

**Morpho-Palynological and Seed Diversity of Invasive Plants
in Lesser Himalaya Pakistan**



By

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Quaid-i-Azam University, Islamabad
Pakistan
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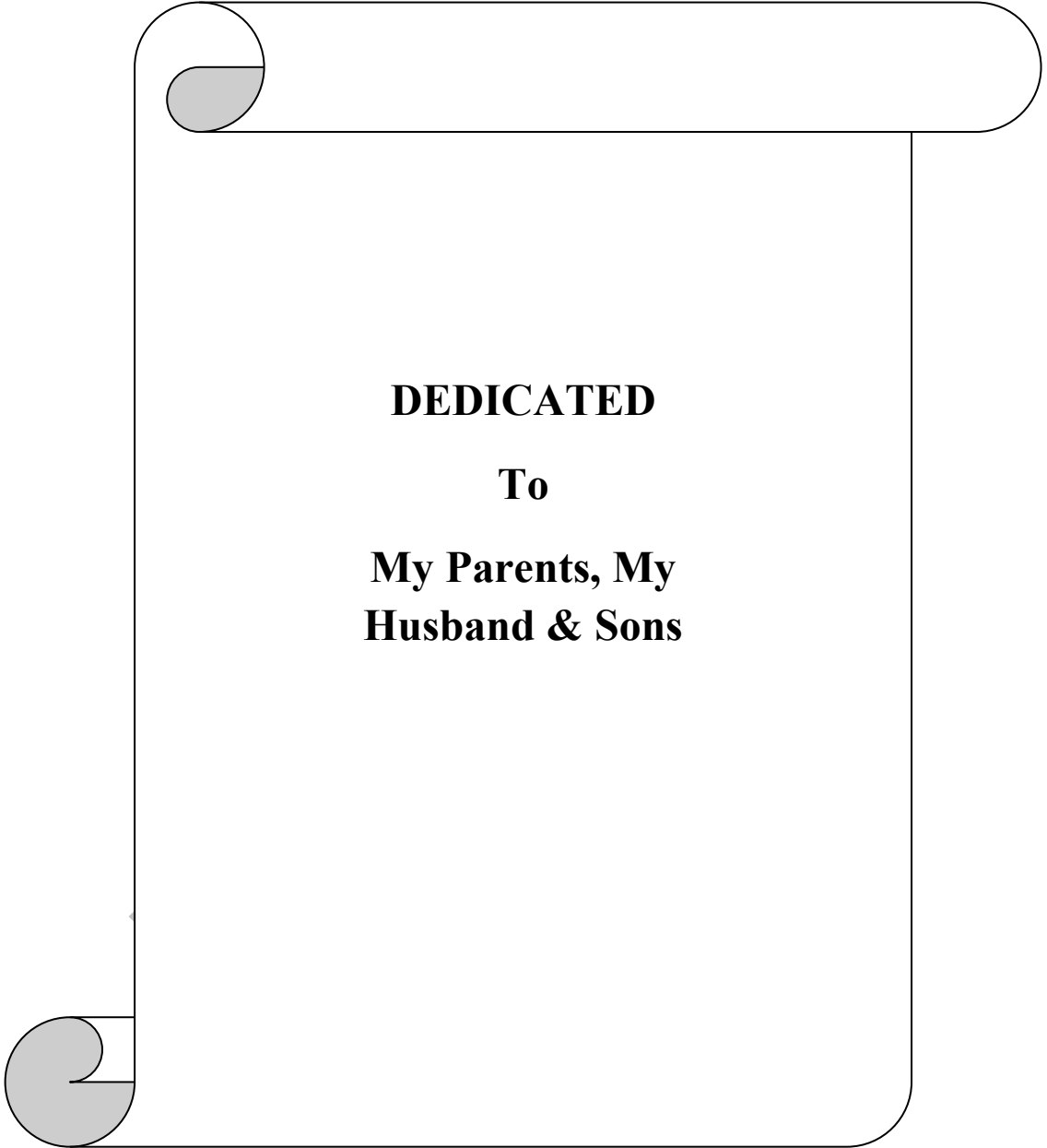
**Morpho-Palynological and Seed Diversity of Invasive Plants in
Lesser Himalaya Pakistan**



**A Thesis Submitted to the Quaid-i-Azam University in Partial
Fulfillment of the Requirements for the Degree of Doctor of
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**In
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**Department of Plant Sciences
Quaid-i-Azam University, Islamabad
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DEDICATED
To
My Parents, My
Husband & Sons

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Farida Anjum

Abstract

The present study planned to the systematic investigation of invasive floral diversity in Lesser Himalaya Pakistan using qualitative and quantitative micromorphological characterization of pollen and seeds. Plants were collected, dried, preserved, identified, mounted on herbarium sheets and deposited in the herbarium of Pakistan (ISL), Quaid-I Azam University, Islamabad. A total of 52 invasive species belonging to 31 families were reported in this study. The most dominant family was Asteraceae (11 species) followed by Fabaceae (04 species), Amaranthaceae, Verbenaceae (03 species each) and rest of the families contain each species respectively. The first section is confined to morpho-palynological characters studied using light and scanning electron microscope. The second section of this study is confined to macro and micro-morphological characters of seeds of invasive plants. The data analysis regarding invasiveness revealed that 29 species were introduced or established unintentionally, 13 species as ornamental, 1 species as food, 1 as fodder, 5 as plantation, and 3 for medicinal purposes. The findings indicates that most dominant life form was herb (31 species) followed by shrubs (11 species) and trees (8 species) while grasses and under shrubs were represented by only 1 species each i.e. *Sorghum halepense* (L.) Pers. and *Cassia occidentalis* L. The pollen micromorphological variations were observed for pollen shape and size, polar equatorial diameter, pore size, P/E ratio, aperture and exine thickness. The tricolporate pollen were most dominant and reported in *Ageratum conyzoides* L., *Anagallis arvensis* L., *Bidens pilosa* L., *Brachychiton acerifolius* (A.Cunn. ex G.Don) F.Muell., *Broussonetia papyrifera* (L.) L'Hér. ex Vent., *Carthamus oxyacantha* M.Bieb., *Cassia occidentalis* L., *Callistemon citrinus* (Curtis) Skeels, *Commelina benghalensis* L., *Lantana camara* L., *Melilotus indicus* (L.) All., *Oxalis corniculata* L., *Parthenium hysterophorus* L., *Prosopis juliflora* (Sw.) DC., *Quisqualis indica* L., *Ranunculus muricatus* L., *Ricinus communis* L., *Taraxacum officinale* (L.) Weber ex F.H.Wigg., *Tecoma stans* (L.) Juss. ex Kunth., *Tropaeolum majus* L., *Verbena officinalis* L., *Verbesina encelioides* (Cav.) Benth. & Hook.f. ex A.Gray. The tricolpate pollen were reported in *Bryophyllum pinnatum* (Lam.) Oken., *Cannabis sativa* L., *Convolvulus arvensis* L., *Datura innoxia* Mill., *Digera muricata* (L.) Mart. and *Jasminum humile* L. The third dominant pollen type was pantoporate which were observed in *Achyranthes aspera*

L., *Chenopodium ambrosioides* L., *Eucalyptus camaldulensis* Dehnh., *Malvestrum coromandelianum* (L.) Garcke., *Tribulus terrestris* L. Similarly, remarkable variation regarding exine sculpturing was observed among invasive plant species from Psilate, echinate, reticulate, psilate-perforate to scabrate. As far as size of pollen is concerned 45% pollen were moderate size, followed by the small (34%) and small to medium (13%). In polar view circular shape was shown by most of the plant species while equatorial view the dominant shape was prolate- spheroidal represented by 54% of collected plant species.

