by Li et al. (2021) under CMIP6's scenarios (SSP1-26, SSP2-45, and SSP5-85) also indicated that the drylands would face the potential threat of severe and longer-lasting drought with expected impacts on global biodiversity, including on plants' structure, compositions, and distributions. Hence, depending on the degree of exposure, sensitivity, and adaptive capacity, a species responds to climate change differently.

The findings can be utilized by policy makers and conservation organizations to inform the development and implementation of conservation plans for *Ziziphus* species in the country. However, it is important to note that this study has few potential limitations. For example, *i*) With the uncertain nature of absence data and its importance in SDM (Lobo et al., 2010), this research did not incorporate absence data as an additional input variable; *ii*) This study was conducted using only one global climate model (GCM), and thus needs further researches by incorporating other GCMs that are also thought to have good performance in Ethiopian environments.

Habitat suitability of Balanites aegyptiaca under climate change in Ethiopia

Under the current climate, the ensemble SDM predicted that 8.6% of the total area of Ethiopia, mainly distributed in the Ethiopian Rift Valley and a few areas of Gambella, Hararge, Bale, and Borena in Oromia and Somalia regions are identified as potential suitable habitats for *B. aegyptiaca*. This finding partly agreed with the preliminary modeling report by Kindt et al. (2019) on the habitat distribution of *B. aegyptiaca* in Ethiopia inferred from the ensemble modeling with *BiodiversityR*. Similarly, Azene Bekele-Tesemma (2007) also reported that in Ethiopia *B. aegyptiaca* normally occurs up to 1800m altitude in the arid and semi-arid agroclimatic zones of the Rift Valley in Gamo Gofa, and in Sidama, Tigray, Wollo, Shewa, Gojam, Ilu Ababora, Arsi, and upland Hararge regions. The ensemble SDM under the current climate indicated that the Central Rift Valley is the most suitable habitat area to *B. aegyptiaca*. These areas will remain suitable in the future under both SSP2-45 and SSP5-85 of HadGEM3-GC31-LL with increasing areas to 8.8% and 9.5% of Ethiopia in 2061–2080, respectively. A recent projection of the maximum entropy distribution of *B. aegyptiaca* for Tigray region of Ethiopia by Yirga Gufi et al. (2023) showed that the habitat suitability of the species will increase by 2070 under RCP4.5 scenarios, while decreasing in mid- and end-century under RCP8.5. A study by Chérif et al. (2022) also indicated that *B. aegyptiaca* may decrease its habitat suitability by 2055 but a significant habitat increases by 2085 beyond its current habitat in the Republic of Chad under CMIP5's RCP8.5 emission scenario. The increasing in habitat suitability for *B. aegyptiaca* is possibly due its morphological and physiological responses and adaptive strategies to cope with drought and increasing temperature, including a significant reduction of biomass, early stomatal closure with small changes in photosynthesis activities (Amer et al., 2002; Khamis et al., 2015).

The Temperature Annual Range (Bio07) and Precipitation Seasonality (Bio15) are the most important variables influencing the distribution of *B. aegyptiaca* in Ethiopia. A recent projection of the maximum entropy distribution of *B. aegyptiaca* for Tigray region of Ethiopia by Yirga Gufi et al. (2023) showed that Temperature Seasonality (Bio04), Mean Diurnal Range (Bio02) and Temperature Annual Range (Bio07) are reported as the most important variables determining the distribution of the species. However, *B. aegyptiaca* has a wider ecological range. In connection to this, for species with wider global distribution, species distribution models of truncated niche (i.e., models based on data pools from wider geographical areas, such as regional or global) are more accurate than models of untruncated niche (i.e., models based on data from restricted geographical ranges, such as below regional scale) under climate changes (Chevalier et al., 2022). Another study of the maximum entropy of *B. aegyptiaca* by Chérif et al. (2022) for the republic of Chad under the changing climate using CMIP5's RCP8.5 indicated that Precipitation of Wettest Month (Bio13) was the most important variable in the model predictions. This showed that variable selection and ensemble models' approach are essential steps in the model calculation to increase model accuracy. In this regard, De Marco & Nóbrega (2018) advised that PCA-derived variable selection was an effective approach for species modeling both to control the negative effects of collinearity and as a more objective solution for the problem of variable selection. In this sense, the current study has the merit of proper variable selection for the modeling purpose of the subject species. Moreover, in addition to bioclimatic variables, incorporating soil and landscape parameters would also result in different but more sounding models, as these variables are determinant factors for plant growth and geographical distribution (Oliveira-Filho & Ratter, 2002; Baldeck et al., 2013). A report by Orwa et al. (2009) also indicated that the ecosystem of *B. aegyptiaca* is predominantly characterized by soil particles of deep sands, sandy clay loams, sandy loams or clays with a physical limit of 0–2,000 m altitude, 20–30°C mean annual temperature, and 250–1,200 mm mean annual rainfall.

Habitat suitability of Balanites rotundifolia under climate change in Ethiopia

The ensemble SDM for *B. rotundifolia* under the current climate indicated that 3.1% of the total area of Ethiopia is suitable habitat for *B. rotundifolia*, and distributed mainly in the Southern Ethiopian Rift Valley region. These areas will remain suitable

in the future under both SSP2-45 and SSP5-85 of HadGEM3-GC31-LL with decreasing areas to 2.2%, and increasing areas to 3.8% of Ethiopia in 2061–2080, respectively. In this regard, the actual field survey also proved the presence of a higher density of the population of *B. rotundifolia* across the designated areas than any other areas surveyed in the study area. Previously, no studies were reported that dealt with the habitat suitability of *B. rotundifolia* in Ethiopia. Hence, no credible findings were available that could be validated with the current study. Therefore, the current study could be taken as a breakthrough in habitat modeling for *B. rotundifolia* in Ethiopia and even be inferred to further habitat modeling of the species in other countries or on a global scale. Nevertheless, study indicated suitable areas that should be strategically delineated for the conservation and management of the species for sustainable use.

In this study, Precipitation of Driest Quarter (Bio17) and Annual Precipitation (Bio12) share the largest contribution to the model's prediction for *B. rotundifolia*. In other words, the distribution of *B. rotundifolia* was mainly influenced by both precipitation and temperature-related environmental variables and by CEC to some extent. In Kenya, *B. rotundifolia* was also reported to be often seen as the only tree on dunes in northern Kenya and observed between 50m and 1350 m altitude and a 150 mm to 400 mm rainfall range (Beentje, 1994; Katende et al., 1995). In Uganda, *B. rotundifolia* was reported to grow on a variety of soils, often found in sandy dry river beds; as well as frequently occurring in very dry savannah (Katende et al., 1995).

Overall, this study presents valuable information on the distribution of *Balanites* species in Ethiopia in the context of climate change. The findings can be utilized by policy makers and conservation organizations to inform the development and implementation of conservation plans for *Balanites* species in the country. Additionally, the study's outputs can serve as crucial inputs for the scientific community, particularly those interested in conducting further research on various aspects of *Balanites*. However, it is important to note that this study has few potential limitations. For example, *i*) With the uncertain nature of absence data and its importance in SDM (Lobo et al., 2010), this research did not incorporate absence data as an additional input variable; *ii*) This research, despite being the first SDM for *B. rotundifolia*, used a smaller sample size of occurrence data to model the habitat

suitability for *B. rotundifolia* due to its rarity; *iii*) This study was conducted using only one global climate model (GCM), and thus needs further researches by incorporating other GCMs such as the second generation Euro-Mediterranean Centre on Climate Change Earth System Model (CMCC-ESM2), and the Goddard Institute for Space Studies Model version E2.1-H (GISS-E2-1-H) (NASA-GISS) that are also thought to have good performance in Ethiopian environments. Modelling the habitat suitability of *Balanites* based on a broadly truncated niche at a regional scale (e.g., East Africa) is recommended for the period between 2081 and 2100 besides the use of this research findings for decision making and policy development.

5.5 Conclusions

The findings of this study identified suitable habitat areas for *Z. spina-christi*, *Z. mucronata*, *B. aegyptiaca* and *B. rotundifolia* under the current and future climates. The models and suitability maps produced in this study seem ecologically sensible considering their present occurrences and the ecophysiology of the study species. The Ethiopian Rift Valley and other semi-arid and arid ecosystems are the most suitable habitats for *Ziziphus* and *Balanites*. These findings highlight potential habitat areas that should be given priority for the long-term conservation and sustainable utilization of each species in Ethiopia. Hence, habitat areas identified as suitable for the species under the changing climate should be prioritized for the long-term conservation and sustainable use of each species in Ethiopia. Therefore, the conservation and management of both species should take these model outputs into consideration. Most importantly, it seems that the highly sensitive and low-adaptive nature of *Z. mucronata* should gear toward a species-targeted conservation approach to tackle the depletion of the population of *Z. mucronata* from its natural habitat. It was also noticed that *B. aegyptiaca* tends to increase its habitat ranges under both future forcing scenarios (SSP2-45 and 5-85). However, it is observed that *B. rotundifolia* tends to have two future possibilities, i.e., decreasing its habitat ranges under SSP2-45 and increasing under SSP5-85. Hence, conservation efforts should focus on maintaining large populations, through domestication, to counter the potential negative effects of climate change and other pressure to maintain the genetic diversity and viable populations of *Ziziphus* and *Balanites* in Ethiopia.

Chapter 6

6. General Discussions

The present study found significant morphology variabilty in *Balanites* and *Ziziphus*. Additionally, it identified potential pre-sowing treatments that improve seed germination of *Z. spina-christi*, *Z. mucronata*, and *B. aegyptiaca*. These changes in seed and plant morphology play a critical role in helping plants adapt to different microclimates (Soheili et al., 2022). Additionally, the study revealed that *Z. spina-christi* and *B. aegyptiaca* are highly effective against certain pathogenic bacteria. Habitat suitability models predict that *Z. spina-christi* and *B. aegyptiaca* have wider habitat ranges in Ethiopia compared to their respective sister species. Furthermore, these models project that increasing climate change (SSP5-85) is likely to favor *Z. spina-christi*, *B. aegyptiaca*, and *B. rotundifolia* in Ethiopia between 2062 and 2080. Therefore, it is important to consider morpho-physiological adaptations to propagate, domesticate, and conserve species in response to climate change (Villeneuve et al., 2016). Overall, considering the morphological and physiological adaptations of species to climate change in species conservation and management is crucial for ensuring the resilience of biodiversity. In relation to this, physiological traits play a significant role in species' adaptation to changing environmental conditions (Seebacher et al., 2023), while morphological diversity within species can enhance their resilience to environmental change (Schulte & Healy, 2022).

***Morphological characterization of Ziziphus and Balanites* in Ethiopia**

Environmental conditions, such as variability in geological, climatic, biome, and socio-cultural environments, affect the morphological and physiological traits of plants (Sultan, 1995; Robakowski et al., 2003; Pescador et al., 2015; Qi et al., 2020), while preserving their adaptability in diverse environments (Berli et al., 2013; Gratani, 2014; Qi et al., 2020). The present study showed that environmental variables, such as Bio01, Bio03, Bio07, Bio16, and Tpi, affect the morphological variability among the populations of *Balanites* (**Figure 60**). Similarly, environmental variables, mainly Bio03, Bio04, Bio06, Bio10, Bio14, Bio17, Bio19, elevation, and Tpi, determine the morphological variability in *Ziziphus* (**Figure 60**).

Figure 60. The specimens of *Z. spina-christi*, *Z. mucronata*, *Z. mauritania*, *Z. abyssinica*, *B. aegyptiaca*, *and B. rotundifolia.*

This study identified two district morphotype clusters of *B. aegyptiaca* (Cluster-2 and Cluster-3). Similarly, the populations of *B. rotundifolia* also formed two morphotype clusters (Cluster-4 and Cluster-5). The two population clusters of *B. aegyptiaca* are markedly differ mainly in their leaf width and pubescence, and spine length and density. Cluster-2 mainly characterized by having leaflet indumentum usually sparse and/or early glabrescent or minutely puberulous, while Cluster-3 is characterized by leaflet indumentum densely tomentellous. Thus, these findings verified the two varieties of *B. aegyptiaca* (i.e., Cluster-2 = *B. aegyptiaca* var. *pallida*; Cluster-3 = *B. aegyptiaca* var. *aegyptiaca*) described for Ethiopia by the Flora of Ethiopia (Hedberg & Edwards, 1989). The present study also indicated that *B. aegyptiaca* showed a remarkable intra-specific morphological variability compared to the other sister species. Similarly, studies showed that *B. aegyptiaca* is widely distributed and dispersed in wider ecological ranges in semi-arid and arid ecosystems and show variability that might be ecological adaptation driven. For instance, Sands (2001) recognized five varieties of *B. aegyptiaca* for specimens collected from Ethiopia, Somalia, Kenya, and Tanzania based on spine length, leaflet shape, indumentum

density of leaflet and flowers, and pedicel length. Similarly, Hamada et al. (2022) identified one and two varieties of *B. aegyptiaca* from Makkah (Saudi Arabia) and Egypt, respectively, using a comparative taxonomic study of the species based on morphological parameters such as leaflet shape, leaflet indumentum density, petiole length, etc. The current study also implicated the possible presence of taxa (or subspecies) under *B. rotundifolia*. However, the Flora of Ethiopia (Hedberg & Edwards, 1989) did not mention any taxonomic rank below the species of *B. rotundifolia*. Hence, this study invites further assessment of the phenetic classification of *B. rotundifolia* in the Flora of Ethiopia. Moreover, molecular phylogenetics is required to clearly check the genetic diversity *Balanites* as a whole.

The current study identified two distinct morphotype clusters for *Z. spina-christi* (Cluster-3 and Cluster-4) and *Z. mauritania* (Cluster-2 and Cluster-5). The two morphotypes of *Z. spina-christi* are related to the two varieties of Z. *spina-christi* (i.e., Z. *spina-christi* var. *mitissima* Chiov. and Z. *spina-christi* var. *microphylla* Hochst. ex A. Rich.) described by the Flora of Ethiopia (Hedberg & Edwards, 1989). The present study also indicated the existence of remarkable morphological variability within *Z. spina-christi* compared to the case in the other sister species. In this regard, Hedberg and Edwards (1989) also suggested that the wider presence and dispersion of *Z. spina-christi* and the occurrence of several morphotypes of *Z. spina-christi* could also be associated with the fact that the fruits are edible, and the stones are often dispersed by the local people and travelers. Dispersal of plant units by different vectors has a fundamental contribution in plant succession and co-existence by avoiding intraspecific competition, avoiding inbreeding and predation (Dirzo & Dominguez, 1986), and so affects the level of gene flow (Young et al., 1996), and influences local adaptations or speciation (Harrison & Hastings, 1996). A study by Zarei et al. (2021) also indicated the presence of higher genetic diversity within *Z. spina-christi* compared to the two other *Ziziphus* species (*Z. mauritiana* and *Z. nummularia*). The current study also indicated for the presence of possible taxonomic ranks below species level under *Z. mauritania*. However, the Flora of Ethiopia (Hedberg & Edwards, 1989) did not mention any taxonomic rank below the species of *Z. mauritania*. Likewise, this study invites further assessment of the phenetic and phylogenetic classification of *Z. mauritania* in the Flora of Ethiopia.

Seed germination of Ziziphus and Balanites under different pre-sowing treatments

This study demonstrated that the techniques of seed extraction and handling, and application of seed treatments would significantly enhance the seed germination of *Ziziphus* species (*Z. spina-christi* and *Z. mucronata*). For instance, cracking seed stone along with soaking in cold-water for a couple of days significantly enhanced the seed germination percentage with faster germination time when compared to uncracked seed stones. A similar finding was reported by Saied et al. (2008). A study report by Wondwossen Gebretsadik (2013), however, showed that higher germination percentage was achieved from seeds treated with hot water at 85°C left to cool at room temperature (25°C) for 12 hours, and soaked with 98% H_2SO_4 for 30 minutes. Nevertheless, it is essential to apply mechanical and biochemical treatments to the seed (or stones) in order to improve its ability to germinate in a shorter period of time. Similarly, for *Z. mucronata*, though a very low germination percentage (<20%) was observed, soaking of cracked seed stones with 98% H_2SO_4 as well as rubbing with sandpaper improved the germination capacity of *Z. mucronata*. This finding partly agrees with the report by Hassen et al. (2005) that scarification with sandpaper and soaking with Sulphuric acid for up to 20 minutes were the most effective techniques in breaking the seed dormancy of *Z. mucronata.* Azene Bekele-Tesemma (1993) also reported that cracking the seed stones and subsequently soaking them in cold water for at least six hours is suggested to enhance the germination of *Z. mucronata*. The seeds of *Z. mucronata* were generally observed to be less sensitive to water stress, without exhibiting noticeable marks of soaking impairment even after an extensive soaking period. This is possibly an adaptation strategy for species adapted to arid and semi-arid ecosystems, helping the seed dispersal along drainage lines after flash floods (Zietsman & Botha, 1987).