Remarkable variations about seeds morphology were reported for seed inner and outer color, seed shape, seed surface/texture, hilum position, seed length and width, and length to width ratio. Most of the seeds were spheroidal followed by obovate and elliptical. Seeds of 12 invasive plants were found with smooth surface followed by 6 with rough surface while ridges with furrows were observed in only one species. As far as position of hilum is concerned 15 invasive plants showed terminal while in 5 plants it was not clear. The seed of *Xanthium strumarium* L. was observed with maximum length of 13mm with width of 8mm. Length by width ratio of seeds were also calculated, it was found that maximum L/W ratio was observed in *Sonchus oleraceus* L. i.e. 2.66. Based on above findings regarding palyno-morphological and seed morphological features, this study is useful addition for write up of invasive flora Atlas for ecologists, botanists and taxonomists. Furthermore this systematic study will provide a base line data for molecular biologists for the better and correct identification of invasive plants. This study further recommends to work on invasive plants with special reference to their management and their impacts on natural biodiversity, economy, human health and environment and conservation of indigenous flora of Lesser Himalaya particularly and world generally .

CHAPTER: 1

Introduction

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1.1 Introduction to Invasive Plant Species

Invasive plant species are non-native species that may cause economic, ecological, and environmental harm and has the ability of self-sustaining and expanding populations. For invasive plants most frequently used terms are alien, exotic, non-native, introduced species (Rai and Singh, 2020). They can be introduced intentionally or purposefully by human in natural or established habitat. According to Wang et al (2011) other main invasion rout is introduction by chance. Alien plant species is aggressively a mediator of change and have become a global threat to many ecosystems, economy and health. Most of non-native plant species possess higher invasive potential and have higher ability to adapt well in variety of conditions, while native plants faces difficulty to compete these non-native species and ultimately they displaces the native species resulting in reduction of plant diversity.

Exotic plant species which invaded successfully have strong reproduction capacity such as higher rate of seed production, short life cycle, better seed dispersal and germinate faster. For example study of Zhao (2003) reveals that one ragweed has a capacity of production of 2000-8000 seeds. Invasive plant species also produce seeds which are small in size and light in weight may help them to disperse with wind easily and travel long distance (Wan and Wang, 1990).

Invasive plants show strong environmental adaptability, when these plants arrive in new environment they change their morphology, genetic characters and behavior which are helpful for their survival in new environment. However not all introduced plant species are invasive as they have lesser potential to establish themselves in new environmental conditions and not harmful to environment and health unlike invasive.

In most of cases, alien plants are introduced for ornamental and horticultural purposes but after some time they become invasive. The same situation is observed in Pakistan as these plants species were accidentally arrived or brought for beautification of the area and horticultural purposes etc., are now invaded in that area (Mahrine et al.,

2014). Intentionally introduced plant species have many tremendous social benefits, on the other hand some intentionally introduced species become invasive and can cause significant economic damages.

1.2 Impacts of Invasive Plants

Invasive plant species have detrimental effects on ecosystem, economy and health as these are spreading rapidly and extensively in the many regions of world (Levine et al., 2003; Schirmel et al., 2016).

1.2.1 Impacts of Invasive Plants on Biodiversity

As ecosystem services and human welfare is inextricably linked with the biodiversity so it is considered as safeguard for environment/ecosystem services. Invasive plants has direct negative or adverse effects on indigenous flora or native communities (Fike and Niering, 1999; Go'mez-Aparicio and Canham, 2008; Hunter and Mattice, 2002; Martin, 1999; Morrison et al., 2007; Yurkonis et al., 2005).

Biodiversity cannot be denied as it is responsible for food production and ensure nutritional security (Aerts et al., 2018; Jones, 2019; Stone et al., 2018). Rai and Lalramnghinglova, (2011) and Aerts et al., (2018) stated that it is source of many herbal medicines which are used to cure diseases like cardiovascular, pulmonary, digestive, dermal and even dreaded cancer.

Qureshi et al., 2019, studied the impacts of *Xanthium strumarium* native to North and South America on the native plants of Pothohar region of Pakistan. This weed adversely effected biodiversity and have negative effects on agricultural yield of raw crops (soybean, cotton, maize and groundnut), host of crop pathogens, cattle poisoning and contamination of sheep wool by lodging of burs. Outcomes of this study proved that there was a reduction in native flora of studied region and appropriate measures should be taken for its control.

Pejchar and Mooney (2009) stated that invasive species are major global change drivers and they are responsible for loss of biodiversity which alter ecosystem

functioning and socio economic conditions. Loss of biodiversity of native plants due to invasive have adverse effect on environment, ecosystem and climate change.

Ameen (1999), reported that ecological community alteration caused by alien invasive plant and animal species influence the overall functioning of ecosystems. Weiner (2004) studied the effects of fast-spreading, well-established alien plants on native species and found that invaded plants species are more competitive than native species in a particular habitat. There is strong competition between native flora and invaded species may results into invasion melt down that helps other nonnative species to invade or establish in that environment easily Studies of Bakker and Wilson (2001) also shows that there is a strong competitive effect on native species and there is a tendency of some invasive plants to form mono specific stands while the population of dominant native species decreases rapidly.

Susana et al., (2017) studied the impacts of invasive plants on animal diversity in South Africa. This review highlighted the research gap in taxonomic and geographic coverage and the IAP impacts on ectotherms mechanisms.

It has been observed that over the past couple of centuries that several thousands of the alien species have become established in different parts of the world. There is deficit in context of invasion biology literature in our country, however the scale of invasive species is not as large as in some other countries. Khan et al., (2010) termed biological invasion as a biological pollution and classified it more disastrous than the chemical pollution. Alien invasive plant species not only reduce land value and cause great loss to agricultural communities but were detected as a source of allergy as well (Khan et al., 2010).

1.2.2 Impacts of Invasive Plants on Environment

In the 21st century a serious global environmental issue to be considered is a biological invasion caused by human beings (Sala et al., 2000). According to Pysěk and Richardson (2012) there are three basic ways by which invasive plants affects ecosystems (a) dropping the diversity of indigenous plants and animals, (b) changes in

physico-chemical characteristics of soils (mostly through allelopathy), and (c) enrichment in ecosystems response towards altered fire regimes. Reduction in biodiversity is one of the most important impact of invasive plants and they also adversely affects the wild life of ecosystem (Gan, 2009). They also adversely affect the fire regime various terrestrial ecosystems.

Invasive plants also have ability to invade in aquatic environments and badly hinder the flow of water and makes the water unfit for drinking and irrigation and also affect the quantity of surface water and ground water (Shackleton et al., 2019). Dudley et al., (2014) reported that exploitations of groundwater resources in arid regions of Hawaii Island occurs due to *Prosopis pallida* which results in adverse effects on the soil. Many invasive plants have profound influence on soil chemistry, microbial functioning, soil stability and soil quality.

1.2.3 Impacts of Invasive Plants on Ecosystem Services

In current era all over the world impacts of invasive plant species on ecosystem services have gain a great attraction as invasive plants are responsible for disturbing ecosystems. Aesthetic, recreational, cultural and regulatory services comes under the umbrella of Ecosystem services. According to Colautti (2006), Pejchar and Mooney (2009) many ecosystem services such as water treatment, pest management, pollination, climate change, hazards mitigation etc. which are linked with agriculture and forestry are affected by these invasive plant species.

Shackleton et al., (2017) reported that *Opuntia stricta* has invaded in African region and adversely affected the environment, economy and livelihood of local people by restraining fodder production and livestock health. The impact of invasive alien *Prosopis* species (mesquite) in different environments of South Africa on native plants was studied by Shackleton et al., (2015) and found that it has extremely affected the ecosystems.

1.2.4 Impacts of Invasive Plants on Economic

Invasive plants has economic impacts on international trade due to impact on agriculture and fisheries. In this context agriculture and health sectors have badly suffered economically due to invaders but this economic loss is more pronounced in agriculture sector as compare to health sector. Sileshi et al., (2019) evaluated the damage to agriculture crops due to invasive plants species and found that it resulted in an estimated economic loss of US\$ 1 billion in the agriculture sector of African countries per year.

Ferdinand et al., (2004) had conducted a study on 14 species of potentially invasive landscape plants of Florida nursery industry and found that these plants species have substantial value to the nursery industry. Souza et al., in 2018 noted the harmful effects of *Acacia mangium*, found in northern Brazilian Amazon as invasive to economy, environment and indigenous people as this species causes alteration of the water quality.