The fresh seeds of *B. aegyptiaca* are believed to germinate readily without treatments. But, germination can be enhanced through application of treatments (Elfeel, 2012). This study demonstrated that the seed germination capacity of *B. aegyptiaca* can be enhanced through application of some pre-sowing treatment techniques. For instance, soaking of the seeds in 98% H_2SO_4 and water generally enhance the seed germination capacity of *B. aegyptiaca*. On the other hand, Hall and Walker (1991) reported that cold and hot water enhance the seed germination of *B. aegyptiaca*. Similarly, Ahmed

et al. (2017) and Elfeel (2012) also reported that soaked with cold distilled water for 18 and 24 hours showed a higher germination percentage compared to seeds soaked for 48 hours. Moreover, Azene Bekele-Tesemma (1993, 2007) also recommended soaking the seed in cold water for 24 to increase the germination rate with 50–70%. Hence, the studies generally indicated that the soaking duration of the seeds of *B. aegyptiaca* is preferred to be less than 48 hours because over-soaking in both 98% H_2SO_4 and water (cold & hot) could damage in both the external and internal parts of the seeds, which ultimately damages the embryonic tissue, resulting in lower germination percentages (Aref et al., 2011; Ahmed et al., 2017). Therefore, increasing the soaking duration in both cold and hot water is very likely to decrease the germination percentage of *B. aegyptiaca* (Elfeel, 2012; Ahmed et al., 2017), which was also observed in our present study. Overall, for successful germination of the seeds of *B. aegyptiaca*, it is important to remove the mesocarp (i.e., the fleshy edible part of the fruit) in order to avoid the delay of germination (Elfeel, 2012). Furthermore, the types of sowing orientations in the soil substrate also affect the germination percentages. A horizontal orientation of the seed stalk of *B. aegyptiaca* is recommended to attain higher germination (Elfeel, 2012) which was the case applied in this study too. The findings of this study can be used as an input for future large-scale propagation, plantation, as well as commercialization of the products of *B. aegyptiaca* in Ethiopia. Overall, the current study indicated the potential of seed pre-sowing treatment technologies to enhance seed germination in *B. aegyptiaca*, which has greater implications for the conservation and sustainable utilization of the species in the semi-arid ecosystems of Ethiopia and beyond.

This study reveals that no pre-sowing treatments tested are able to enhance the seed germination capacity of *B. rotundifolia*. This might be related to the presence of abundant mesocarp along with soft endocarp. The presence of much mesocarp likely abrupt water and oxygen entrance affecting the embryonic physiology to germinate. On the other hand, the presence of softer and delicate endocarp increases the possibility of injury of embryo when applying pre-sowing treatments (i.e., either mechanical or biochemical). The fruits of *B. rotundifolia* are brittle and smooth on the outside, with a fibrous and oily endocarp and high moisture content. This probably helped the species maintain the sufficient moisture required for germinating in dryland. That is why the species is a very drought-resistant tree, even more so than *B.*

aegyptiaca (Beentje, 1994; Ruffo et al., 2002). Therefore, the outcome of >60% germination percentage without application of pre-sowing treatments showed that the species can be easily propagated when quality and mature seeds are used. Therefore, the application of treatments to the fruit (seed) of *B. rotundifolia* is not necessary (Ruffo et al., 2002). In this regard, plant propagation without treatment application is by far the most cost-effective approach.

Antibacterial properties of selected species of Ziziphus and Balanites

Z. spina-christi is the most common traditional medicinal plants used by the local communities and cultures in Ethiopia (Mirutse Giday & Tilahun Teklehaymanot, 2013; Asmare Talie et al., 2018). The current findings also demonstrated that Z. *spina-christi* has high degree of antimicrobial properties against a wide range of bacterial pathogens including *E. faecalis*, *P. aeruginosa, S. aureus, E. coli, S. typhimurium*, and *K. pneumoniae*. In this regard, previous studies by Al-Kaabi et al. (2021), Elaloui et al. (2022) and Al-Azawi et al. (2022) also reported a high degree of susceptibility of *Klebsiella sp., S. aureus*, *E. coli,* and *P. aeruginosa* against alcoholic extracts of leaves of *Z. spina-christi*. Therefore, further study of fractionation of the leaves extracts from *Z. spina-christi* could result in a higher level of potency at lower concentrations of fractionates. The high degree of potency of *Z. spina-christi* is likely emanate from the presence of multiple bioactive compounds such as alkaloids, sterols like β-sitosterol, flavonoids, triterpenoids, sapogenins, and saponins in it (Hussein, 2019; Taghipour et al., 2020; Abdulrahman et al., 2022).

Moreover, this study verified the traditional medicinal values of *B. aegyptiaca* claimed by the different communities in many parts of Ethiopia (Mirutse Giday & Tilahun Teklehaymanot, 2013; Tilahun Teklehaymanot, 2017; Ali Zeynu et al., 2021). *B. aegyptiaca* showed inhibitory effects against two pathogenic bacteria, namely *S. typhimurium* and *K. pneumoniae*. A study by Koko et al. (2017) also showed that a disc diffusion assay of leaves extracts of *B. aegyptiaca* showed inhibitory effects against *S. aureus*, *Bacillus subtilis, E. coli,* and *P. aeruginosa*. The antibacterial property of *B. aegyptiaca* is strongly related to the presence of saponins, phenolic acids, flavonoids, coumarins, alkaloids, and poly-sterols (Murthy et al., 2021).

Leaves extracts of *Z. mucronata* and *B. rotundifolia* did not show potency against the test bacterial strains up to the 500 mg/ml concentration. However, Nemudzivhadi and

Masoko (2015) reported that leaves extract of *Z. mucronata* showed inhibitory effects against *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* using the agar-well diffusion method. The difference in the outputs between the current and the above-mentioned reports might be due to the fact that accumulations of secondary metabolites with bioactive properties are highly dependent on environmental factors such as light, temperature, soil water, soil fertility, and salinity (Jan et al., 2021). The success of antimicrobial effects of isolated compounds from plant materials is dependent on the solvent used for extraction (Masoko et al., 2008). For example, Nemudzivhadi and Masoko (2015) reported that leave extract of *Z. mucronata* using hexane and chloroform showed higher inhibitory effects against *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa*.

Habitat suitability of Ziziphus and Balanites under climate change in Ethiopia

The SDMs under the current climate predicted that *Z. spina-christi* occurs in extensive habitat areas across the country, with projected expansion in its distribution ranges with the increasing changing climate scenarios in the future. The SSP5-85 climate scenario (1.9 ºC higher than SSP2-45) likely favours *Z. spina-christi* to expanding its habitat ranges. Hence, the finding of this study can be taken as an input for the restoration of dryland ecosystems in Ethiopia using *Z. spina-christi* as the key species. A study by Ksiksi et al. (2019) also revealed that the habitat suitability of *Z. spina-christi* will potentially increase its habitat distribution with climate change across the United Arab Emirate (UAE) in the 2050s and 2070s. Both SSP2-45 and SSP5-85 scenarios project the possibility of a warmer climate compared to the current average. The temperature-loving nature of *Z. spina-christi* with adaptive strategies for photosynthesis and drought under the changing climate open the windows of opportunity and ecological advantages to increase its habitat ranges under the changing climate (Zait & Schwartz, 2018). The distribution of *Z. spina-christi* in Ethiopia is mainly affected by pH, Bio17, Bio13, and elevation. Except elevation, the distribution of *Z. spina-christi* positively correlated with pH, Bio17 and Bio13. This finding provides very essential information for propagation and conservation of the species. A study by Ksiksi et al. (2019) using MaxEnt models indicated that the distribution of *Z. spina-christi* in UAE is mainly influenced by the Bio13, Bio19, and Bio12. Model outputs resulting from using one algorithm (e.g., only MaxEnt) cannot be reliable unless multiple models are run in ensemble to significantly reduce

potential biases in model performance and so increase the robustness of SDM models (Zhang et al., 2015). In fact, different provenances of the same species from diverse ecological ranges may show variable responses to environmental variables due to the adaptation evolution of the different ecotypes.

Similarly, under the current climate, the SDMs for *Z. mucronata* showed that it has narrower habitat ranges compared to *Z. spina-christi*. The increasing CO_2 under both emission scenarios will very likely negatively affect the distribution of *Z. mucronata* in the future. Therefore, it appeared that *Z. mucronata* is high sensitivity and low adaptive capacity to climate change. Its distribution is its distribution is largely affected by Clay, Sand, Silt and BD. Hence, species with high sensitivity and low adaptive capacity to climate change are most likely at risk of extinction (Dawson et al., 2011). For that reason, it is very important to consider selective or species-specific conservation (Ahrens et al., 2022), and management of *Z. mucronata* in nexus of climate change in the future.

The ensemble SDM under the current climate showed that the Ethiopian Rift Valley and a few areas of Gambella, Hararge, Bale, and Borena in Oromia and Somalia regions are suitable habitats for *B. aegyptiaca*, accounting for that 8.6% of the areas of Ethiopia. Similarly, Azene Bekele-Tesemma (2007) indicated that *B. aegyptiaca* normally occurs up to 1,800m altitude in the arid and semi-arid agroclimatic zones of the Rift Valley in Gamo Gofa, and in Sidama, Tigray, Wollo, Shewa, Gojam, Ilu Ababora, Arsi, and upland Hararge regions. Under the future climate of HadGEM3-GC31-LL both SSP2-45 and SSP5-85, *B. aegyptiaca* is expected to increase its habitat range to 8.8% and 9.5% of the area of Ethiopia in 2061–2080, respectively. The increasing in habitat suitability for *B. aegyptiaca* is possibly due to its morphological and physiological responses and adaptive strategies to cope with drought and increasing temperature, including a significant reduction of biomass, early stomatal closure with small changes in photosynthesis activities (Amer et al., 2002; Khamis et al., 2015). The distribution of *B. aegyptiaca* in Ethiopia is mainly determined by Bio07, and Bio15. Moreover, Orwa et al. (2009) also indicated that the habitats of *B. aegyptiaca* are mainly attributed with deep sands, sandy clay loams, sandy loams or clays with a physical limit of 0–2000 m altitude, 20–30°C mean annual temperature, and 250–1,200 mm mean annual rainfall. Therefore, it is

very essential to consider such environmental parameters and species-niche matching when considering forestry development and conservation of *B. aegyptiaca*.

Similarly, the ensemble SDM under the current climate indicated mainly the southern Ethiopian Rift Valley region is the most suitable habitat for *B. rotundifolia*, accounting for 3.1% of the total area of Ethiopia. Hence, habitat areas identified as suitable for *B. rotundifolia* should be protected for long term conservation and sustainable utilization. Under the future climate of HadGEM3-GC31-LL, the species is expected to decrease its habitat coverage to 2.2% of Ethiopia under SSP2-45, while increasing its habitat coverage to 3.8% of Ethiopia under SSP5-85. Hence, it can be generally inferred that *B. rotundifolia* will possibly increase its habitat coverage with increasing climate change at the end of the century. The distribution of *B. rotundifolia* is assumed to be affected by the Precipitation of Driest Quarter (Bio17) and annual precipitation (Bio12), and CEC to some extent. Generally, these findings can play important role in the conservation and management of *B. rotundifolia* under its natural habitat as well as *ex-situ*.

Overall, the present study emphasizes the importance of recognizing the interconnectedness between plant morphology, seed physiology, antimicrobial properties, and habitat suitability of *Ziziphus* and *Balanites* for the goals of biodiversity conservation and sustainable use. It demonestrates the importance of selecting, domesticating, and conserving phenotypically superior populations of both genera based on their respective habitat suitability. This selection should aim for optimal growth and production of *Ziziphus* and *Balanites*, including high-quality edible fruits, as well as enhancing the biosynthesis of bioactive compounds with medicinal value. Habitat conditions have a significant influence on plant morphology (Thuiller et al., 2010), while seed physiology also contributes to species' habitat affinities (Belluau & Shipley, 2018). Thefrefore, understanding these connections can help researchers assess how plants adapt to different habitats and respond to stressors such as drought through physiological traits (Belluau & Shipley, 2018). Moreover, these connections can also influence antimicrobial properties of *Ziziphus* and *Balanites*.

Chapter 7

7. General Conclusions and Recommendations

7.1 General Conclusions

This study clearly demonstrates the inter- and intra-specific morphological variability in *Balanites* and *Ziziphus* in Ethiopia. Morphological variability in *Balanites* and Ziziphus is mainly constrained by Bio01, Bio03, Bio04, Bio06, Bio07, Bio10, Bio14, Bio16, Bio17, Bio19, elevation, and Tpi. Therefore, the domestication, conservation, and management of *Ziziphus* and *Balanites* should consider suitable environmental variables that enhance sustainable production of the species. In relation to this, it is also recommended to consider innovative ways of maintaining the genetic quality of the genera by selecting phenotypically superior populations and domesticating them on a wider scale of ecological and social environment. Furthermore, it is recommended to study the genetic diversity of *Balanites* and *Ziziphus* using molecular approaches to support the current findings of phenetic study.

The current study also identified the best pre-sowing seed treatments that could enhance the germination of *Z. spina-christi*, *Z. mucronata*, and *B. aegyptiaca*. The cracked seed stones, coupled with soaking in water, and to some extent in 98% H_2SO4, generally enhance the germination of *Z. spina-christi* and *Z. mucronata*. The seed germination of *B. aegyptiaca* is observed to improve when soaked in 98% H_2SO4 and water (hot and cold). Yet, the untreated seeds of *B. aegyptiaca* and *B. rotundifolia* showed good germination (>50%), showing an implication of the high degree of regeneration and subsequent ecological succession under the natural conditions of recurrent climate change and habitat fragmentation. Overall, the findings of this study provide important inputs for the wider propagation and production of *Ziziphus* and *Balanites* to foster the restoration and rehabilitation of the dryland and pastoral communities in Ethiopia.

Moreover, the current study has confirmed that *Z. spina-christi* and *B. aegyptiaca* have significant antibacterial properties against pathogenic bacteria. Generally, the methanol extracts of the leaves of *Z. spina-christi* and *B. aegyptiaca* are more effective against the tested bacterial strains. Therefore, it is necessary to further

investigate the phytochemical composition of *Ziziphus* and *Balanites* in order to search for potential antibiotic agents from *Z. spina-christi* and *B. aegyptiaca*.

Furthermore, the results of the habitat suitability modeling for *Z. spina-christi*, *Z. mucronata, B. aegyptiaca,* and *B. rotundifolia* provide valuable insights into potential areas that should be prioritized for long-term conservation and sustainable use of these species in Ethiopia. The models and suitability maps generated in this study align well with the species' current distribution patterns and ecological characteristics. In Ethiopia, *Z. spina-christi* and *B. aegyptiaca* exhibit a broader distribution compared to their respective sister species. Environmental factors, such as pH, Bio17, Bio13, and Silt, strongly influence the distribution of *Z. spina-christi*, while Bio07 positively influences the distribution of *B. aegyptiaca*. Conversely, the distribution of *Z. mucronata* is primarily affected by clay, bulk density, silt, sand, and Bio16, while cation exchange capacity has a positive impact. Therefore, any efforts related to the propagation, plantation, domestication, and conservation of *Z. mucronata* should take into account its environmental suitability. Given the species' high sensitivity and low adaptability, a targeted conservation approach is crucial to address the decline of *Z. mucronata* populations in their natural habitats.

Overall, it is recommended to practice on-farm domestication and in-situ conservation of the species as a solution to the declining populations of each species in their natural habitats, which is primarily caused by changes in climate. The conservation efforts for both genera should primarily be focused on the areas where they are most commonly found, such as the *Vachellia-Commiphora* woodland and bushland, the Vachellia wooded grassland of the Ethiopian Rift Valley, and the *Combretum-Terminalia* woodland and wooded grassland areas of Ethiopia. In order to maintain the genetic diversity of Ziziphus and Balanites in their natural habitat, it is necessary to select and breed phenotypically superior populations of both genera. Additionally, the domestication and conservation of these populations should be undertaken based on their respective habitat suitability, as identified in the present study. This approach will ensure optimal growth and production of Ziziphus and Balanites, resulting in a greater quantity and quality of edible fruits that can supplement the nutritional needs of people. It has been observed that plants growing in suitable areas tend to produce higher quality and less dormant seeds, compared to seeds produced in nutrient-poor

areas. Furthermore, cultivating these species in their ideal habitat enhances their ability to biosynthesize and accumulate bioactive compounds that hold medicinal value. This, in turn, supports increased bioprospecting and commercialization of plant products derived from Ziziphus and Balanites, such as medicines and foods.

7.2 Recommendations

Following the findings of this study, the following recommendations are forwarded:

- Selection of phenotypically superior populations of *Ziziphus* and *Balanites* must be done for the further breeding and improvement of both genera, while maintaining their genetic diversity in their natural habitats.
- Most potential pre-sowing treatments identified in this study should be employed for the large-scale propagation and domestication of *Ziziphus* and *Balanites*.
- Further elucidation of the phytochemical composition of *Z. spina-christi* and *B. aegyptiaca* is required for the purpose of prospecting for antibiotic agents from them.
- Based on habitat suitability, on-farm domestication and in-situ conservation of the species are recommended to counter the depopulation of the species due to climate change and land use and land cover changes.

8. References

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9. Annexes

Annex 1. List of morphological characters and coding procedures used (adapted from Hedberg & Edwards, 1989; Sands, 2001; Islam & Simmons, 2006).

General characters		**Code**	**Coding procedure**	***Ziziphus* (13)**	***Balanites* (19)**
1	Growth habit type	HabTy	0 = shrub, 1= tree	√	√
2	Spine/ thorns presence	SpPre	0 = absent, 1 = present	√	√
Bark/stem characters					
3	Bark/stem color	BarkCol	0=Grey, 1=Grey to brown, 2= Grey to dark-brown, 3=Dark grey.	√	
4	Branchlets color	BrnchCol	0=Whitish, 1=Whitish to pale-yellow, 2=Yellowish, 3=Reddish-brownish, 4=Dark-brownish, 5=Orange-brown (rusty).	√	
Leaf characters					
5	Leaf (leaflet) surface pubescence	LSurPub	0 = abaxial pubescent, 1 = adaxial pubescent, 2 = abaxial pubescent-tomentose/ adaxial subglabrous, 3 = abaxial subglabrous / adaxial pubescent, 4 = abaxial glabrous/adaxial glabrous, 5 = abaxial subglabrous/adaxial glabrous, 6= abaxial subglabrous/adaxial subglabrous, 7 = only petiole and/or midrib (nerve) pubescent, 8 = densely pubescent (tomentose).	√	√
6	Leaf (leaflet) shape	LSh	0= oval (round), 1 = ovate, 2 = lanceolate, 3= cordate, 4= elliptical, 5 = spathulate, 6 = obovate, 7 = oblong/cylindrical, 8 = obcordate	√	√
7	Leaf (leaflet) margin	LMar	0 = entire, 1 = serrate/serrulate, 2 = crenate/crenulate, 3 = dentate, 4=serrulate/crenulate.	√	
8	Leaf marginal tooth	LMarT	0 = without tuft of fine hairs, 1= with tuft of fine hairs.	√	
9	Leaf (leaflet) apex shape	LASh	0 = acuminate, 1 = acute, 2 = rounded/ovate, 3 = mucronate, 4 = retuse, 5 = elliptical, 6 = spathulate, 7= obtuse, 8= cuneate	√	√
10	Leaf (leaflet) base shape	LBSh	0 = oval (round), 1= obtuse, 2 = sub-cordate/oblique, 3 = cordate, 4 = cuneate/acute/ acuminate.	√	√
11	Leaf (leaflet) base symmetry	LBSym	0 = All leaves symmetric, 1 = most leaves symmetric, 2 = most leaves asymmetric.	√	√

12	Stipule presence	StipPre	0 = absent, 1 = present.		√
13	Stipule persistence	StipPer	0 = none, 1 = deciduous, 2 = persistent.		√
14	Leaf (leaflet) length	LL	***Ziziphus***: 0 = less than 4.6cm, 1 = 4.6cm, 2 = 4.6–9.1cm, 3= greater than 9.1cm. ***Balanites***: 0 = less than 3.5cm, 1 = 3.5–6cm, 2= greater than 6cm.	√	√
15	Leaf (leaflet) width	LW	***Ziziphus***: 0 = less than 3cm, 1 = 3–5.4cm, 2 = greater than 5.4cm. ***Balanites***: 0 = less than 2.5cm, 1 = 2.5–4.3cm, 2 = greater than 4.3cm.	√	√
16	Petiole length	PL	0 = less than 2.9mm, 1 = 2.9–5.7mm, 2 = greater than 5.7mm.		√
17	Inter-node length	INL	0 = up to 2cm, 1 = greater than 2cm.		√
Flower/ fruit characters					
18	Flower merosity	FloM	0 = 3-merous (3-petal & 3-sepal), 1= 4-merous (4-petal & 4-sepal), 2 = 5-merous (5-petal & 5-sepal), 3= 4 or 5-merous occasionally, 4= 6-merous (6-petal & 6-sepal).		√
19	Ovary surface	OvSur	0 = naked (glabrous), 1= covered with hairs (sub-glabrous, pubescent).		√
20	Fruit shape	FrSh	0 = globose (round or oval), 1= ovate (conical), 2= elongated (elliptical).		√
Spines characters					
21	Spine complexity (density)	SpCom	0 = no spine, 1 = simple and less dense, 2 = complex, dense and occasionally with subordinate branches.		√
22	Spine bearing	SpB	0 = no spine, 1 = spines bearing flowers or normal leaves, 2 = spines not bearing flowers or normal leaves		√
23	Spine's length	SpL	0 = no spine, 1 = less than 7.7cm, 2 = 7.7–12.2cm, 3 = greater than 12.2cm.		√

Annex 2. Summary of the geographical distributions, and population coding procedures.

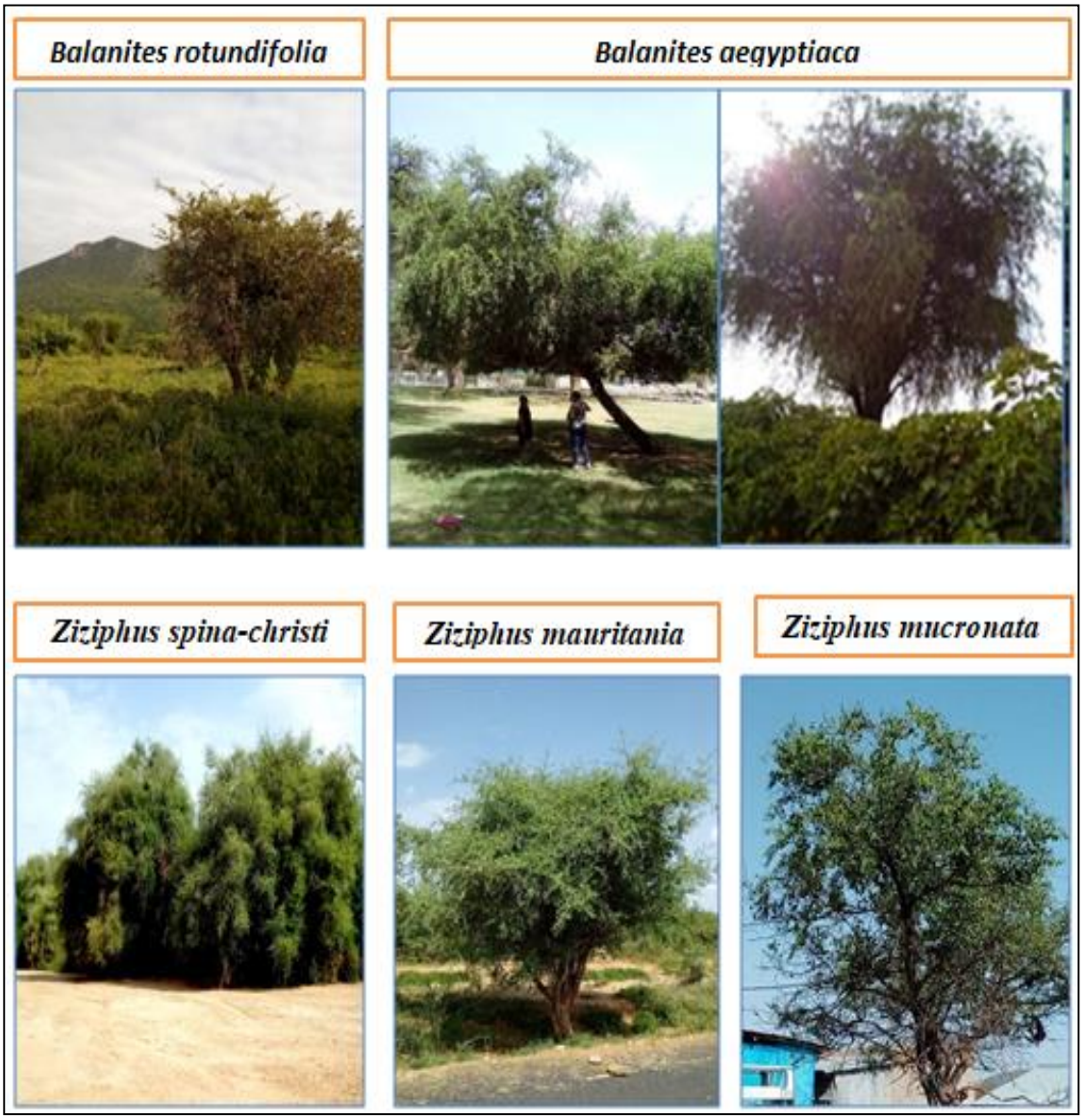
Population code	Woreda	Zone	Region	Lat. (Y)	Long. (X)
Z.aby_ArsNeg_W.Ars_Oro	Arsi-Negele	West Arsi	Oromia	7.4602777	38.680555
Z.aby_Boset_E.shew_Oro	Boset	East Shewa	Oromia	7.9658333	38.721944
Z.ham_SN633218_Liben_Som	Dollo	Liben	Somali	4.203049	42.074016
Z.mau_Das_S.Omo_SNP	Dasenech	South Omo	SNNPR	4.749	36.17329
Z.mau_Dima_Anuak_Gam_1	Dima	Anuak	Gambella	6.47332	35.06402
Z.mau_Dima_Anuak_Gam_2	Dima	Anuak	Gambella	6.43677	35.098
Z.mau_Dima_Anuak_Gam_3	Dima	Anuak	Gambella	6.48617	35.19855
Z.mau_Dima_Anuak_Gam_4	Dima	Anuak	Gambella	6.618	35.18161
Z.mau_Dima_Anuak_Gam_5	Dima	Anuak	Gambella	6.64146	35.18439
Z.mau_Ham_S.Omo_SNP_1	Hamer	South Omo	SNNPR	5.04247	36.49316
Z.mau_Ham_S.Omo_SNP_2	Hamer	South Omo	SNNPR	4.74564	36.34702
Z.mau_Ham_S.Omo_SNP_3	Hamer	South Omo	SNNPR	4.77128	36.40012
Z.mau_Ham_S.Omo_SNP_4	Hamer	South Omo	SNNPR	4.92805	36.46693
Z.mau_Ham_S.Omo_SNP_5	Hamer	South Omo	SNNPR	4.99703	36.47423
Z.mau_Humbo_Wolait_SNP_1	Humbo	Wolaita	SNNP	6.6963888	37.786666
Z.mau_Humbo_Wolait_SNP_2	Humbo	Wolaita	SNNP	6.5852777	37.838333
Z.mau_Kucha_Gam_SNP	Kucha	Gamo	SNNPR	6.54177	37.52843
Z.mau_M.Abay_Gam_SNP_1	Mirab-Abaya	Gamo	SNNP	6.3988888	37.743611
Z.mau_M.Abay_Gam_SNP_2	Mirab-Abaya	Gamo	SNNP	6.5252777	37.784444
Z.mau_Ofa_Wolait_SNP	Ofa	Wolaita	SNNPR	6.64538	37.57098
Z.mau_Zala_Wolait_SNP	Zala	Wolaita	SNNPR	6.3412	37.0739
Z.muc_A.MinZur_Gam_SNP_1	A.Minch Zuria	Gamo	SNNPR	6.12204	37.62743
Z.muc_A.MinZur_Gam_SNP_2	A.Minch Zuria	Gamo	SNNP	5.894401	37.492368
Z.muc_A.MinZur_Gam_SNP_3	A.Minch Zuria	Gamo	SNNP	5.7761111	37.449722
Z.muc_Afdem_Shini_Som_1	Afdem	Shinile	Somali	9.28526	40.82568
Z.muc_Afdem_Shini_Som_2	Afdem	Shinile	Somali	9.37621	40.92058
Z.muc_Alle_Kons_SNP_1	Alee	Konso	SNNP	5.415	37.205555
Z.muc_Alle_Kons_SNP_2	Alee	Konso	SNNP	5.3952777	37.233333
Z.muc_Bat_E.shew_Oro_1	Batu	East Shewa	Oromia	7.5302777	38.546388
Z.muc_Bat_E.shew_Oro_2	Batu	East Shewa	Oromia	8.1316666	38.808611
Z.muc_B.Tsem_S.Omo_SNP	Bena-Tsemay	South Omo	SNNP	5.4733333	36.788611
Z.muc_Boset_E.shew_Oro	Boset	East Shewa	Oromia	8.1058333	38.774722
Z.muc_Dima_Anuak_Gam	Dima	Anuak	Gambella	6.64147	35.18337
Z.muc_DireDawa_1	Dire-Dawa	Dire-Dawa	Dire-Dawa	9.52263	41.88146
Z.muc_DireDawa_2	Dire-Dawa	Dire-Dawa	Dire-Dawa	9.52263	41.88146
Z.muc_Fent_E.Shew_Oro	Fentale	East Shewa	Oromia	8.8283333	39.716388
Z.muc_Ham_S.Omo_SNP	Hamer	South Omo	SNNPR	4.96782	36.49123
Z.muc_Lome_E.shew_Oro	Lome(Mojo)	East Shewa	Oromia	8.5148	39.22408
Z.muc_Maeso_W.Har_Oro_1	Maeso	West Hararge	Oromia	9.25843	40.79266
Z.muc_Maeso_W.Har_Oro_2	Maeso	West Hararge	Oromia	9.17381	40.58287
Z.muc_Maeso_W.Har_Oro_3	Maeso	West Hararge	Oromia	9.15365	40.53701
Z.muc_UDT_Gofa_SNP_1	Ubadeberetsehay	Gofa	SNNPR	6.11977	36.9503
Z.muc_UDT_Gofa_SNP_2	Ubadeberetsehay	Gofa	SNNPR	6.19399	36.9647
Z.pub_SN63244_Anuak_Gam	Abobo	Anuak	Gambella	7.6166666	34.5
Z.spi_Adam_E.Shew_Oro_1	Adama	East Shewa	Oromia	8.54799	39.20774
Z.spi_Adam_E.Shew_Oro_2	Adama	East Shewa	Oromia	8.53156	39.2293
Z.spi_Adam_E.Shew_Oro_3	Adama	East Shewa	Oromia	8.53863	39.29878
Z.spi_Adam_E.Shew_Oro_4	Adama	East Shewa	Oromia	8.58546	39.34726
Z.spi_Afdem_Shini_Som_1	Afdem	Shinile	Somali	9.28513	40.8245
Z.spi_Afdem_Shini_Som_2	Afdem	Shinile	Somali	9.37655	40.92005
Z.spi_Afdem_Shini_Som_3	Afdem	Shinile	Somali	9.47564	41.00498
Z.spi_Afdem_Shini_Som_4	Afdem	Shinile	Somali	9.52079	41.09964
Z.spi_Afdem_Shini_Som_5	Afdem	Shinile	Somali	9.54898	41.18208
Z.spi_Asay_AwsRas_Afa_1_1	Asayta	Awsi Rasu	Afar	11.524166	41.471388
Z.spi_Asay_AwsRas_Afa_1_2	Asayta	Awsi Rasu	Afar	11.526388	41.470555
Z.spi_Bordede_W.Har_Oro	Bordede	West	Oromia	9.12714	40.47335