1.2.5 Impacts of Invasive Plants on Human Health

Human wellbeing is also affected by the invasive plant species often in negative but sometimes in positive manners. The effects of invasive plants on human health are indeed a major issue but very few studies have conducted to analyze it. Invasive plants species may effects human health in verities of ways such as it may cause diseases or infections, may expose humans to bio toxins, allergens or toxicants, may facilitate diseases, injuries or death; and may inflicting other negative effects on human livelihood.

Rai and Lalramnghinglova (2011) and Rai et al., (2018) studied positive implications of invasive plants which includes their applications in vector borne control and ethno-medicinal uses so exotic plant species play an important part as medicinal remedies to treat different human diseases e.g. *Lantana camara* is used to extract a mosquito repellent (Mng'ong' et al., 2011; Stone et al., 2018). *Amorpha fruticose* a

shrub that is native to North America is useful against diabetes and metabolic diseases (Ekaterina et al., 2017).

Invasive plants are the source of environmental contamination which affects the human health. Loss of host plants due to invasive results in a spurt in the growth of pathogen population that facilitates the outbreak of several human diseases such as Tick-borne diseases, Tuberculosis (multidrug-resistant), severe acute respiratory syndrome (SARS), acquired immunodeficiency syndrome (AIDS) and virulent Malaria (Daszak et al., 2000; Hulme, 2014; Pysěk and Richardson, 2010; Stone et al., 2018; Young et al., 2017). Invasive plants are also responsible for allergy in humans. In this perspective the most severely affected area from the allergic immune responses such as asthma and other respiratory and skin diseases is the European continent (Bayliss et al., 2017; Muller-Scharer et al., 2018; Schindler et al., 2015). For managing human health issues occurred due to such allergic invasive plants, the most effective tool is Allergen-specific immunotherapy (AIT) (Chen et al., 2018). The management of invasive plants is important to improve public health and biodiversity (de Wit et al., 2017).

1.3 Invasion Mechanism of Invasive Plants

A wide array of trait strategies are utilized by invasive plants to establish in new ecosystem. Exotic plant species possesses many characteristic which favor their successful invasion in new ecosystem like their ability to produce of large number of seeds, small sized and light weight seeds, grow rapidly from germination to reproductive stage, environmental adaptability.

There are numerous hypotheses regarding successful invasion of exotic plants in new habitat such as Enemy Release Hypothesis (ERH), Novel Weapon Hypothesis (NWH) and Empty Niche (EM) (Prabhat and Singh, 2020). Studies of Blumenthal in 2006 and Rai in 2015 shows that invasive plants are more successful in new environment as they are away from their natural enemies like pathogens and herbivores which results in rapid increase and spread of exotic plants. But research findings of

Feng (2008), reveals that enemy release hypothesis is not only main reason for invasion of exotic plants.

1.4 Allelopathy: A Potential Mechanism for Successful Invasion

Recently allelopathy is considered as potential mechanism for which results in successful invasion of plants. According to Bao Ming et al., (2017), there are several aspects of plant invasion but role of allelopathy in successful invasions cannot be deny in plant invasion. Exotic plants are more successful in new ecosystems when they produce evolutionary new compounds. Molish (1937) was scientist who considered Allelopathy as a mechanism of invasion for exotic plants. Allelopathy is the production of chemicals by the one plant species that affects the other plant species in harmful way (Lawrence, et al., 1991; Rice, 1984). Allelochemicals are the secretions of plants, identified as a novel weapon, responsible for the inhibition of native plants and helps in invasion of exotic plant species in new habitat (Pinzone et al., 2018). Allelochemicals are secondary metabolites, most common types includes phenolic acids, terpenoid and sesquiterpenes, these compounds have a negative effect on growth of native plant species (Pinzone et al., 2018; Singh et al., 2014a, b; Uddin and Robinson, 2017).

Hierro et al., (2005) indicates that as per novel weapon hypothesis, allelochemicals released by exotic plant species are relatively less effective against well adapted neighbors in original range but these highly suppress native flora in introduced environment.

Findings of Einhellig (1994) shows that seed germination of native plants is inhibited by the phenolic acids released by alien plants as these chemicals inhibit phosphorylate, a key enzyme required for seed germination.

Ma et al., (2020) conducted research studies on assessment of allelopathic effects of two invasive plants *Mikania micrantha* and *Ipomoea cairica* which seriously damage Hainan Island. Their findings shows that benzoic acid and cinnamic acid were main forms of phenolic acids, responsible for allelopathic effects of these invasive plants.

Due to allelopathic effects, most of exotic/alien plants make it possible to survive in new habitat and attain dominant position in that habitat (Latif et al., 2017). Allelopathy has gained a prominence in the field of invasion biology as it has adverse effects on the growth of neighboring plants. Analysis of Kalisz et al., in 2021 indicates that allelopathy is an important characteristics of invasive plants and it is common mechanism across the plant phylogeny. Nikki et al., (2010), studied four invasive plant species i.e. *Ailanthus altissima*, *Alliaria petiolata*, *Celastrus orbiculatus*, and *Microstegium vimineum* and results shows that all these plants have a strong germination inhibition of native flora due to allelopathic potential.

For determining the allelopathic effects of invasive plants, the evolutionary experiences of native flora to the allelochemicals is also important (Abhilasha et al., 2008; Thorpe et al., 2009). By the release chemical compounds in their environment, exotic plant species suppress the native flora, this facilitates the successful invasion of these plants (Bais et al., 2003; Hierro et al., 2003; Ridenour and Callaway 2001). Samuel et al., in 2005, assessed the negative impacts of two exotic invasive plants species i.e. *Lolium arundinaceum* (tall fescue) and *Elaeagnus umbellata* (autumn olive) in North America, on three successional tree species *Acer saccharinum* (silver maple), *Populus deltoides* (eastern cottonwood), and *Platanus occidentalis* (sycamore).