		Hararge			
Z.spi_Boset_E.shew_Oro_1	Boset	East Shewa	Oromia	8.64831	39.41765
Z.spi_Boset_E.shew_Oro_2	Boset	East Shewa	Oromia	8.6775	39.489722
Z.spi_Boset_E.shew_Oro_3	Boset	East Shewa	Oromia	8.6775	39.489722
Z.spi_Dima_Anuak_Gam_1	Dima	Anuak	Oromia	6.43635	35.11494
Z.spi_Erer_Shini_Som_1	Erer	Shinile	Somali	9.49122	41.26941
Z.spi_Erer_Shini_Som_2	Erer	Shinile	Somali	9.50946	41.31136
Z.spi_Erer_Shini_Som_3	Erer	Shinile	Somali	9.52699	41.34547
Z.spi_Erer_Shini_Som_4	Erer	Shinile	Somali	9.54446	41.45547
Z.spi_Erer_Shini_Som_5	Erer	Shinile	Somali	9.57136	41.53803
Z.spi_Fent_E.Shew_Oro_1	Fentale	East Shewa	Oromia	8.8722222	39.775555
Z.spi_Fent_E.Shew_Oro_2	Fentale	East Shewa	Oromia	8.6891666	39.506388
Z.spi_Ham_S.Omo_SNP	Hamer	South Omo	SNNPR	4.83858	36.44756
Z.spi_Haramay_E.Har_Oro_1	Haramaya	East Hararge	Oromia	9.4847	41.91668
Z.spi_Haramay_E.Har_Oro_2	Haramaya	East Hararge	Oromia	9.49178	41.90239
Z.spi_Haramay_E.Har_Oro_3	Haramaya	East Hararge	Oromia	9.50541	41.8866
Z.spi_Maeso_W.Har_Oro_1	Maeso	West Hararge	Oromia	9.24908	40.77328
Z.spi_Maeso_W.Har_Oro_2	Maeso	West Hararge	Oromia	9.17336	40.58248
Z.ham_SN633219_Liben_Som	Dollo	Liben	Somali	4.2333333	42.05
Z.ham_SN63320_Boren_Oro	Moyale	Borena	Oromia	4.1	41.366666
Z.aby_SN63343_S.East_Tig	Hagere Selam	South Eastern	Tigray	13.516666	39.383333
Z.aby_SN63342_Anuak_Gam	Gambella	Anuak	Gambella	8.1666666	34.716666
Z.aby_SN63344_N.East_Tig	Tahtay Adiyabo	North Eastern	Tigary	14.183333	37.583333
Z.aby_SN63347_E.Goja_Amh	Baso Liben	East Gojam	Amhara	10	37.6
Z.pub_SN63247_Anuak_Gam	Abobo	Anuak	Gambella	7.991516	34.565373
Z.pub_SN63246_Anuak_Gam	Abobo	Anuak	Gambella	7.929014	34.54901
Z.pub_SN63250_Anuak_Gam	Gog	Anuak	Gambella	7.921505	34.141525
Z.pub_SN63219_Anuak_Gam	Gog	Anuak	Gambella	7.6166666	34.366666
Z.pub_SN63249_S.Omo_SNP	Hamer	South Omo	SNNP	5.4166666	36.216666
Z.pub_SN63252_Anuak_Gam	Abobo	Anuak	Gambella	7.757682	34.365076
Z.spi_Jilee_Oro_Amh_1	Jile Dhumuga	Oromo zone	Amhara	10.330428	39.9816
Z.spi_Farsi_Oro_Amh_1	Artuma Farsi	Oromo zone	Amhara	10.548934	39.944424
Z.spi_Farsi_Oro_Amh_2	Artuma Farsi	Oromo zone	Amhara	10.615781	39.920059
Z.spi_Kewt_N.Shew_Amh_1	Kewt	North Shewa	Amhara	10.021353	39.92855
Z.spi_Jilee_Oro_Amh_2	Jile Dhumuga	Oromo zone	Amhara	10.055809	39.947645
Z.spi_Jilee_Oro_Amh_3	Jile Dhumuga	Oromo zone	Amhara	10.192747	39.979449
Z.spi_Kewt_N.Shew_Amh_2	Kewt	North Shewa	Amhara	9.906434	39.837258
Z.spi_Kewt_N.Shew_Amh_3	Kewt	North Shewa	Amhara	9.919519	39.843844
Z.spi_Erebti_KilRas_Afa_1	Erebti	Kilbet Rasu	Afar	13.221304	40.054117
Z.spi_Erebti_KilRas_Afa_2	Erebti	Kilbet Rasu	Afar	13.254116	40.012155
Z.spi_G.Lafto_N.Wolo_Amh	Guba Lafto	North Wollo	Amhara	11.839351	39.623937
Z.spi_Kobo_N.Wolo_Amh_1	Kobo	North Wollo	Amhara	12.046844	39.632645
Z.spi_Habru_N.Wolo_Amh_1	Habru	North Wollo	Amhara	11.675703	39.903593
Z.spi_Habru_N.Wolo_Amh_2	Habru	North Wollo	Amhara	11.734844	39.856125
Z.spi_Habru_N.Wolo_Amh_3	Habru	North Wollo	Amhara	11.820023	39.792806
Z.spi_Kobo_N.Wolo_Amh_2	Kobo	North Wollo	Amhara	12.130003	39.628119
Z.spi_Habru_N.Wolo_Amh_4	Habru	North Wollo	Amhara	11.762498	39.596275
Z.spi_Habru_N.Wolo_Amh_5	Habru	North Wollo	Amhara	11.68981	39.652044
Z.spi_Habru_N.Wolo_Amh_6	Habru	North Wollo	Amhara	11.608635	39.672308
Z.spi_Ambsel_N.Wolo_Amh_1	Ambasel	North Wollo	Amhara	11.544009	39.607877
Z.spi_Ambsel_N.Wolo_Amh_2	Ambasel	North Wollo	Amhara	11.457119	39.620553
Z.spi_Kobo_N.Wolo_Amh_3	Kobo	North Wollo	Amhara	11.958131	39.662532
Z.spi_Erebti_KilRas_Afa_3	Erebti	Kilbet Rasu	Afar	13.27898	39.854505
Z.spi_Erebti_KilRas_Afa_4	Erebti	Kilbet Rasu	Afar	13.310592	39.960544
Z.spi_Erebti_KilRas_Afa_5	Erebti	Kilbet Rasu	Afar	13.312285	39.981126
Z.spi_Tehule_S.Wolo_Amh_1	Tehuledere	South Wollo	Amhara	11.377138	39.645954
Z.spi_Tehule_S.Wolo_Amh_2	Tehuledere	South Wollo	Amhara	11.335332	39.676621
Z.spi_Habru_N.Wolo_Amh_7	Habru	North Wollo	Amhara	11.739802	39.855245
Z.muc_G.Lafto_N.Wolo_Amh	Guba Lafto	North Wollo	Amhara	11.885109	39.7072
B.aeg_A.MinZur_Gam_SNP_1	A.Minch Zuria	Gamo	SNNP	6.1097222	37.574444
B.aeg_A.MinZur_Gam_SNP_2	A.Minch Zuria	Gamo	SNNP	5.7761111	37.449722
B.aeg_Adar_AwsRas_Afa	Adaar	Awsi Rasu	Afar	11.585555	40.258611
B.aeg_Adam_E.Shew_Oro_1	Adama	East Shewa	Oromia	8.53565	39.22144
B.aeg_Adam_E.Shew_Oro_2	Adama	East Shewa	Oromia	8.5355	39.2216

B.aeg_Afdem_Shini_Som_1	Afdem	Shinile	Somali	9.28512	40.82555
B.aeg_Afdem_Shini_Som_2	Afdem	Shinile	Somali	9.30948	40.85315
B.aeg_Afdem_Shini_Som_3	Afdem	Shinile	Somali	9.34283	40.88668
B.aeg_Afdem_Shini_Som_4	Afdem	Shinile	Somali	9.37269	40.90837
B.aeg_Afdem_Shini_Som_5	Afdem	Shinile	Somali	9.43552	40.98116
B.aeg_Afdem_Shini_Som_6	Afdem	Shinile	Somali	9.47564	41.00499
B.aeg_Afdem_Shini_Som_7	Afdem	Shinile	Somali	9.50203	41.04451
B.aeg_Afdem_Shini_Som_8	Afdem	Shinile	Somali	9.54897	41.18227
B.aeg_ArsNeg_W.Ars_Oro_1	Arsi-Negele	West Arsi	Oromia	7.5397222	38.689722
B.aeg_ArsNeg_W.Ars_Oro_2	Arsi-Negele	West Arsi	Oromia	7.4602777	38.680555
B.aeg_B.Tsem_S.Omo_SNP_1	Bena-Tsemay	South Omo	SNNPR	5.50377	36.70976
B.aeg_B.Tsem_S.Omo_SNP_2	Bena-Tsemay	South Omo	SNNPR	5.46977	36.67359
B.aeg_B.Tsem_S.Omo_SNP_3	Bena-Tsemay	South Omo	SNNPR	5.32657	36.55381
B.aeg_B.Tsem_S.Omo_SNP_4	Bena-Tsemay	South Omo	SNNP	5.5772222	36.701666
B.aeg_B.Tsem_S.Omo_SNP_5	Bena-Tsemay	South Omo	SNNP	5.3744444	37.013055
B.aeg_B.Tsem_S.Omo_SNP_6	Bena-Tsemay	South Omo	SNNP	5.3966666	37.225555
B.aeg_Bat_E.shew_Oro_1	Batu	East Shewa	Oromia	7.9688888	38.721111
B.aeg_Bat_E.shew_Oro_2	Batu	East Shewa	Oromia	7.9808333	38.724444
B.aeg_Bat_E.shew_Oro_3	Batu	East Shewa	Oromia	8.0511111	38.717777
B.aeg_Bat_E.shew_Oro_4	Batu	East Shewa	Oromia	8.0941666	38.767222
B.aeg_Bordede_W.Har_Oro	Bordede	West Hararge	Oromia	9.06348	40.4165
B.aeg_Boset_E.shew_Oro_1	Boset	East Shewa	Oromia	8.8283333	39.716388
B.aeg_Boset_E.shew_Oro_2	Boset	East Shewa	Oromia	8.8069444	39.6725
B.aeg_Chfra_AwsRas_Afa_1	Chifra	Awsi Rasu	Afar	11.673333	40.000277
B.aeg_Chfra_AwsRas_Afa_2	Chifra	Awsi Rasu	Afar	11.618611	40.113333
B.aeg_D.Fango_Wolait_SNP	Diguma-Fango	Wolaita	SNNPR	6.93554	38.12734
B.aeg_D.Woyde_Wolait_SNP	Damot-Woyde	Wolaita	SNNPR	6.8772	37.93678
B.aeg_Deras_Kons_SNP_1	Derashe	Konso	SNNP	5.9788888	37.596111
B.aeg_Deras_Kons_SNP_2	Derashe	Konso	SNNP	5.8641666	37.411388
B.aeg_Deras_Kons_SNP_3	Derashe	Konso	SNNP	5.4902777	37.434444
B.aeg_Dima_Anuak_Gam_1	Dima	Anuak	Gambella	6.47333	35.0636
B.aeg_Dima_Anuak_Gam_2	Dima	Anuak	Gambella	6.43324	35.17637
B.aeg_Dima_Anuak_Gam_3	Dima	Anuak	Gambella	6.55876	35.18076
B.aeg_Dima_Anuak_Gam_4	Dima	Anuak	Gambella	6.59881	35.18589
B.aeg_Dima_Anuak_Gam_5	Dima	Anuak	Gambella	6.64147	35.18337
B.aeg_DireDawa	Dire-Dawa	Dire-Dawa	Dire-Dawa	9.5314	41.87879
B.aeg_Dodola_W.Ars_Oro_1	Dodola	West Arsi	Oromia	7.2597222	38.5075
B.aeg_Dodola_W.Ars_Oro_2	Dodola	West Arsi	Oromia	7.2561111	38.504722
B.aeg_Dodola_W.Ars_Oro_3	Dodola	West Arsi	Oromia	7.2788888	38.465
B.aeg_Dodola_W.Ars_Oro_4	Dodola	West Arsi	Oromia	7.2944444	38.395277
B.aeg_Erer_Shini_Som_1	Erer	Shinile	Somali	9.49014	41.27298
B.aeg_Erer_Shini_Som_2	Erer	Shinile	Somali	9.54444	41.45608
B.aeg_Erer_Shini_Som_3	Erer	Shinile	Somali	9.57142	41.53827
B.aeg_Erer_Shini_Som_4	Erer	Shinile	Somali	9.57136	41.53804
B.aeg_Erer_Shini_Som_5	Erer	Shinile	Somali	9.61041	41.63226
B.aeg_Fent_E.Shew_Oro_1	Fentale	East Shewa	Oromia	8.9186111	39.916111
B.aeg_Fent_E.Shew_Oro_2	Fentale	East Shewa	Oromia	8.9244444	39.8525
B.aeg_Fent_E.Shew_Oro_3	Fentale	East Shewa	Oromia	8.9297222	39.839166
B.aeg_Ham_S.Omo_SNP_1	Hamer	South Omo	SNNPR	5.13694	36.51731
B.aeg_Ham_S.Omo_SNP_2	Hamer	South Omo	SNNPR	4.99781	36.47393
B.aeg_Hawasa_Sidama_1	Hawassa	Hawassa	Sidama	8.52474	39.21437
B.aeg_Humbo_Wolait_SNP	Humbo	Wolaita	SNNP	6.6844444	37.786666
B.aeg_M.Abay_Gam_SNP_1	Mirab-Abaya	Gamo	SNNP	6.1894444	37.703333
B.aeg_M.Abay_Gam_SNP_2	Mirab-Abaya	Gamo	SNNP	6.5108333	37.78
B.aeg_Maeso_Shini_Som	Maeso	Shinile	Somali	9.01404	40.33185
B.aeg_Maeso_W.Har_Oro_1	Maeso	West Hararge	Oromia	9.26196	40.79818
B.aeg_Maeso_W.Har_Oro_2	Maeso	West Hararge	Oromia	9.17379	40.58303
B.aeg_Male_S.Omo_SNP	Malee	South Omo	SNNPR	5.85422	36.71789
B.aeg_Segen_Konso_SNP_1	Segen	Konso	SNNP	5.4555555	37.445
B.aeg_Segen_Konso_SNP_2	Segen	Konso	SNNP	5.4555555	37.445
B.aeg_UDT_Gofa_SNP_1	Ubadeberetsehay	Gofa	SNNPR	5.92461	36.76703
B.aeg_UDT_Gofa_SNP_2_1	Ubadeberetsehay	Gofa	SNNPR	5.99764	36.82414
B.aeg_UDT_Gofa_SNP_2_2	Ubadeberetsehay	Gofa	SNNPR	5.99764	36.82414

B.aeg_UDT_Gofa_SNP_3	Ubadeberetsehay	Gofa	SNNPR	6.19399	36.9647
B.glab_A.Tulu_W.Ars_Oro_1	Adami-Tulu	W.Arsi	Oromia	7.7952777	38.672222
B.glab_A.Tulu_W.Ars_Oro_2	Adami-Tulu	W.Arsi	Oromia	7.7036111	38.646666
B.glab_ArsNeg_W.Ars_Oro	Arsi-Negele	W.Arsi	Oromia	7.6636111	38.681666
B.rot_A.MinZur_Gam_SNP	A.Minch Zuria	Gamo	SNNP	5.7761111	37.449722
B.rot_Adar_AwsRas_Afa	Adaar	Awsi Rasu	Afar	11.585555	40.258611
B.rot_BT_S.Omo-SNP_1_1	Bena-Tsemay	South Omo	SNNP	5.4930555	36.755833
B.rot_BT_S.Omo-SNP_1_2	Bena-Tsemay	South Omo	SNNP	5.4930555	36.755833
B.rot_BT_S.Omo_SNP_2	Bena-Tsemay	South Omo	SNNP	5.4730555	36.815833
B.rot_BT_S.Omo_SNP_3	Bena-Tsemay	South Omo	SNNP	5.4930555	36.755833
B.rot_Das_S.Omo_SNP_1	Dasenech	South Omo	SNNPR	4.76124	36.2487
B.rot_Das_S.Omo_SNP_2	Dasenech	South Omo	SNNPR	4.80648	36.1195
B.rot_Das_S.Omo_SNP_3	Dasenech	South Omo	SNNPR	4.749	36.17329
B.rot_Das_S.Omo_SNP_4	Dasenech	South Omo	SNNPR	4.75978	36.25886
B.rot_Deras_Kons_SNP_1	Derashe	Konso	SNNP	5.5672222	37.426944
B.rot_Deras_Kons_SNP_2	Derashe	Konso	SNNP	5.4902777	37.434444
B.rot_M.Abay_Gam_SNP_1	Mirab-Abaya	Gamo	SNNP	6.3075	37.768333
B.rot_M.Abay_Gam_SNP_2	Mirab-Abaya	Gamo	SNNP	6.3988888	37.743611
B.rot_M.Abay_Gam_SNP_3	Mirab-Abaya	Gamo	SNNP	6.5252777	37.784444
B.rot_M.Abay_Gam_SNP_4	Mirab-Abaya	Gamo	SNNP	6.1894444	37.703333
B.rot_UDT_Gofa_SNP_1	Ubadeberetsehay	Gofa	SNNPR	5.92465	36.76659
B.rot_UDT_Gofa_SNP_2	Ubadeberetsehay	Gofa	SNNPR	6.19399	36.9647
B.aeg_Jilee_Oro_Amh	Jile Dhumuga	Oromo zone	Amhara	10.055809	39.947645
B.aeg_Hbru_N.Wolo_Amh_1	Habru	North Wollo	Amhara	11.739802	39.855245
B.aeg_G.Laf_N.Wolo_Amh_1	Guba Lafto	North Wollo	Amhara	11.885109	39.7072
B.aeg_Kobo_N.Wolo_Amh_1	Kobo	North Wollo	Amhara	12.046844	39.632645
B.aeg_Jilee_Oro_Amh_1	Jile Dhumuga	Oromo zone	Amhara	10.192747	39.979449
B.aeg_Jilee_Oro_Amh_2	Jile Dhumuga	Oromo zone	Amhara	10.178261	39.97867
B.aeg_Kobo_N.Wolo_Amh_2	Kobo	North Wollo	Amhara	12.130003	39.628119
B.aeg_Hbru_N.Wolo_Amh_2	Habru	North Wollo	Amhara	11.608635	39.672308
B.aeg_G.Laf_N.Wolo_Amh_2	Guba Lafto	North Wollo	Amhara	11.877613	39.683926
B.rot_Erebti_KilRas_Afa	Erebti	Kilbet Rasu	Afar	13.254116	40.012155

Annex 3. The trees of *Balanites* and *Ziziphus* species observed from different ecosystems (Photo taken by Mohammed Adefa).



Annex 4. Growing seedlings of *Z. spina-christi* in nursery pots.

Annex 5. Growing seedlings of *Z. mucronata* in nursery pots.

Annex 6. Germination measurement of *B. aegyptiaca* under different pre-sowing treatments.

Factor	GP	R	MGT	MGR	CV_t	CVG	GI	U	Z	T_{10}	T_{25}	T_{50}	T_{75}	T_{90}	T_{10-90}	T_{25-75}	MDG	Peak value	G value
Ctrl	59	64	27.1	0.0	37.1	3.7	0.7	2.8	0.1	10.6	16.3	24.0	33.0	39.0	28.4	16.7	1.2	1.4	1.7
Ctrl	59	64	31.4	0.0	27.7	3.2	0.6	2.6	0.1	17.0	21.3	29.7	36.3	40.3	23.3	15.0	1.2	1.4	1.7
Ctrl	59	64	27.1	0.0	37.1	3.7	0.7	2.8	0.1	10.6	16.3	24.0	33.0	39.0	28.4	16.7	1.2	1.4	1.7
Ctrl	56	60	25.7	0.0	38.2	3.9	0.7	2.7	0.1	7.0	14.9	22.2	31.1	36.8	29.8	16.3	1.2	1.4	1.6
RuSP	67	72	30.5	0.0	28.8	3.3	0.6	2.6	0.1	16.0	20.5	28.0	35.5	40.0	24.0	15.0	1.4	1.6	2.2
RuSP	56	60	28.0	0.0	32.4	3.6	0.6	2.7	0.1	13.8	17.6	25.5	33.4	37.2	23.3	15.8	1.2	1.4	1.6
RuSP	52	56	28.0	0.0	33.6	3.6	0.6	2.7	0.1	13.7	17.2	25.5	33.8	37.3	23.7	16.7	1.1	1.3	1.4
RuSP	59	64	27.1	0.0	37.1	3.7	0.7	2.8	0.1	10.6	16.3	24.0	33.0	39.0	28.4	16.7	1.2	1.4	1.7
CW24h	59	64	24.3	0.0	34.9	4.1	0.8	2.6	0.1	7.4	14.7	21.3	29.0	34.0	26.6	14.3	1.2	1.6	2.0
CW24h	63	68	24.8	0.0	34.2	4.0	0.8	2.6	0.1	7.8	15.1	22.2	29.3	33.8	26.0	14.2	1.3	1.7	2.2
CW24h	70	76	28.0	0.0	34.2	3.6	0.8	2.8	0.1	12.4	17.6	25.5	33.4	38.3	25.9	15.8	1.5	1.7	2.4
CW24h	56	60	26.3	0.0	35.6	3.8	0.7	2.7	0.1	10.0	15.9	23.8	31.1	36.8	26.8	15.2	1.2	1.4	1.6
CW48h	63	68	26.2	0.0	39.3	3.8	0.8	2.8	0.1	7.8	15.1	22.2	32.4	38.8	31.0	17.3	1.3	1.5	1.9
CW48h	52	56	23.4	0.0	35.1	4.3	0.7	2.5	0.1	6.6	13.8	20.5	27.2	32.0	25.4	13.3	1.1	1.5	1.6
CW48h	48	52	23.8	0.0	33.1	4.2	0.6	2.5	0.1	8.8	15.1	20.5	27.4	32.3	23.5	12.3	1.0	1.4	1.4
CW48h	52	56	24.8	0.0	34.1	4.0	0.6	2.6	0.1	9.4	15.5	21.3	29.3	34.5	25.1	13.8	1.1	1.4	1.5
HW65d	59	64	27.1	0.0	37.1	3.7	0.7	2.8	0.1	10.6	16.3	24.0	33.0	39.0	28.4	16.7	1.2	1.4	1.7
HW65d	56	60	26.0	0.0	36.2	3.8	0.7	2.7	0.1	10.0	15.9	22.2	31.1	36.8	26.8	15.2	1.2	1.4	1.6
HW65d	59	64	27.4	0.0	35.2	3.7	0.7	2.7	0.1	13.8	16.8	24.0	33.0	39.0	25.3	16.3	1.2	1.4	1.7
HW65d	78	84	25.4	0.0	34.5	3.9	0.9	2.7	0.1	9.4	15.8	22.4	29.3	35.3	25.9	13.4	1.6	2.0	3.3
HW75d	81	88	27.3	0.0	29.5	3.7	0.9	2.6	0.1	14.5	18.5	24.3	31.1	36.0	21.5	12.6	1.7	2.1	3.5
HW75d	70	76	25.9	0.0	33.6	3.9	0.8	2.7	0.1	12.4	16.4	22.4	30.1	35.8	23.4	13.7	1.5	1.8	2.6
HW75d	70	76	25.6	0.0	37.0	3.9	0.9	2.7	0.1	8.6	15.2	22.2	31.1	36.5	27.9	15.9	1.5	1.8	2.6
HW75d	67	72	27.7	0.0	33.0	3.6	0.7	2.7	0.1	14.0	17.4	24.7	32.2	38.5	24.5	14.8	1.4	1.6	2.2
98HSO10m	74	80	24.5	0.0	32.5	4.1	0.9	2.6	0.1	9.0	15.5	21.8	28.7	34.0	25.0	13.2	1.5	2.0	3.1
98HSO10m	93	100	25.6	0.0	32.5	3.9	1.1	2.6	0.1	11.0	16.3	22.5	30.2	34.9	23.9	13.9	1.9	2.4	4.7
98HSO10m	89	96	25.5	0.0	33.7	3.9	1.1	2.5	0.1	10.6	15.5	22.3	30.5	35.0	24.4	15.0	1.9	2.3	4.3
98HSO10m	93	100	25.6	0.0	32.9	3.9	1.1	2.5	0.1	11.0	15.7	23.5	30.2	34.9	23.9	14.5	1.9	2.4	4.7
98HSO20m	89	96	25.7	0.0	29.8	3.9	1.0	2.5	0.1	13.4	17.0	23.0	29.3	34.0	20.6	12.3	1.9	2.4	4.4
98HSO20m	93	100	25.2	0.0	31.4	4.0	1.1	2.6	0.1	11.0	16.3	22.5	28.9	33.8	22.8	12.7	1.9	2.5	4.8

98HSO20m	78	84	25.1	0.0	30.5	4.0	0.9	2.5	0.1	13.1	16.3	22.4	28.9	32.9	19.8	12.7	1.6	2.1	3.5
98HSO20m	70	76	20.9	0.0	32.2	4.8	1.0	2.3	0.2	5.6	12.4	17.5	23.4	28.3	22.7	11.0	1.5	2.3	3.3

Key

Parameter	**Parameter description**	**Unit**
GP	Germination percentage	%
R	Relativized percentage	%
MGT	Mean germination time	day
MGR	Mean germination rate	day^{-1}
CV_t	Coefficient of variation of germination time	%, seed day^{-1}
CVG	Coefficient of velocity of germination	%
GI	Germination index	day
U	Uncertainity of germination process	bit
Z	Synchronization index	unit less
T_{10}	Time to 10% germination	day or hour
T_{25}	Time to 25% germination	day or hour
T_{50}	Time to 50% germination	day or hour
T_{75}	Time to 75% germination	day or hour
T_{90}	Time to 90% germination	day or hour
T_{10-90}	Time from 10 to 90% germination	day or hour
T_{25-75}	Time from 25 to 75% germination	day or hour
MGD	Mean daily germination Percent	%
Peak value	Peak value for germination	day^{-1} or $hour^{-1}$
G value	Germination value	

Annex 7. Germination measurement of *B. rotundifolia* under different pre-sowing treatments.

Factor	GP	R	MGT	MGR	CV_t	CVG	GI	U	Z	T_{10}	T_{25}	T_{50}	T_{75}	T_{90}	T_{10-90}	T_{25-75}	MDG	Peak value	G value
Ctrl	70	90	8.2	0.1	46.1	12.3	2.8	1.8	0.3	1.8	3.1	5.4	9.3	12.5	10.6	6.1	2.6	6.7	17.4
Ctrl	56	71	9.0	0.1	42.4	11.1	2.0	2.0	0.2	2.2	4.0	6.9	10.3	13.0	10.8	6.3	2.1	4.2	8.6
Ctrl	59	76	7.9	0.1	43.5	12.6	2.3	1.8	0.3	1.9	3.3	5.6	9.3	11.8	9.9	6.0	2.2	5.6	12.2
Ctrl	78	100	8.1	0.1	43.8	12.3	3.0	1.8	0.3	1.9	3.3	5.8	8.8	12.2	10.3	5.4	2.9	6.9	20.0
RuSP	48	62	7.1	0.1	36.2	14.1	2.1	1.5	0.4	1.7	2.9	4.7	7.8	9.7	8.0	4.9	1.8	5.2	9.3
RuSP	44	57	6.8	0.1	35.2	14.8	2.0	1.4	0.4	1.7	2.7	4.4	7.0	9.2	7.5	4.3	1.7	5.2	8.5
RuSP	44	57	7.6	0.1	43.4	13.2	1.8	1.7	0.3	1.8	3.0	5.7	8.6	10.7	8.9	5.6	1.7	4.4	7.3
RuSP	37	48	8.1	0.1	41.7	12.3	1.4	1.9	0.2	2.0	3.5	6.0	8.8	12.7	10.7	5.3	1.4	3.2	4.5
CW24h	37	48	6.8	0.1	30.9	14.7	1.6	1.4	0.4	1.8	3.0	4.9	6.9	9.8	8.0	3.9	1.4	4.2	5.7
CW24h	37	48	6.2	0.2	33.8	16.1	1.7	1.2	0.5	1.6	2.4	3.9	5.8	9.0	7.4	3.3	1.4	5.2	7.1
CW24h	56	71	7.7	0.1	40.9	13.0	2.2	1.8	0.3	1.9	3.1	5.4	8.3	10.5	8.6	5.1	2.1	5.2	10.7
CW24h	48	62	8.6	0.1	40.6	11.6	1.7	1.9	0.2	2.3	4.3	6.5	9.1	12.4	10.1	4.9	1.8	4.2	7.4
CW48h	33	43	7.4	0.1	47.5	13.4	1.4	1.7	0.3	1.7	2.8	4.6	7.6	11.4	9.7	4.8	1.2	3.7	4.6
CW48h	48	62	7.4	0.1	43.7	13.5	2.0	1.7	0.3	1.7	2.9	4.7	7.8	10.6	8.8	4.9	1.8	5.2	9.3
CW48h	67	86	7.2	0.1	34.6	14.0	2.8	1.5	0.3	1.8	3.0	5.5	7.7	9.7	7.9	4.7	2.5	6.7	16.5
CW48h	56	71	7.4	0.1	31.4	13.5	2.2	1.5	0.3	2.0	3.5	5.8	7.6	9.5	7.5	4.1	2.1	5.6	11.4
HW65d	48	62	7.2	0.1	46.0	14.0	2.1	1.6	0.4	1.7	2.6	4.3	7.6	10.6	8.9	5.0	1.8	5.9	10.6
HW65d	44	57	7.0	0.1	33.4	14.3	1.9	1.5	0.3	1.8	3.0	5.2	7.3	9.2	7.4	4.3	1.7	4.6	7.6
HW65d	44	57	7.3	0.1	42.5	13.6	1.9	1.6	0.3	1.8	3.0	5.2	7.3	10.4	8.6	4.3	1.7	4.6	7.6
HW65d	44	57	7.3	0.1	45.9	13.6	1.9	1.6	0.4	1.7	2.7	4.4	8.0	10.7	9.0	5.3	1.7	5.2	8.5
HW75d	30	38	8.5	0.1	42.7	11.8	1.1	1.9	0.2	2.1	3.7	6.5	9.5	11.8	9.7	5.8	1.1	2.4	2.6
HW75d	33	43	8.4	0.1	40.2	11.8	1.2	1.9	0.2	2.2	4.0	6.5	9.1	11.4	9.2	5.1	1.2	2.8	3.4
HW75d	44	57	8.9	0.1	41.5	11.2	1.6	2.0	0.2	2.2	4.0	7.0	10.0	12.6	10.4	6.0	1.7	3.4	5.5
HW75d	30	38	8.5	0.1	42.7	11.8	1.1	1.9	0.2	2.1	3.7	6.5	9.5	11.8	9.7	5.8	1.1	2.4	2.6
98HSO10m	59	76	8.1	0.1	41.4	12.3	2.3	1.8	0.3	2.1	3.7	6.0	9.5	11.8	9.7	5.8	2.2	5.6	12.2
98HSO10m	56	71	8.3	0.1	40.5	12.0	2.1	1.8	0.3	2.2	4.0	6.3	8.4	12.0	9.8	4.4	2.1	5.1	10.5

98HSO10m	30	38	8.5	0.1	42.7	11.8	1.1	1.9	0.2	2.1	3.7	6.5	9.5	11.8	9.7	5.8	1.1	2.4	2.6
98HSO10m	33	43	8.4	0.1	40.2	11.8	1.2	1.9	0.2	2.2	4.0	6.5	9.1	11.4	9.2	5.1	1.2	2.8	3.4
98HSO20m	44	57	7.6	0.1	39.9	13.2	1.8	1.7	0.3	2.0	3.4	5.6	7.4	10.4	8.4	4.0	1.7	4.6	7.6
98HSO20m	37	48	8.1	0.1	41.7	12.3	1.4	1.9	0.2	2.0	3.5	6.0	8.8	12.7	10.7	5.3	1.4	3.2	4.5
98HSO20m	33	43	8.4	0.1	40.2	11.8	1.2	1.9	0.2	2.2	4.0	6.5	9.1	11.4	9.2	5.1	1.2	2.8	3.4
98HSO20m	33	43	8.4	0.1	40.2	11.8	1.2	1.9	0.2	2.2	4.0	6.5	9.1	11.4	9.2	5.1	1.2	2.8	3.4

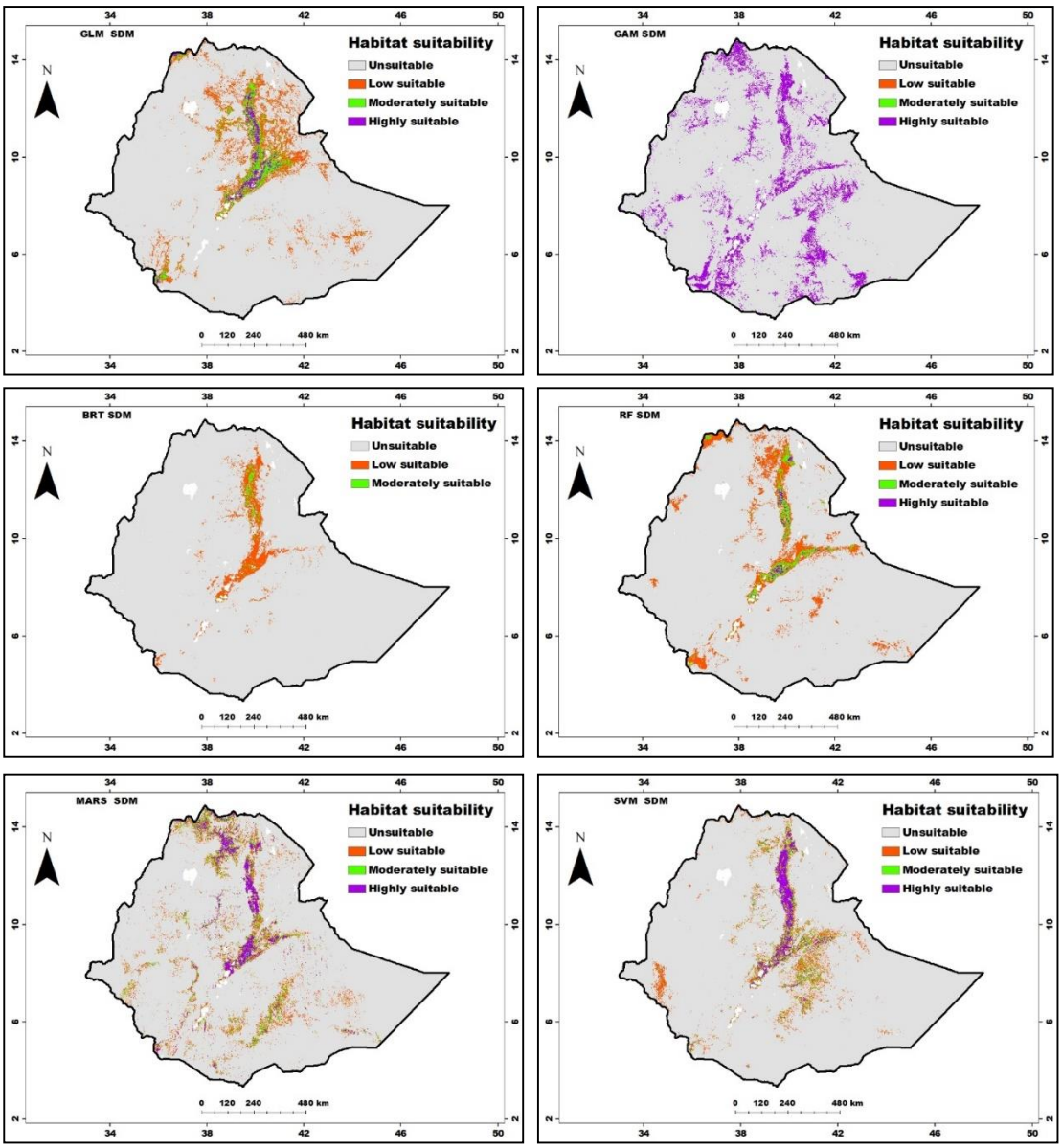
Key

Parameter	**Parameter description**	**Unit**
GP	Germination percentage	%
R	Relativized percentage	%
MGT	Mean germination time	day
MGR	Mean germination rate	day^{-1}
CV_t	Coefficient of variation of germination time	%, seed day^{-1}
CVG	Coefficient of velocity of germination	%
GI	Germination index	day
U	Uncertainity of germination process	bit
Z	Synchronization index	unit less
T_{10}	Time to 10% germination	day or hour
T_{25}	Time to 25% germination	day or hour
T_{50}	Time to 50% germination	day or hour
T_{75}	Time to 75% germination	day or hour
T_{90}	Time to 90% germination	day or hour
T_{10-90}	Time from 10 to 90% germination	day or hour
T_{25-75}	Time from 25 to 75% germination	day or hour
MGD	Mean daily germination Percent	%
Peak value	Peak value for germination	day^{-1} or $hour^{-1}$
G value	Germination value	