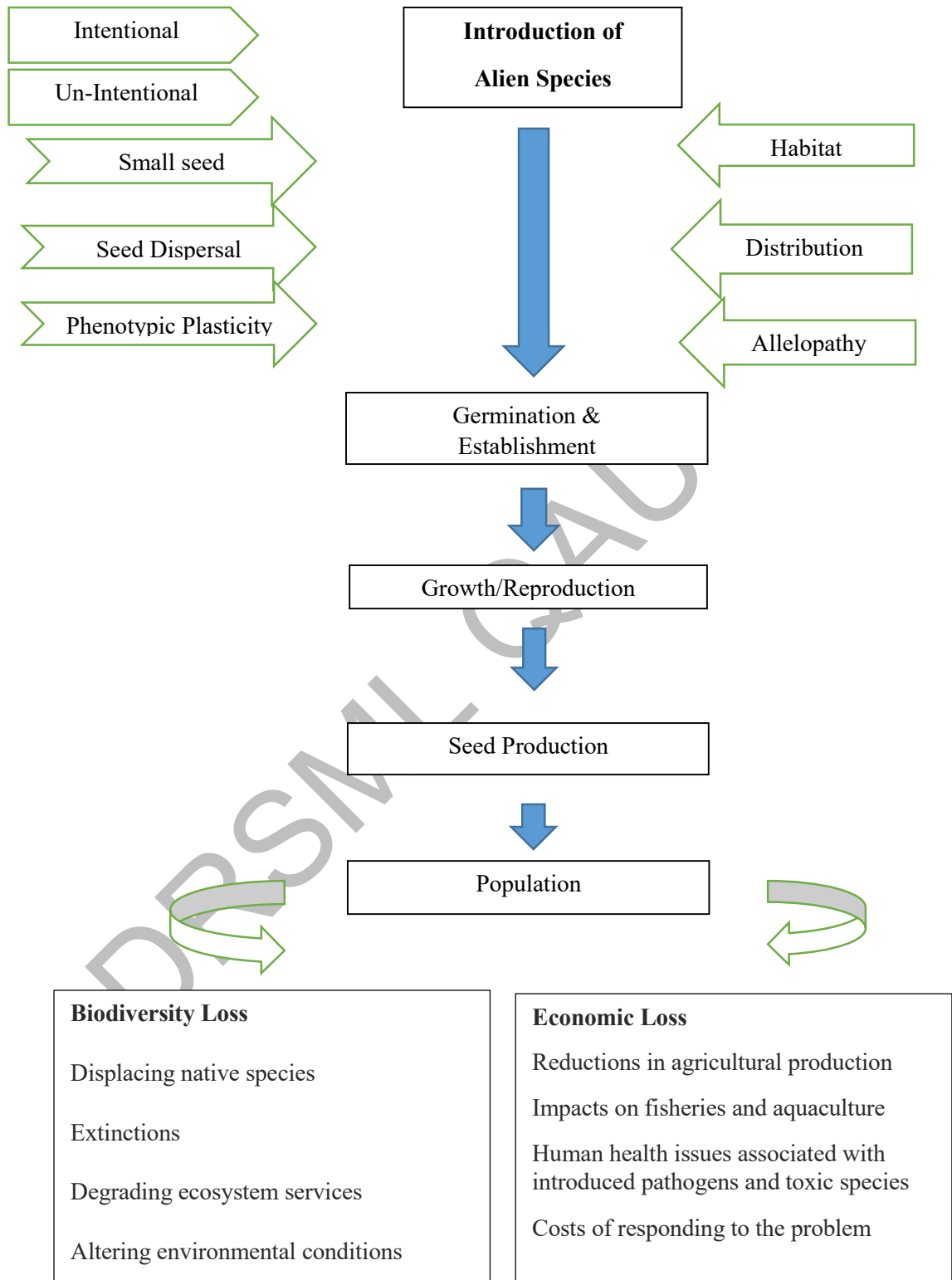


Figure 1: Flow Chart Showing Invasion Mechanism

1.5 Significance of Morpho-Palynological Features in Invasiveness

The morphological structure of pollen grains has revolutionized the study of pollen and spores as used in identification of plants under palynological area. Variations in pollen biology in invasive plants can be related to various evolutionary adaptations of the flowers to make them more attractive to pollinators. There is competition among invasive species and native plants for resources like nutrients, water, light, space and also exert the indirect pressure on native plants by competing pollinators. Invasive species have an ability to compete effectively with native species for pollination services and represent significant threat on reproductive success of indigenous species. Invasive species produce numerous large size attractive flowers with large amount of pollen. According to Titze (2000) these characteristics increases the visitation of pollinators and so does the probability of pollen deposition and seed production and ultimately play a crucial role in successful invasion. The high percentage of fertile pollen in invasive species is closed linked with distribution success and successful invasion mechanism.

Study and research on pollen morphology depends upon the use of appropriate microscopy techniques. Multiple and different techniques can be used while studying the pollen morphology and pollen ultrastructure. LM and SEM can be used to study the recent and fossils pollens grains while recent pollen grains are also studied by TEM. SEM is appropriate when there is need to get specific information regarding pollens while LM is mostly used to gather the data very quickly. But it is important to study the pollen grains in both LM and SEM to obtain accurate taxonomic description. One of main advantage of using light microscope is convenience and of SEM is that it provides increased resolution and significantly more data can be assessed. Methodology involved in LM and SEM for sample analysis are more or less similar and required same procedures and time consumption (Gretchen and Vaughn 2007).

Pollen features play significant role in the correct identification at genus and species level and provide sufficient information to taxonomists so LM and SEM is useful to get such type of information (Butt et al., 2018). According to Ulrich et al.,

2012, in delimiting taxa the palynological features play a very important role furthermore these features also play role to explain the evolutionary evidences. Morphological features of pollen are helpful at specific and generic level for correct plant identification and classification, these features can also provide solutions for critical and disputed taxonomic problems (Shaheen et al., 2018).

Stephen et al., (2017) studied pollen morphology of five invasive *Jatropha* species *J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *J. multifida* and *J. tanjorensis* belongs to family Euphorbiaceae from India by using Light Microscope (LM). They found that pollen grains of *Jatropha* are typically crotonoid, uniformly spheroidal, in aperture/omniaperturate, and hexagonally arranged exinous knobs. Findings of research indicates that pollen characters are important to understand the evolutionary and systematic relationships.

Pavlova and Glogov (2021) studied the invasive success of *Impatiens* species growing Bulgaria through pollen morphology and pollen production. The findings of this study showed great variation in pollen morphology of invasive and native *Impatiens* species and these characters can be used for identification of species due to presence of great variations established in invasive and native species.

The pollen morphology of two invasive species i.e. *Pelargonium endlicherianum* and *Pelargonium quercetorum* (Geraniaceae) in Turkey was studied by Başer et al., (2016). The results of study indicates that pollen features like size, shape, exine ornamentation proved to be useful characters for the systematics of the taxa.

Vyas and Gohil (2019) investigated the pollen morphology of some invasive plants of Caesalpinaceae that is sub family of Leguminosae found in Bardoli, South Gujrat, India. Their palynology based findings are useful at the generic and specific levels on the basis of apocolpium, mesocolpium and tectum features. The data obtained on the basis of these characters is also useful to prepare the pollen calendar of invasive plants.

Wrońska-Pilarek et al., (2019) first time investigated the interspecific and inter individual variability of the pollen grains of highly invasive *Spiraea tomentosa* from Poland. The pollen morphology at species level is not taxonomic characters in their findings. However morphological feature of pollens could have taxonomic value at sectional level (Jun-Ho et al., 2017) and interspecific level (Liu et al., 2010). Palynomorphological characters and pollination biology of invasive plant species are helpful to evaluate their success as an invasive nature.

1.6 Significance of Morphological Characters of Seed in Invasiveness

Seed coat morphology as a potential taxonomic character was proposed by Davis and Heywood (1963). In plants seed morphological studies are of high taxonomic value and facilitates not only the identification and delimitation at taxa different taxonomic level. These studies are also valuable in a better understanding of the evolution of taxa too. Features of seed morphology are useful in understanding the phylogenetic relationships as characteristics of seed surface is less influenced by environmental conditions (Haridasan and Mukherjee 1988). The shape, ornamentation, size and outline of seeds are valuable characters for authentication, identification and classification of species. Seed morphological characteristics like seed size, shape, color, surface shape, hilum length etc. are the basis of seed identification (Al-Gramdi and Al-Zahrani, 2010; Gunn, 1970, 1971, 1982; Morozowska et al., 2011). According to Karihaloo and Malik, (1994); Koul et al., (2000); Segarra and Mateu, (2001), outer surface features of seeds under SEM have been commonly used in solving various problems in systematics and phylogenetic relationship of species.

Invasive process is facilitated because of quick seed germination than native species. Seed germination is an important part of life cycle of higher plants due to their impacts on fitness, population dynamics and their evolutionary direction (Muscarella et al., 2013). Establishment and expansion based on successful germination of invasive plants increase their invasion potential (Gioria and Pyšek 2017). Several studies have revealed that invasive plants may germinate earlier or more rapidly than their

noninvasive competitors. Hirsch et al., (2012) investigated that seeds of introduced populations of *Ulmus pumila* germinates at faster rate than those of native populations. Various findings have shown positive relationship between seed germination and plant invasion success (Colautti et al., 2006). The rate of seed germination depends on physical properties like size, shape, and thickness. The faster germination reduces resource availability to surrounding native plants and facilitate the invasion process.

Seed size reflects the genetics investment and environmental factors. It varies between, within species and in a single plant. The seed size of invasive plant increases from native to introduced ranges in various investigations (Daws et al., 2012). Graebner et al., (2012) stated that seeds of invasive plant *Centaurea solstitialis*, native to Spain has larger seeds in invaded region (California) while noninvasive species of *Centaurea calcitrapa* and *Centaurea sulphurea* did not show increasing trend in seed size.

Seeds of invasive plants grow at faster rate than native ones facilitates the invasion rate of invaded species. Thus linking the seed morphological characters to the invasion process is very important in future to better understand the invasion mechanism in introduced plant species.

In case of classification of angiosperm taxa the information and data related to seeds at both micro and macro level is very significant and plays an important role (Abdel Khalik and Osman, 2007). It has also achieved a major position in the modern synthetic systems of angiosperms (Dahlgren, 1983). According to Manos and Miller (2001), various species can be distinguished on the basis of seed morphological characters and these characters are also supported the placement of species in different clades. Abdel Khalik and Osman (2007), carried out investigations on the seed morphology of 31 species of six genera of family Convolvulaceae including the invasive plant *Convolvulus arvensis* from Egypt by using LM and SEM to explain the systematic significance of seed morphological characters as one of the factor for correct identification of invasive plants.

Invasiveness is associated with formation of seed banks in soil through the prolific production of seeds. Marchante et al., (2010) analysis on seed ecology of an invasive alien plant species *Acacia longifolia* from Portuguese dune ecosystems indicated production of extensive number of seeds and form seed bank facilitating the successful invasion of *Acacia longifolia*.

The morphological features of seeds have played a critical role to solve the taxonomic and evolutionary dilemmas. Luqman et al., (2019) carried out a micro-morphological observation on the seed coat of *Eucalyptus* species to determine the seed morphological characters such as seed surface, periclinal and anticlinal wall, as an additional tool for correct identification. This plant is considered as highly invasive plant and there is dire need to manage this plant as has growth rate.

To identify and distinguish plant species, study of micro-morphological characteristics of seeds under SEM are of significant value (Barreto et al., 2013 & Song et al., 2015) and these characters also provide insight to discriminate between authentic herbal medicines and their adulterants (Song et al., 2018). Rewicz et al., (2020) studied the seed morphology and sculpture of invasive *Impatiens capensis* from different habitats of Poland. They first time describe the seed coat ornamentation in *Impatiens capensis*, findings showed that seed morphological characters are important tool for correct identification of closely related invasive taxa and helpful for better understanding of evolutionary relationships.

The high structural diversity of seed coat offers the most valuable criteria for classification of plants at species and family levels. So a comprehensive investigation on the morphological characters of seeds greatly increases our knowledge of individual invasive species and may be helpful in better understanding the phylogenetic relationships of the invasive taxa. Therefore, seed characters are useful to generate valuable information in the identification and delimitation of invasive species.

1.7 Background Justification of the Project

The invasive species are introduced intentionally or sometimes accidentally to a new ecosystem. Alien and exotic plant invasions affect negatively ecological processes and considered as major threats to the floral diversity worldwide. Rate of adaptability of alien species is high in a new region due to their rapid growth and by producing high biomass than native flora. Introduction of alien species are likely to cause economic or environmental harm or harm to human health. Introduction of foreign plant and animal species in Pakistan is practiced since previous decades to fill the gap of timber, fuel wood and fodder (Hussain and Zarif, 2003) as per country demands. These species were also introduced for horticultural purposes and for the beautification of country.

While introducing these alien species in Pakistan, no attention had been made their invasive behavior and no proper cataloging of invasive species yet exists at national level. Furthermore there is no dataset available that could provide comprehensive information and impact of invasive species on native species composition and diversity. Review of literature revealed that data deficit in the invasion biology literature is the result of insufficient and inadequate research efforts. The research base on this subject in the country is very weak.

As literature revealed that currently, a detailed list of alien species of plants introduced to Pakistan is not available. Present research study in this context is very important as this will be a first document covering the morpho-palynological and seed diversity of invasive flora found in Lesser Himalayas Pakistan. To best of our knowledge based upon literature there is no comprehensive studies exists on invasive plants of lesser Himalayas Pakistan with special emphasis on pollen and seed diversity, It is a dire need to explore the invasive plants of Lesser Himalayas Pakistan to develop check list and also studies the diverse features of pollens and seeds. Keeping the scenario into consideration, the current studies were undertaken with the objective of making an inventory of invasive species of the area. Pilot scale investigations are required for systematic exploration of invasive plants for gathering national data of

palynological features and seed morphology for the correct identification and delimitation in the area.

This research study is designed to explore the alien flora introduced to Lesser Himalayas Pakistan over the last few decades. This study will be helpful in the compilation of invasive flora data and diversity of the region. In future further research studies reveal the allelopathic effects of invasive flora on indigenous flora. The present research study will be useful for preparation of pollen calendar of invasive plants particularly. Data obtained on the basis of pollen characters will provide an additional tool to evaluate the invasion success in invasive plants.

The current study of macro and micromorphology of pollen and seeds will be used as an important tool for correct identification and delimitation of invasive taxa for future planning by Botanists, Taxonomists, Policy makers, Climate experts and by Biodiversity specialists to protect indigenous flora of this region.

1.8 Aims & Objectives of the Study

- The present study is focused to prepare detailed inventory of invasive plants in Lesser Himalaya including altitudinal attributes and life form.
- The study is based on a comprehensive qualitative and quantitative characters of pollen of invasive plants using Light and Scanning Microscopy.
- The present study is concentrated to learn the seed coat morphology of invasive plants growing in Lesser Himalaya using Light and Scanning Electron Microscope to indicate the importance of morphological characters for taxonomy and identification of invasive plant species and to understand the invasion process.
- The study aims to calculate the pollen fertility and sterility of invasive plants in order to obtain the data for their invasion success.

CHAPTER: 2

Materials and Methods

2.1. Lesser Himalaya: Study Area

Pakistan is gifted with peculiar resources of rich and diverse plant heritage. The Himalaya is comprised of world's highest mountains with beauty and unique biodiversity nature, attracting tourist around the globe (Sekar, 2012). The Himalayas are known as one of the largest mountain ranges on this planet. The Himalayan is classified as three equivalent ranges often referred as the Greater Himalayas, the Lesser Himalayas, and the Outer Himalayas (Zareen et al., 2018).

In Pakistan Himalaya range occupies the regions of Kashmir, Kaghan, Kohistan, Deosai and Chilas. The elevation of lesser Himalaya is 12,000 to 15,000 feet and it lies between 33°-44' and 35°-35' north latitude and between 72°-33' and 74°-05' east longitude. The total area of Lesser Himalayas in Pakistan is proximately 1700 to 1800 km (Hussain and Ilahi 1991) and the total population of this region is proximately 10 million (Pakistan Bureau of Statistics, 2008). There is a tremendous variation in the topography, altitudes aspects and vegetation cover, the climate of Lesser Himalayas ranges. The key categories of Lesser Himalayas are Subtropical continental lowland and subtropical continental highland. Subtropical continental lowland comprised of plain and foot hills zone while subtropical continental highland comprised of outer and middle Himalayas, Siwalik Hills, Murree Hills and entire Hazara Hills.

As far as rain fall is concerned the Southern parts of Lesser Himalayas receive 70-90 mm average rainfall whereas it varies from 100-130 mm in northern parts. A large part of the winter precipitation from the western disturbance is received in the form of snow. The northern parts receive little rain but heavy snowfall in the winter (Hussain and Ilahi 1991, Khan et al., 2010). The vegetation of Lesser Himalaya falls within the subtropical, temperate, sub-alpine and alpine zones.