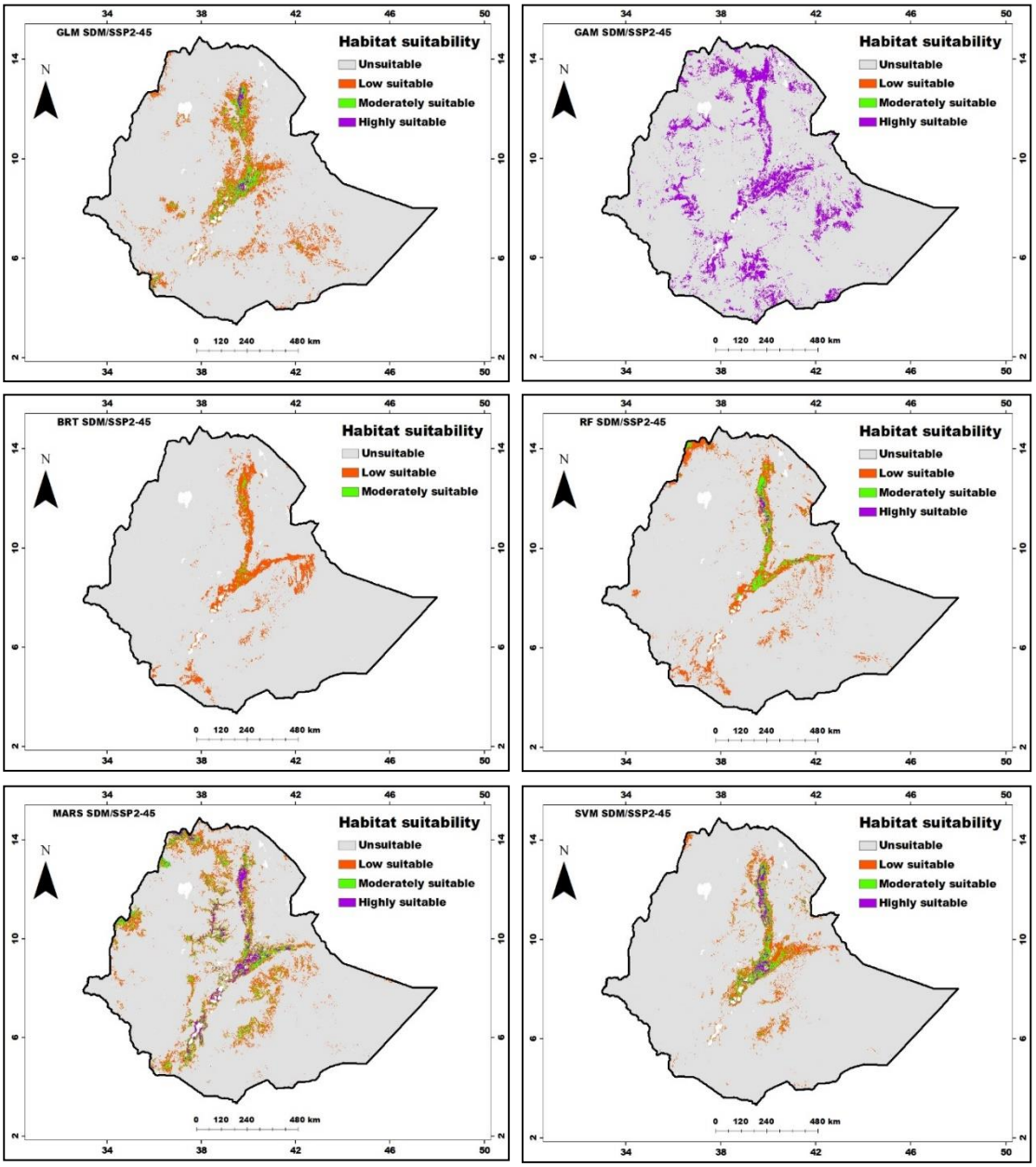
Annex 8. Growing seedlings of *B. aegyptiaca* under nursery pots.

Annex 9. Growing seedlings of *B. rotundifolia* in nursery pots.

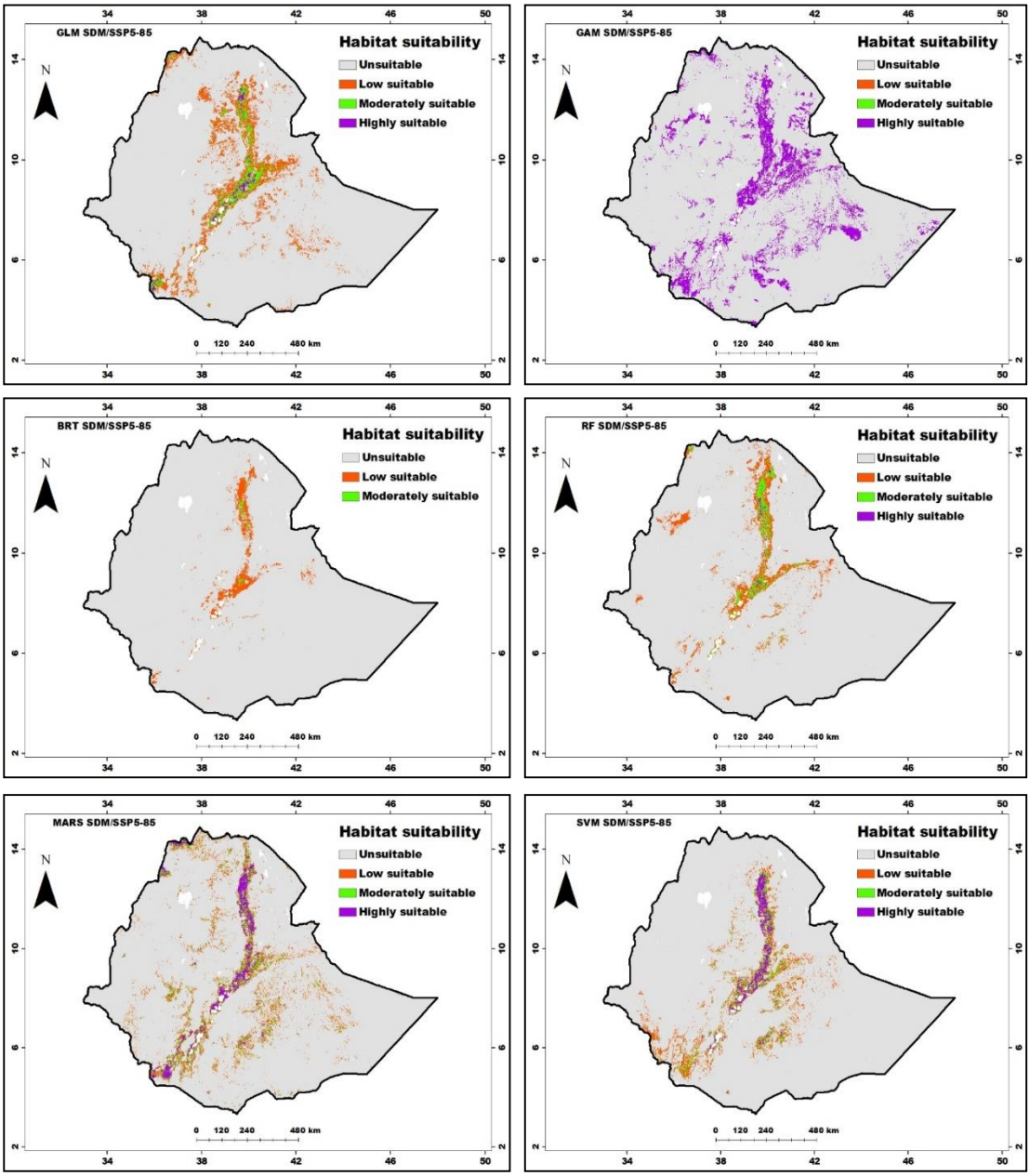
Annex 10. Current habitat suitability models of *Z. spina-christi* (GLM, GAM, BRT, RF, MARS and SVM algorithms).



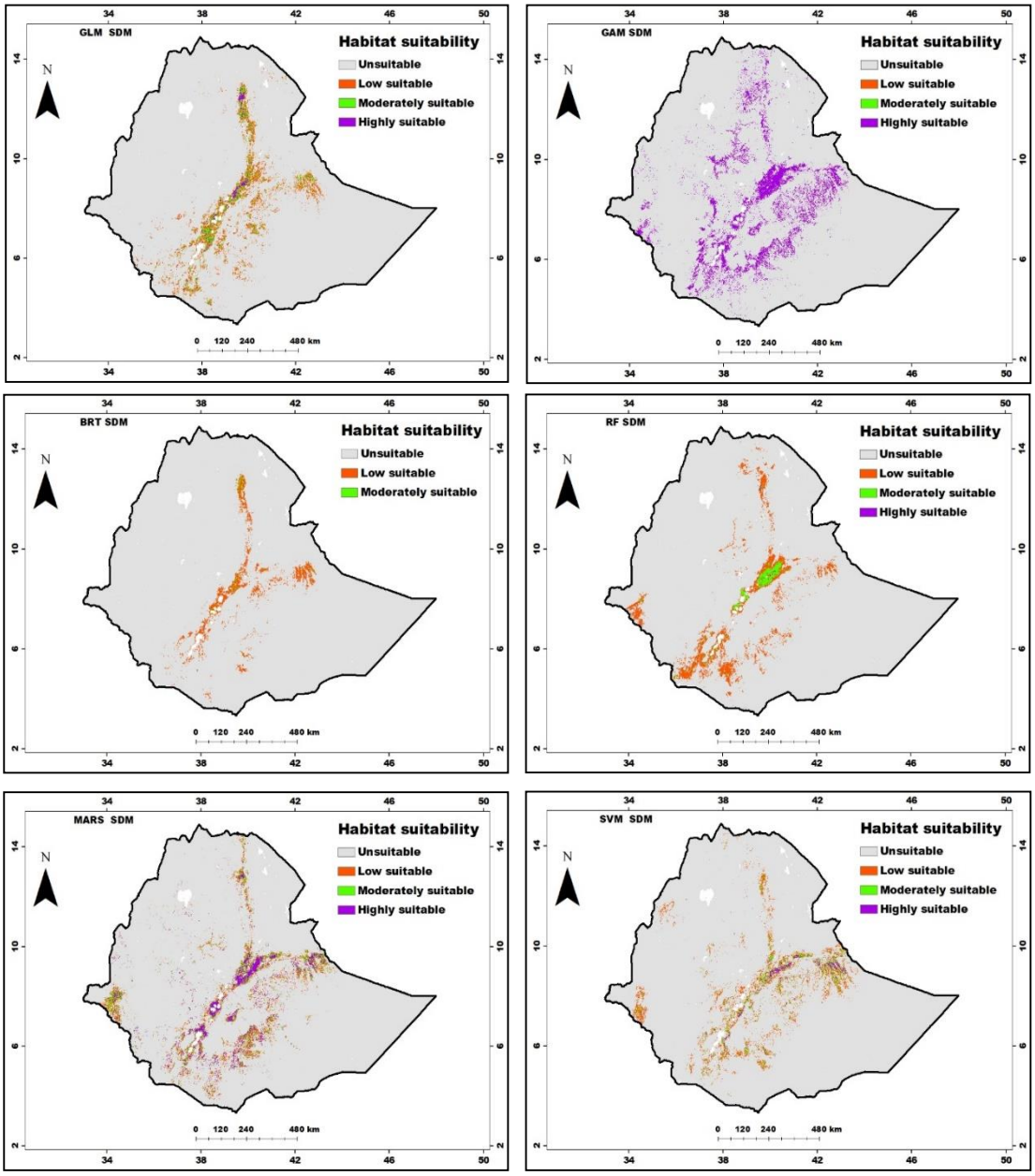
Annex 11. Projected habitat suitability models of *Z. spina-christi* under SSP2-45 of HadGEM3-GC31-LL (GLM, GAM, BRT, RF, MARS and SVM algorithms).



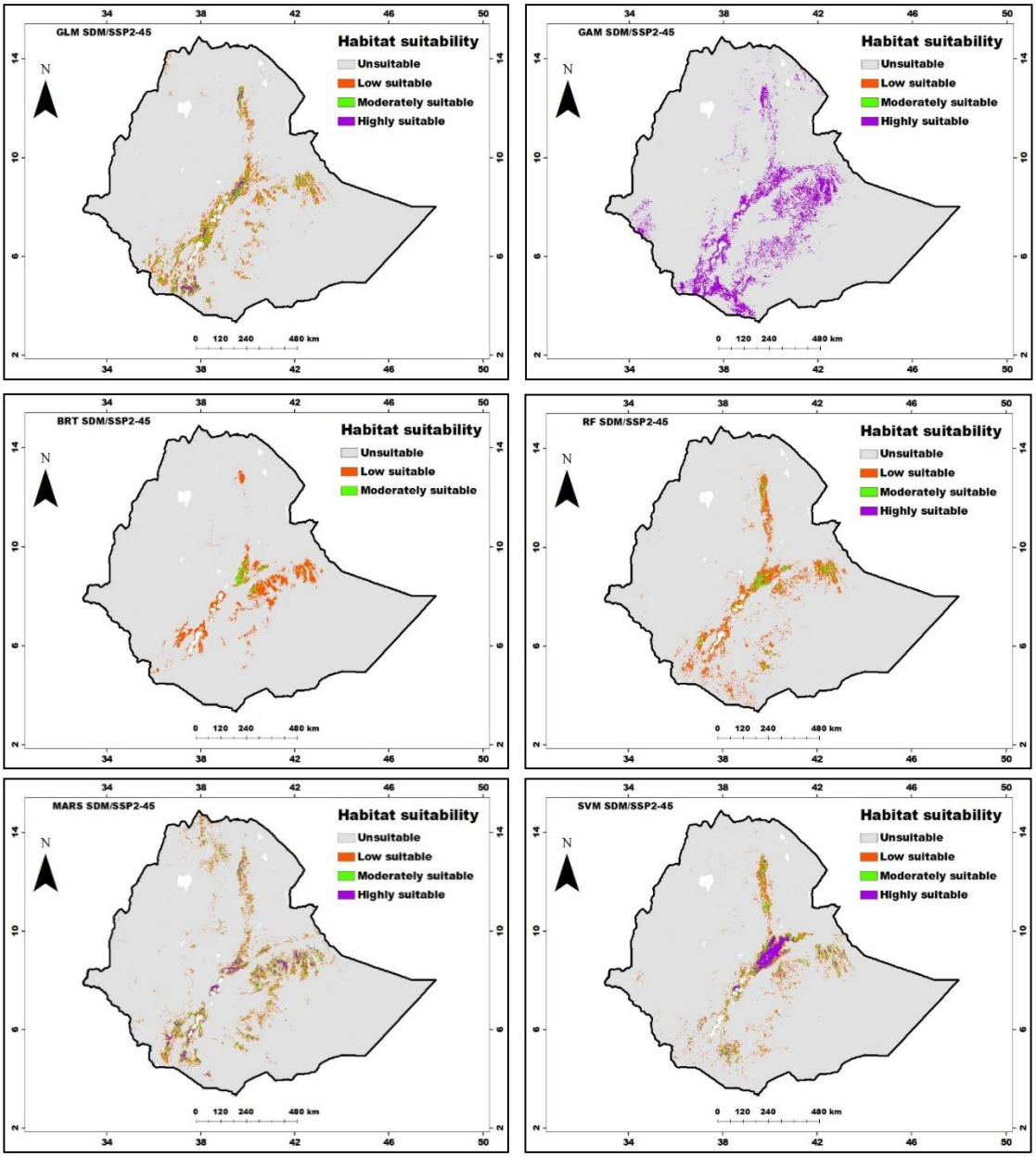
Annex 12. Projected habitat suitability models of *Z. spina-christi* under SSP5-85 of HadGEM3-GC31-LL (GLM, GAM, BRT, RF, MARS and SVM algorithms).



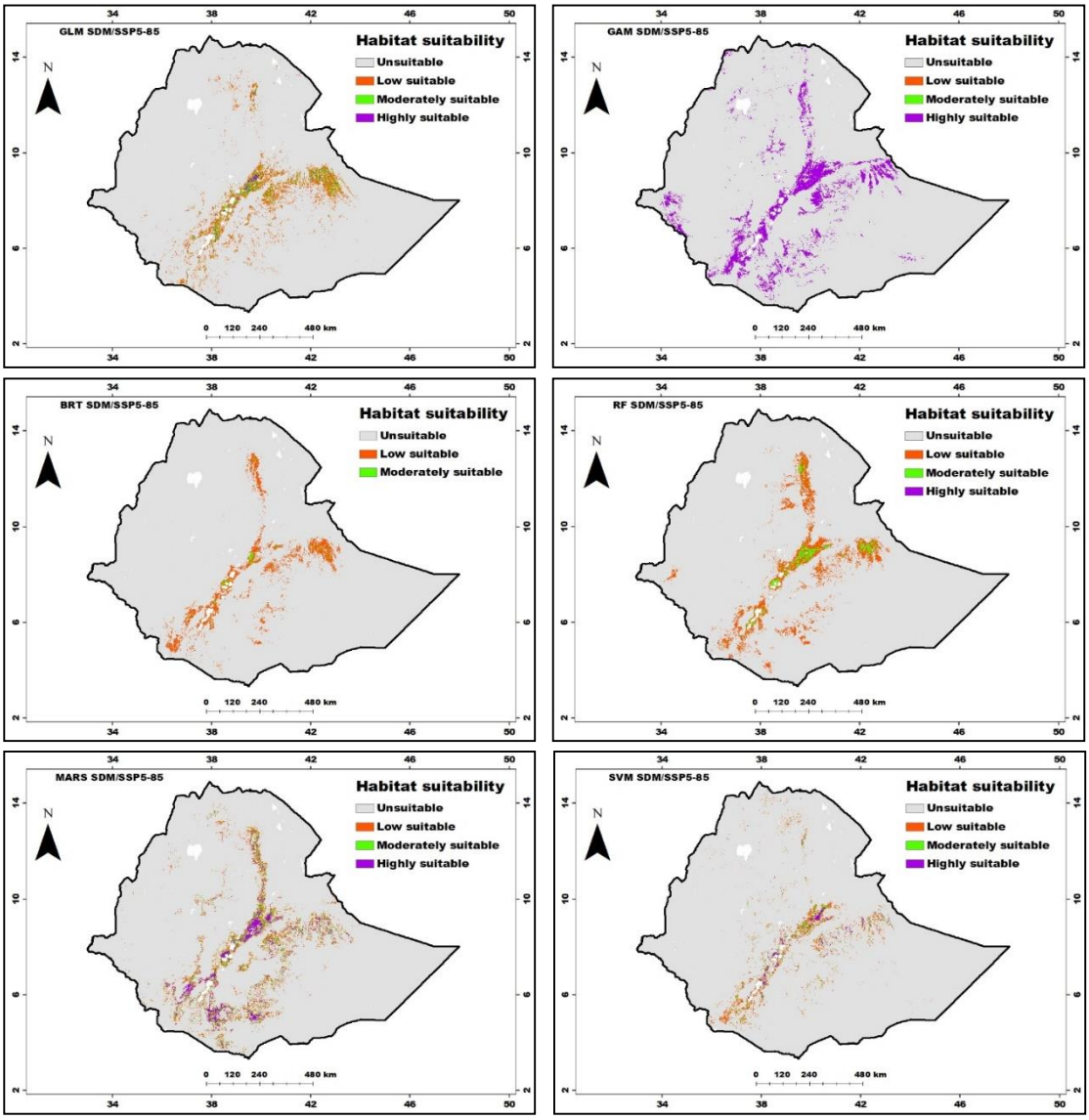
Annex 13. Current habitat suitability models of *Z. mucronata* (GLM, GAM, BRT, RF, MARS and SVM algorithms).