2.2. Schematic Sketch of Present Study

The overall design for current research study is presented in given Figure 2.1

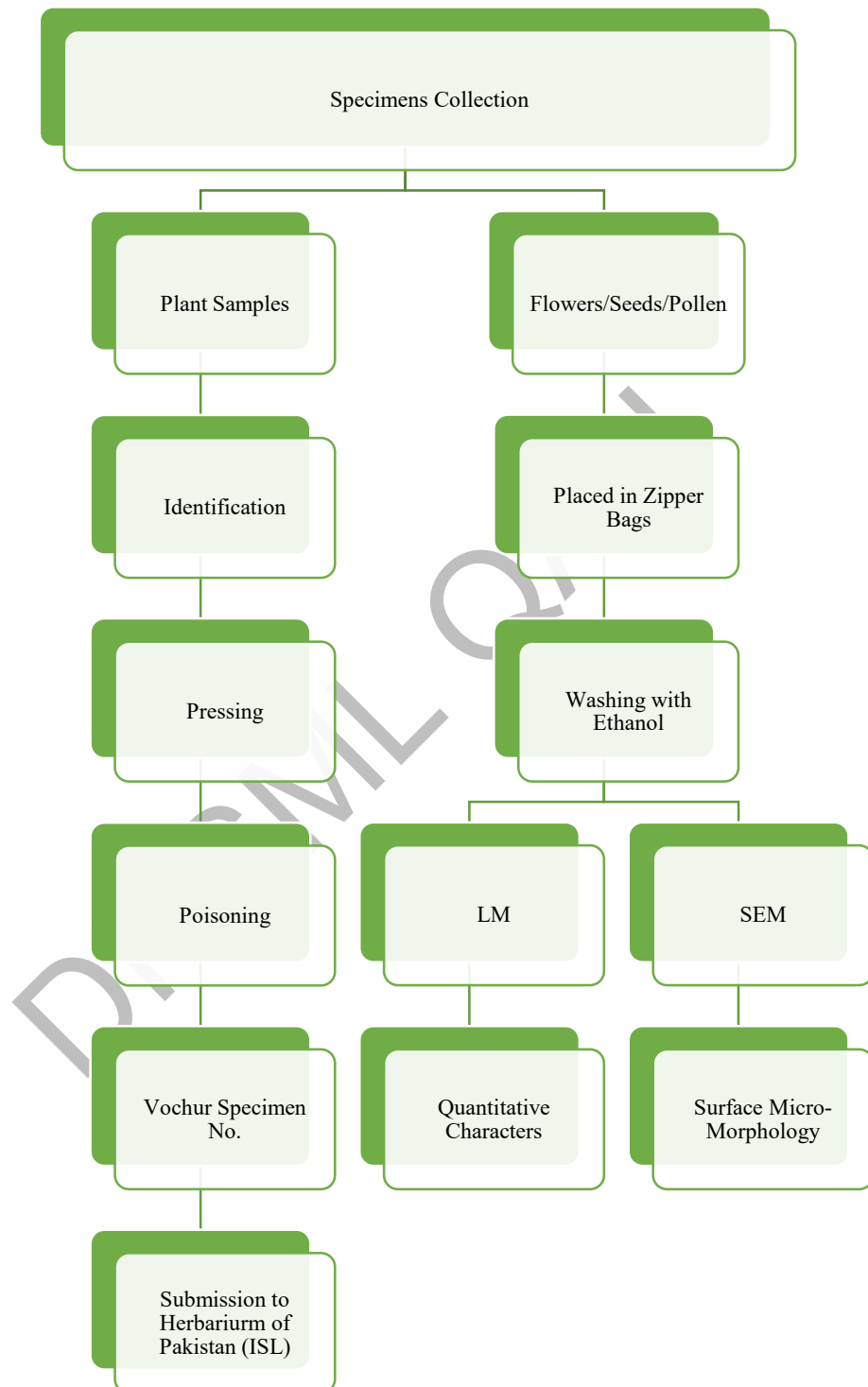


Figure 2: Schematic Sketch of Present Project

2.3. Field survey and Plants Collection

Different field visits were arranged from March 2020 to December 2021 in various sites of Lesser Himalaya, especially in flowering and fruiting stage. The areas/regions includes Margalla Hills National Park Islamabad, Murree Hills and its allied areas in the province of Punjab, Abbottabad, Haripur, Mansehra districts of Khyber Pakhtunkhwa province (KPK) respectively. The particulars of source and collection with GPS data of the collected invasive plant species are presented in Table 1.

DRSML QAU

Table 1: Check List of Invasive Plant Species with Collection Site and Field Attributes

S. No.	Taxa	Family	Locality in Lesser Himalaya	Coordinates	Voucher Specimen No.
1.	<i>Achyranthes aspera</i> L.	Amaranthaceae	Damn-e-Koh	33°44'38.00"N 73°01'22.00"E	IAS-ISL-15
2.	<i>Ageratum conyzoides</i> L.	Asteraceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-22
3.	<i>Ailanthus altissima</i> (Mill.) Swingle	Simaroubaceae	Bhurban Murree	33°56'26.24N 73°26'59.48E	IAS-ISL-18
4.	<i>Anagallis arvensis</i> L.	Primulaceae	Sarban hills Abbottabad	33°08'14.34"N 73°13'19.13"E	IAS-ISL-26
5.	<i>Argemone ochroleuca</i> Sweet, Brit. Fl. Gard.	Papaveraceae	Tret Murree	33°52'27.47N 73°17'31.48E	IAS-ISL-02
6.	<i>Azadirachta indica</i> A.Juss.	Meliaceae	Islamabad (Chak Shahzad)	31° 2'14.01"N 73° 4'32.18"E	IAS-ISL-08
7.	<i>Bidens pilosa</i> L.	Asteraceae	Tarnawai Abbottabad	33°16'16.74"N 73°17'47.18"E	IAS-ISL-27
8.	<i>Brachychiton acerifolius</i> (A.Cunn. ex G.Don) F.Muell.	Malvaceae	Bansra Galli	33°53'08.51N 73°21'23.84E	IAS-ISL-24
9.	<i>Broussonetia papyrifera</i> (L.) L'Hér. ex Vent.	Moraceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-28
10.	<i>Bryophyllum pinnatum</i> (Lam.) Oken	Crassulaceae	Bagnetar Abbottabad	33°07'37.72"N 73°20'34.33"E	IAS-ISL-29
11.	<i>Calotropis procera</i> (Aiton) Dryand.	Apocynaceae	QAU Colony	33°44'44.71"N 73°9'26.55"E	IAS-ISL-10
12.	<i>Cannabis sativa</i> L.	Cannabaceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-07
13.	<i>Callistemon citrinus</i> (Curtis) Skeels.	Myrtaceae	QAU Colony	33°44'44.71"N 73°9'26.55"E	IAS-ISL-31
14.	<i>Carthamus oxyacantha</i> M.Bieb.	Asteraceae	Chemistry Hut Parking, QAU	33.4446° N 73.0759° E	IAS-ISL-30
15.	<i>Cassia occidentalis</i> L.	Fabaceae	Margalla hills (Rumli Village)	33°44'51.55"N 73°08'13.95"E	IAS-ISL-06
16.	<i>Chenopodium ambrosioides</i> L.	Amaranthaceae	Angoori Murree	33.47'56.37° N 73.21'00.86° E	IAS-ISL-21
17.	<i>Commelina benghalensis</i> L.	Commelinaceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-32

			(Rumli Stream)		
18.	<i>Convolvulus arvensis</i> L.	Convolvulaceae	Margalla Hills	33°44'51.55"N 73°08'13.95"E	IAS-ISL-33
19.	<i>Conyza canadensis</i> L.	Asteraceae	Oghi Manshera	33°25'26.36"N 72°58'10.84"E	IAS-ISL-34
20.	<i>Datura innoxia</i> Mill.	Asteraceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-19
21.	<i>Digera muricata</i> (L.) Mart.	Amaranthaceae	Islamabad (Chak Shahzad)	31° 2'14.01"N 73° 4'32.18"E	IAS-ISL-35
22.	<i>Dodonaea viscosa</i> (Linn.) Jacq.	Spindaceae	Margalla Hills	33°44'51.55"N 73°08'13.95"E	IAS-ISL-09
23.	<i>Eucalyptus camaldulensis</i> Dehnh	Myrtaceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-37
24.	<i>Rosa alba</i> L.	Roseaceae	QAU Isb (Rumli Stream)	33°44'40.00"N 73°01'33.00"E	IAS-ISL-25
25.	<i>Jasminum humile</i> L.	Oleaceae	Margalla hills	33°44'51.55"N 73°08'13.95"E	IAS-ISL-39
26.	<i>Justicia adhatoda</i> L.	Acanthaceae	QAU Isb	33°45'40.28"N 73°08'03.95"E	IAS-ISL-40
27.	<i>Lantana urticoides</i> Hayek	Verbenaceae	Tarnawai Abbottabad	33°16'16.74"N 73°17'47.18"E	IAS-ISL-17
28.	<i>Lantana camara</i> L.	Verbenaceae	Margalla Hills	33°44'51.55"N 73°08'13.95"E	IAS-ISL-13
29.	<i>Malvastrum coromandelianum</i> (L.) Gareke	Malvaceae	Chemistry Hut, QAU	33.4447° N 78.0804° E	IAS-ISL-41
30.	<i>Melilotus indicus</i> (L.) All.	Fabaceae	Social Hut QAU	33.4457° N 73.0814° E	IAS-ISL-42
31.	<i>Nasturtium officinale</i> R.Br.	Brassicaceae	Tret Murree	33°52'27.47N 73°17'31.48E	IAS-ISL-03
32.	<i>Oxalis corniculata</i> L.	Oxalidaceae	Battal Manshera	33.35'12.49° N 73.08'58.96° E	IAS-ISL-44
33.	<i>Parthenium hysterophorus</i> L.	Asteraceae	Social Hut QAU	33.4457° N 73.0814° E	IAS-ISL-45
34.	<i>Prosopis juliflora</i> (Sw.) DC.	Fabaceae	Islamabad (Chak Shahzad)	31° 2'14.01"N 73° 4'32.18"E	IAS-ISL-46
35.	<i>Quisqualis indica</i> L.	Combretaceae	Murree Road	33°44'38.00"N 73°01'22.00"E	IAS-ISL-47
36.	<i>Ranunculus muricatus</i> L.	Ranunculaceae	Girwal Manshera	33.25'57.01° N 73.14'54.68° E	IAS-ISL-36
37.	<i>Ranunculus arvensis</i> L.	Ranunculaceae	Angoori Murree	33.47'56.37° N 73.21'00.86° E	IAS-ISL-38
38.	<i>Ricinus communis</i> L.	Euphorbiaceae	QAU Isb (Near Quaidian Hut)	33°45'40.28"N 73°08'03.95"E	IAS-ISL-11

39.	<i>Robinia pseudoacacia</i> L.	Fabaceae	Bagnetar Abbottabad	33°07'37.72"N 73°20'34.33"E	IAS-ISL-01
40.	<i>Solanum surattense</i> Burm. f.	Solanaceae	Margalla Hills	33°44'51.55"N 73°08'13.95"E	IAS-ISL-14
41.	<i>Sapium sebiferum</i> (L.) Roxb.	Euphorbiaceae	Islamabad (H8)	30°44'58.73"N 73°12'37.27"E	IAS-ISL-16
42.	<i>Sesamum indicum</i> L.	Pedaliaceae	Battal Manshera	33.35°12.49° N 73.08°58.96° E	IAS-ISL-04
43.	<i>Silybum marianum</i> (L.) Gaertn.	Asteraceae	Tret Murree	33°52'27.47N 73°17'31.48E	IAS-ISL-20
44.	<i>Sonchus oleraceus</i> L.	Asteraceae	Chemistry Hut Parking, QAU	33.4446° N 73.0759° E	IAS-ISL-05
45.	<i>Sorghum halepense</i> (L.) Pers.	Poaceae	Islamabad (H8)	30°44'58.73"N 73°12'37.27"E	IAS-ISL-48
46.	<i>Taraxacum campylodes</i> G.E.Haglund	Asteraceae	Oghi Manshera	33°25'26.36"N 72°58'10.84"E	IAS-ISL-43
47.	<i>Tecoma stans</i> (L.) Juss. ex Kunth	Bignoniaceae	Social Hut QAU	33.4457° N 73.0814° E	IAS-ISL-49
48.	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Islamabad (H-10/1)	31° 2'14.01"N 73° 4'32.18"E	IAS-ISL-50
49.	<i>Tropaeolum majus</i> L.	Tropaeolaceae	Margalla Hills (Rumli Village)	33°44'51.55"N 73°08'13.95"E	IAS-ISL-23
50.	<i>Verbena officinalis</i> L.	Verbenaceae	Chemistry Hut Parking, QAU	33.4446° N 73.0759° E	IAS-ISL-51
51.	<i>Verbesina encelioides</i> (Cav.) Benth. & Hook.f. ex A.Gray	Asteraceae	Battal Manshera	33.35°12.49° N 73.08°58.96° E	IAS-ISL-52
52.	<i>Xanthium strumarium</i> L.	Asteraceae	Girwal Manshera	33.25°57.01° N 73.14°54.68° E	IAS-ISL-12

2.4. Herbarium Preparation and Plant Identification

The collected plants were pressed, dried, poisoned, labelled and mounted on herbarium sheets and deposit in Herbarium of Pakistan (ISL), Quaid-i-Azam University Islamabad for future reference. In case of any ambiguities voucher specimens were compared with type specimens available at Herbarium of Pakistan (ISL), QAU Islamabad. Voucher specimens of all the documented species were properly processed and submitted to Herbarium of Pakistan (ISL), QAU Islamabad. The collected plants were identified based on their different morphological characteristics and cross verified with the samples already record documented in the herbarium and consulting Flora of Pakistan given by Ali and Qaiser, (1993-2010).

2.5. Systematics Studies of Invasive Species

2.5.1 Palynological Studies (LM & SEM)

Palynological studies were carried out by adopting following procedures.

a) Pollen Material

The pollen material in this research study was obtained from fresh collection containing flowers collected from various regions of Lesser Himalaya Pakistan. The details and origin of studied invasive plant species have been presented in Table 2.1. In order to separate the pollen grains from anthers, stereo microscope was used.

b) Glycerin Jelly Preparation

Preparation of glycerin jelly for palynological studies was carried out by using the methodology of Meo and Khan (2005). For glycerin jelly preparation about 30 gm of gelatin was boiled in 50 ml of distilled water. After boiling for ½ an hour, 40 gm of glycerin and few drops of phenol crystals were added to the mixture. For pink color 0.1 % safranin was added and then mixture was stabilized at room temperature for later use.

c) Light Microscopic (LM) analysis of Pollen

Mature flowers were used for pollen morphological studies. The anthers were acetolyzed with 99.9% pure acetic acid then cursed to release pollen from

anthers and debris was removed. Then drop of glycerin jelly was put on acetolysed pollen and slides were covered with cover slips and after these slides were permanently sealed with transparent nail paint. Tagging tape was used for giving slide number or name (Khan et al., 2020). Prepared slides were observed under light microscope (Meiji Techno MT4300H) and microphotography was carried out using Leica microscope fitted camera Meiji Infinity 1. Both qualitative and quantitative features were studied (Ahmad et al., 2013; Bahadur et al., 2018; Khan et al., 2021). Terminologies for pollen morphology were described according to Barthlott (1984), Erdtman (1960) and Ronald (2000).

d) Scanning Electron Microscopic Analysis (SEM) of Pollen

For SEM studies mature anthers were placed in the middle of clean glass slide, 1-2 drops of acetic acid were added and left for a minute. Anthers were then crushed to release the pollen from anthers, debris was removed and extracted pollen were transferred to stubs. Coating with gold was done in sputtering chamber using SPI-MODEL™. After gold coating, stubs were placed in Jeol Vacuum –evaporator for 15 minutes and observed under Scanning Electron Microscope (Model JEOL JSM- 5910) (Butt et al., 2018; Hussain et al., 2019). Pollen exine ornamentation was studied following the terminally of Bahadur et al., (2018)

2.5.2 Pollen Fertility and Sterility Estimation

Estimation of pollen fertility and sterility percentage was calculated by using formula adapted by Ullah et al., (2018).

$$\text{Percentage of fertility} = \text{Fertility} / F + S \times 100$$

$$\text{Percentage of Sterility} = \text{Sterility} / S + F \times 100$$

Where F represents fertile pollen numbers and S represents sterile pollen numbers on slide.