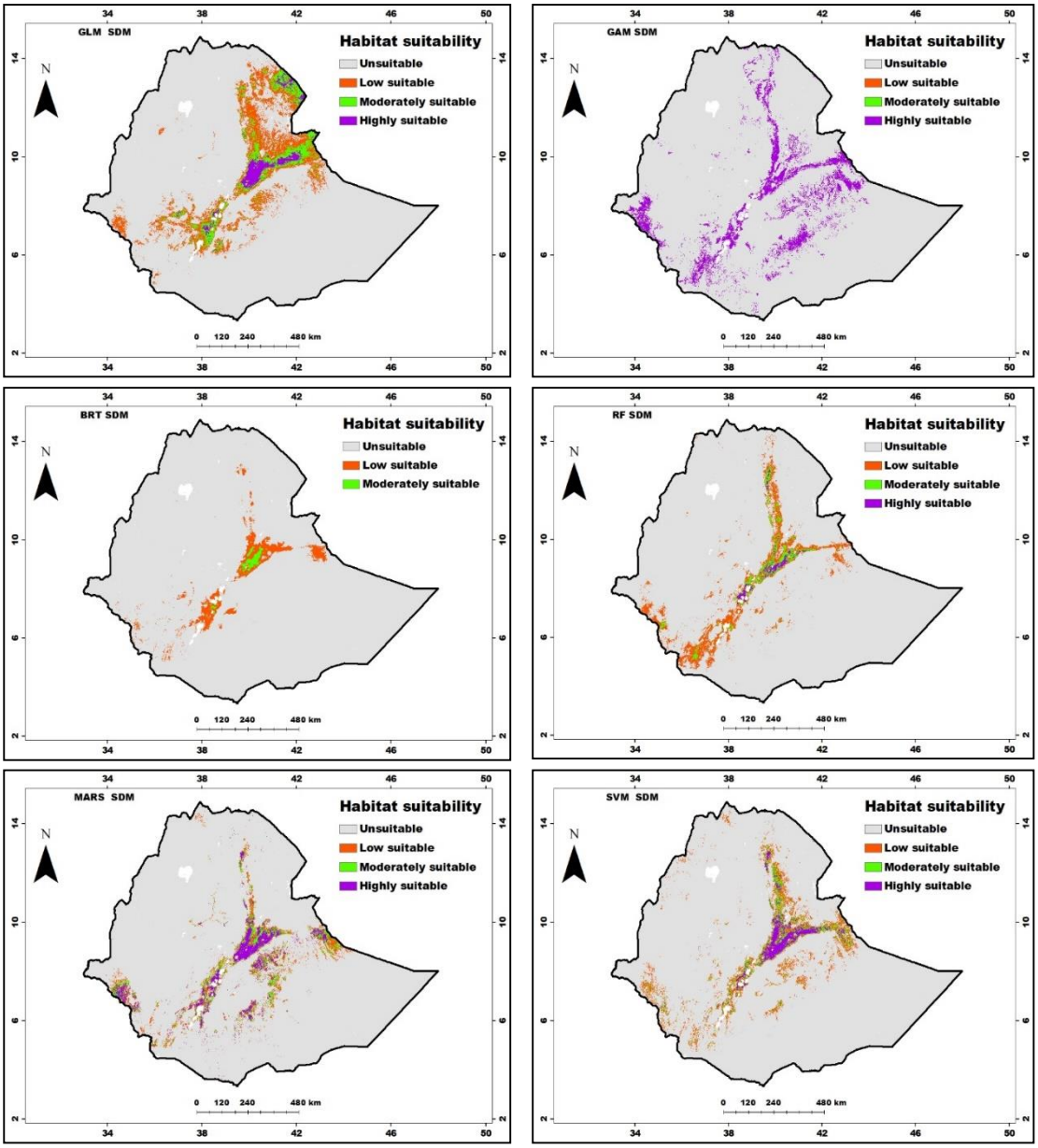
Annex 14. Projected habitat suitability models of *Z. mucronata* under SSP2-45 of HadGEM3-GC31-LL (GLM, GAM, BRT, RF, MARS and SVM algorithms).



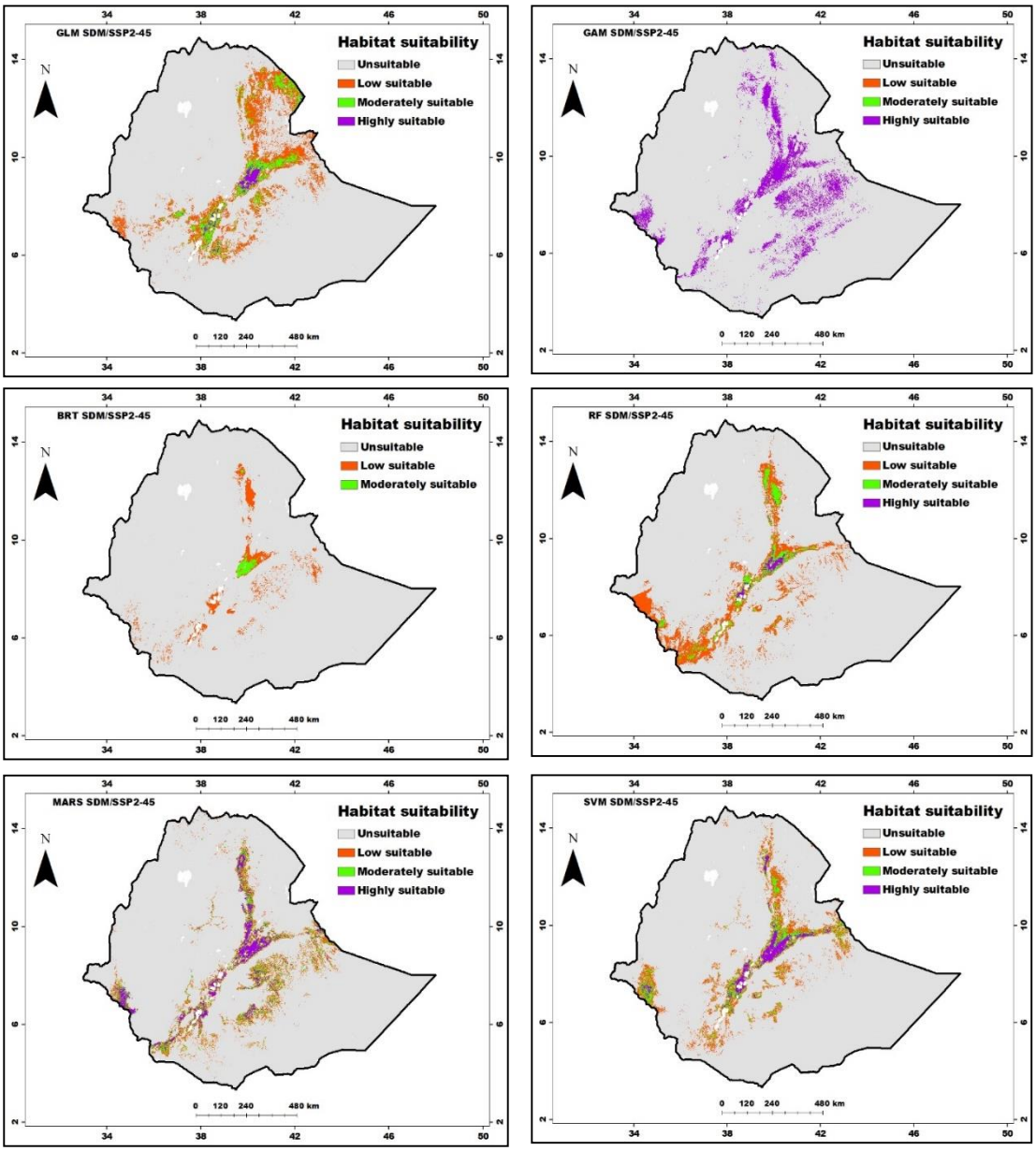
Annex 15. Projected habitat suitability models of *Z. mucronata* under SSP5-85 of HadGEM3-GC31-LL (GLM, GAM, BRT, RF, MARS and SVM algorithms).



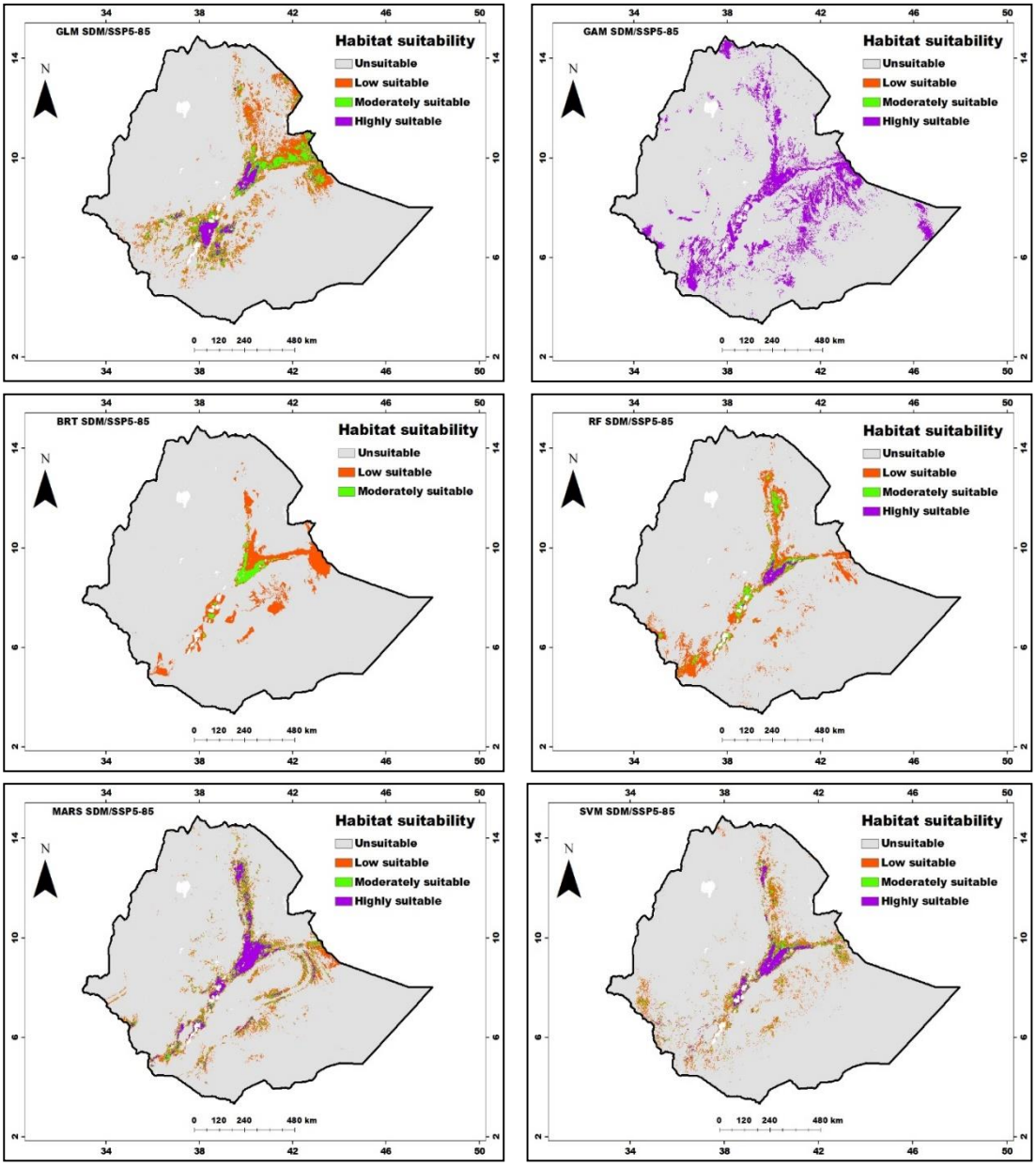
Annex 16. Current habitat suitability models for *B. aegyptiaca* in Ethiopia (GLM, GAM, BRT, RF, MARS and SVM algorithms).



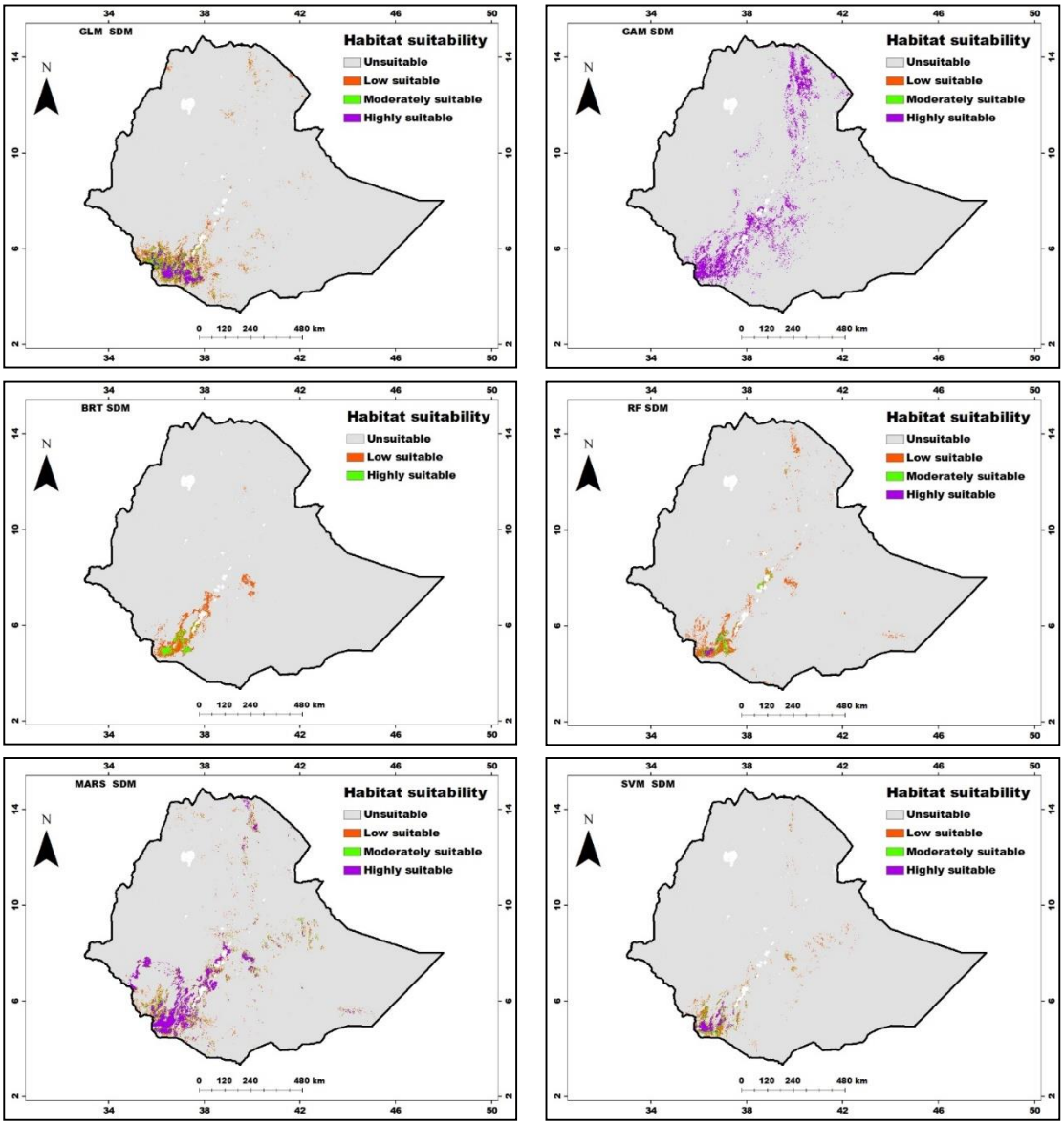
Annex 17. Future potential habitats of *B. aegyptiaca* under SSP2-45/ HadGEM3-GC31-LL climate change scenarios in Ethiopia (GLM, GAM, BRT, RF, MARS and SVM algorithms).



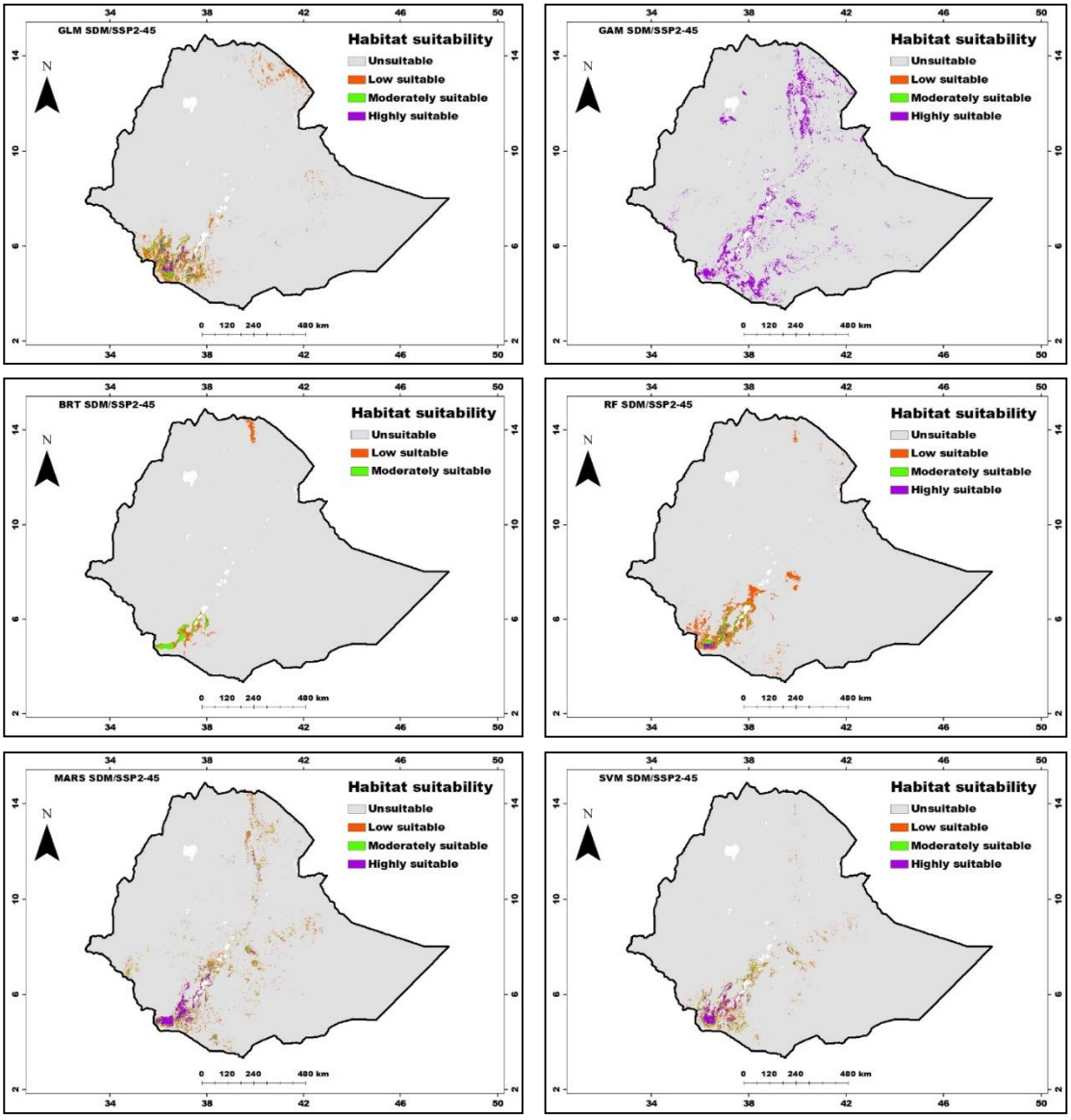
Annex 18. Projected habitat suitability models of *B. aegyptiaca* under SSP5-85/ HadGEM3-GC31-LL in Ethiopia (GLM, GAM, BRT, RF, MARS and SVM algorithms).



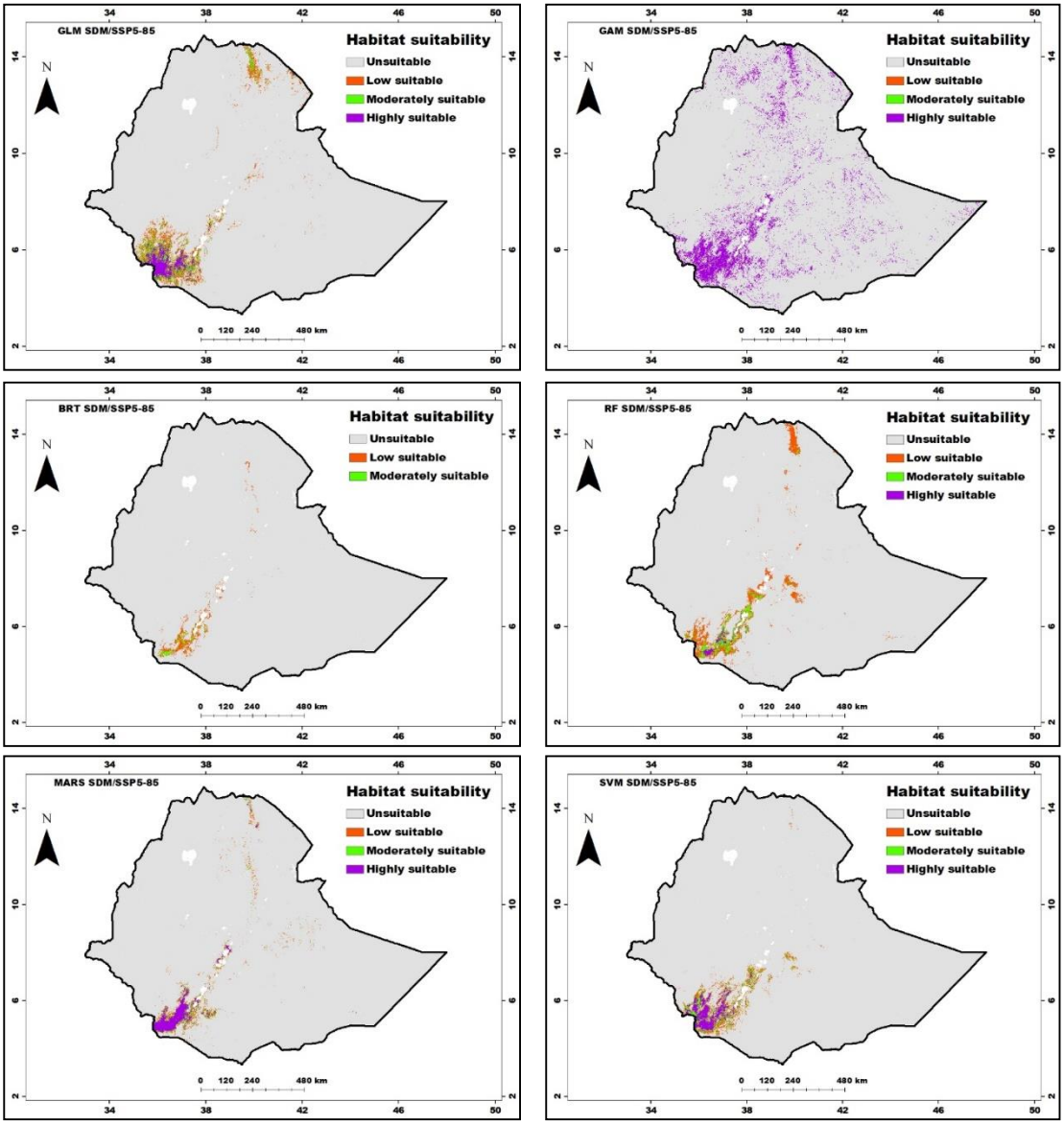
Annex 19. Current habitat suitability models of *B. rotundifolia* in Ethiopia (GLM, GAM, BRT, RF, MARS and SVM algorithms).



Annex 20. Future potential habitats of *B. rotundifolia* under SSP2-45/ HadGEM3-GC31-LL climate change scenarios in Ethiopia (GLM, GAM, BRT, RF, MARS and SVM algorithms).



Annex 21. Future potential habitats of *B. rotundifolia* under SSP5-85/ HadGEM3-GC31-LL climate change scenarios in Ethiopia (GLM, GAM, BRT, RF, MARS and SVM algorithms).



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Morphological Characterization, Seed Physiology, Antimicrobial Properties, and Habitat Suitability of Two Plant Genera (Zygophyllum Mill. and Balanites Del.) Co-occurring in Semi-Arid Ecosystems of Ethiopia

By

MOHAMMED ADEFA SEID

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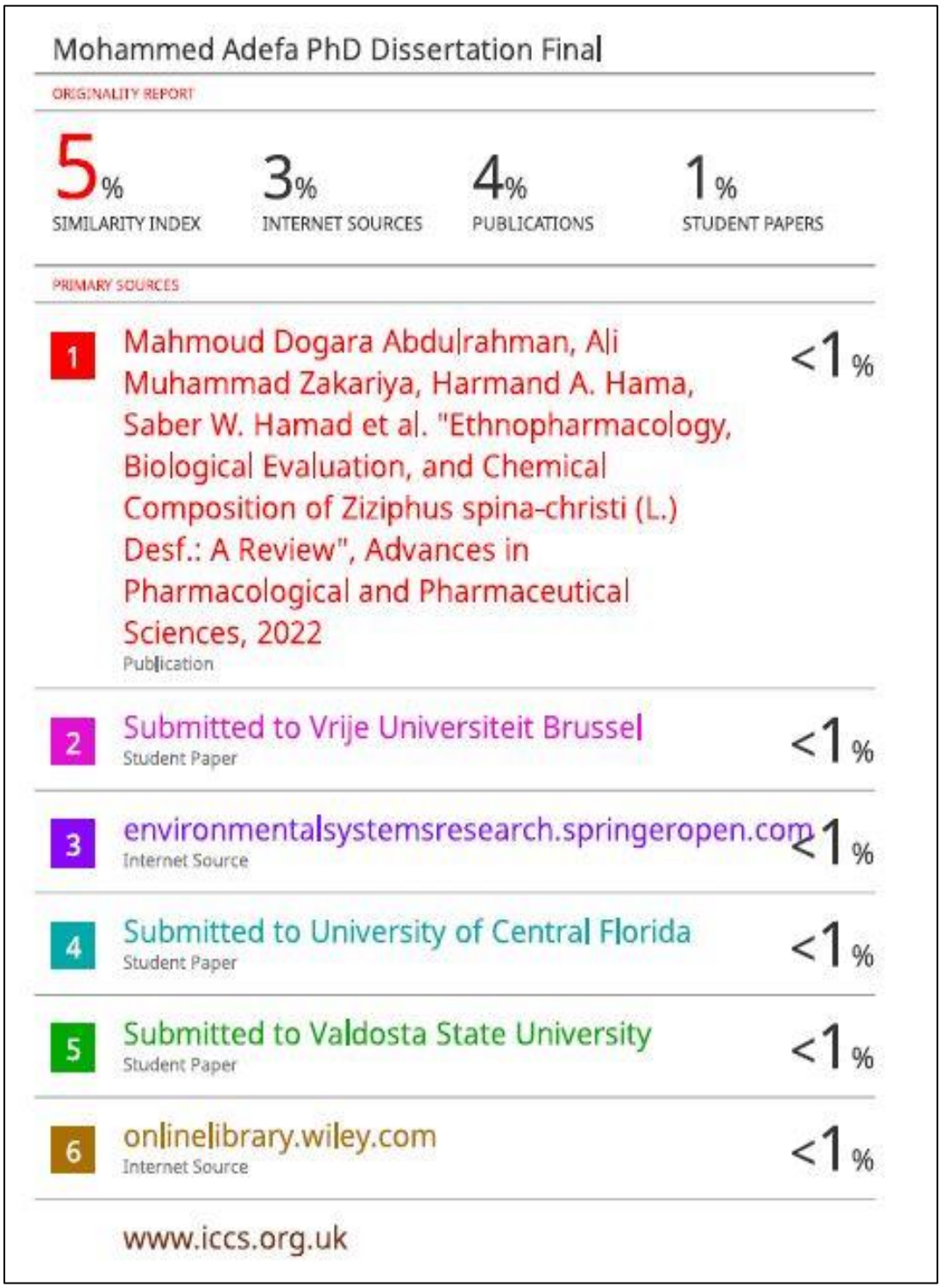

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10.3 Resume (curriculum vitae)

PERSONAL INFORMATION		
Full Name	**MOHAMMED ADEFA SEID**	
Educational profile	**Ph.D**. in Plant Biology and Biodiversity Management; **M.Sc**. in Tropical Biodiversity and Ecosystems; **M. Sc**. in Biology; **B. Sc**. in Biology	
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Orcid Id	*http://orcid.org/0000-0002-8972-7745*	
LinkedIn	*https://www.linkedin.com/in/mohammed-adefa-seid-8100bb69/*	
Scopus ID	*https://www.scopus.com/authid/detail.uri?authorId=57794690400*	

EDUCATIONAL BACKGROUND				
Degree	**Major subjects**	**Year attended**	**University**	**Final Grade/CGPA**
Ph.D.	Plant Biology and Biodiversity Management	2020-2024	Addis Ababa University	3.83/4.0 Dissertation = Excellent
M.Sc. (Joint)	Tropical Biodiversity and Ecosystems	2013-2015	ULB & VUB (Belgium, UniFI (Italy)	15.21/20 Thesis = very Good
M.Sc.	Biology	2008-2010	Bahir Dar University	4.00/4.00 Thesis = Excellent
B.Sc.	Applied Biology	2004-2007	Addis Ababa University	3.80/4.00

WORK EXPERIENCE

Position Held	*Service Year*	*Institution / Organization*
Researcher I (Tree seed and forestry research)	July 2023 – to date	Ethiopia Forestry Development (EFD)
Associate researcher (Tree seed and forestry research)	February 2019 – July 2023	Ethiopia Forestry Development (EFD) previously was known as the "Ethiopian Environment and Forest Research Institute (EEFRI)"
Associate researcher II (Ethnobotany)	Nov 2015 – June 2017	Ethiopian Biodiversity Institute (EBI)
Lecturer (Biology)	Sep 2007 – Aug 2013	Department of Biology, Arba Minch University

ADDITIONAL CERTIFICATES OF ACHIEVEMENTS

- Certificate of completion of "Knowledge Sharing Workshop on Landscape Restoration" jointly offered by EFD and IUFRO-SPDC, Nov. 2022.
- Certificate of completion of a training "Selection of individuals of forest species for wood production, conservation and management", jointly offered by EFD, Brazilian Agricultural Research Cooperation and Brazilian Cooperation Agency, June 2022.
- Certificate of completion of an online course "Research Writing in the Sciences (2022)", offered by *INASP* (an international development organisation based in the UK), May 2022.
- Certificate of completion of "Tissue culture training on Bamboo", offered by INBAR, August 2019.
- Certificate of completion for e-course "Introducing Conservation" awarded by "United for Wildlife", October 2017.
- Certificate of completion for e-course "UN-REDD++ e-academy", 2016
- Certificate of completion of an "Erasmus Mundus Masters Course in Tropical Biodiversity and Ecosystems – TROPIMUNDO", 2015
- Certificate of completion for e-course "UN:CC learning introductory on climate change", 2014
- Certificate of completion for "Higher Diploma Programme" as a "Certified Professional Education" in higher education, granted by Arba Minch University, 2010/2011.

AWARDS/GRANTS/SCHOLARSHIPS

- Tropimundo Erasmus Mundus Masters Course Scholarship in *Tropical Biodiversity and Ecosystems*: By European Union/Commission, 2013-2015
- Dean's Honor Award Certificate for Academic Excellence in Biology: Addis Ababa University, 2006
- The Aklilu Lemma fund for scholarships and research grants: Addis Ababa University, 2006

COMPETENCIES

Language proficiency	- **Amharic (***Mother Tongue*) - **English** (Excellent) - **Arabic** (Beginner)
Computer skills and Competences	- Basic and advanced computer skill - Software skills: R-stat, Python, ArcGis, Diva-GIS, SPSS, PAST, etc.

PUBLICATION (S)

♦ **JOURNAL ARTICLES:**

1. **Seid, M.A.**, Hailu, B.T., Wondimu, T., & Nemomissa, S. (2024). Modelling and Mapping Habitat Suitability for *B. aegyptiaca* (L.) Del. and *B. rotundifolia* (Tiegh.) Blatt. under Climate Change in Ethiopia. *International Journal of Ecology*, vol. 2024, Article ID 6656529, 18 pages. https://doi.org/10.1155/2024/6656529.
2. **Seid, M.A**., Wondimu, T., Degu, A., & Assefa, A. (2023). Seed Germination Enhancement of Two *Balanites* Species (*B. aegyptiaca* (L.) Del. and *B. rotundifolia* (Tiegh.) Blatt.) Using Different Presowing Treatments in Ethiopia. *Scientifica*, vol. 2023, Article ID 5571489, 10 pages. https://doi.org/10.1155/2023/5571489.
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