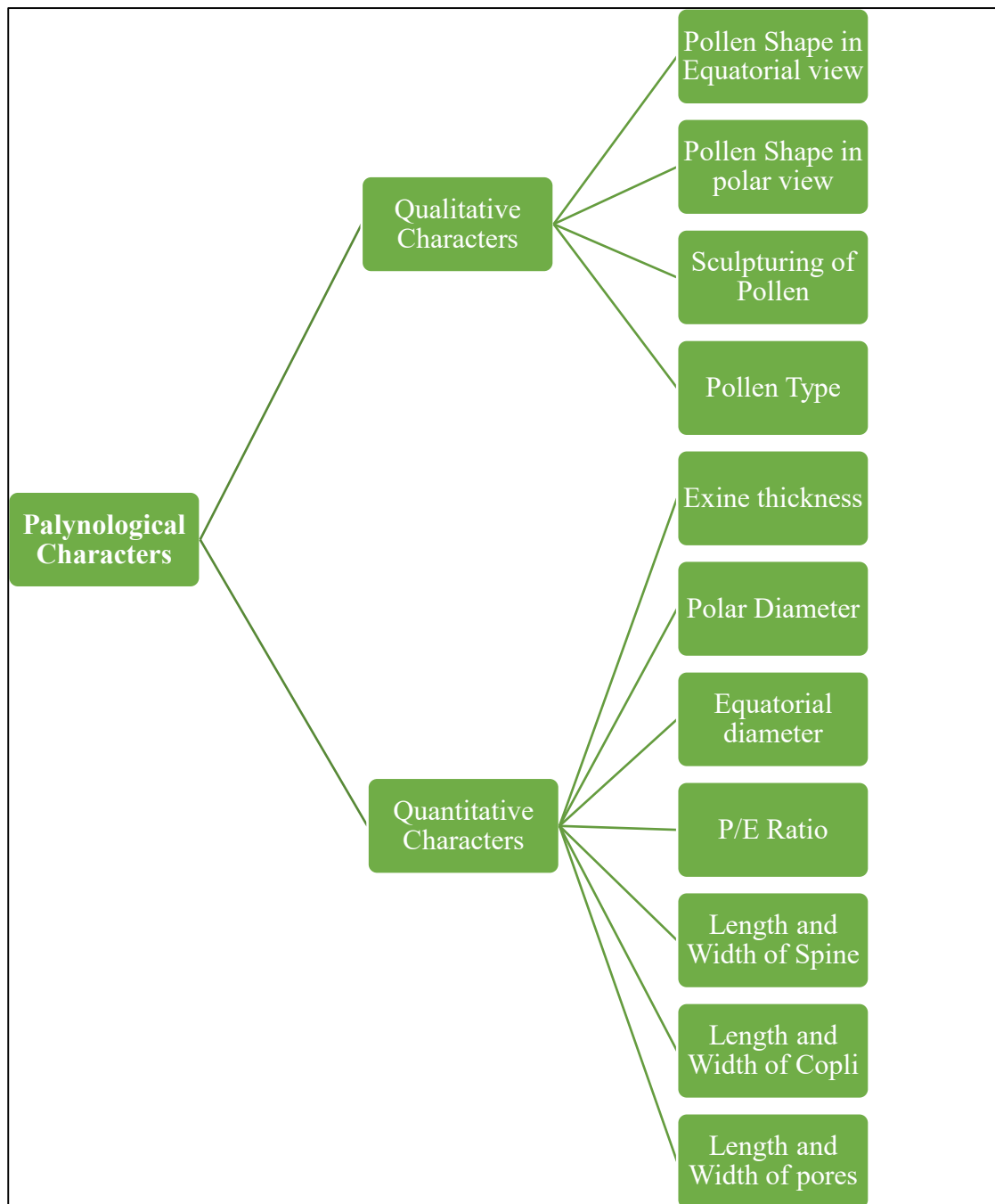


Figure 3: Schematic Representation of Pollen Parameters Characterization

2.6. Seed Morphological Studies

2.6.1 Taxon Sampling

In order to obtain seeds, mature seeds of invasive plants were removed from fruits collected during field visits in fruiting season.

2.6.2 Light Microscopic Studies of Seed Morphology

Mature seeds of invasive plants were washed through different grades of ethanol (60-80%) for 1 to 3 minutes to remove the debris on their surface. External color, Shape, Size (length & width), Hilum were observed using Binocular Dissecting microscope, placed in plant anatomy Lab Department of Plant sciences Quaid-i-Azam University, Islamabad, Pakistan.

2.6.3 Scanning Electron Microscopic Studies of Seed Morphology

For SEM studies seeds were immersed in absolute ethanol and left for a minute to completely dry. This step was carried out for detailed study of invasive seed morphology. Then seeds were mounted on stubs in different positions and gold coating was performed in sputtering chamber using SPI-MODEL™. In all cases seeds of at least 10 samples of each species were analyzed, characterized and photographed with a Scan Electron Microscope (Model JEOL JSM- 5910). The seed characters observed were length of main axis, position of hilum, surface sculpture.

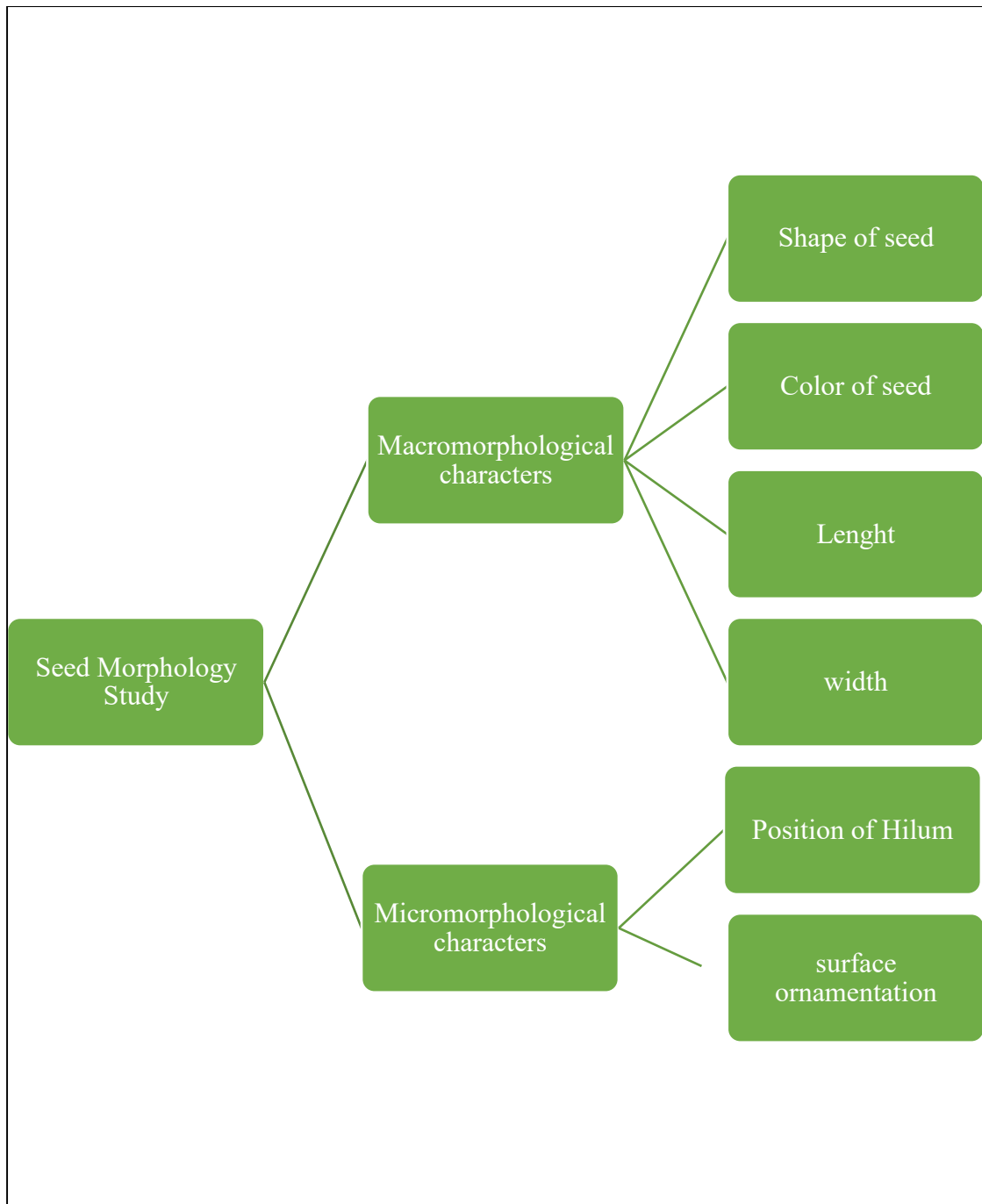


Figure 4: Schematic Demonstration of Study Parameters for Invasive Seed Characterization



Plate 1: Field collection of invasive plants of Lesser Himalayas Pakistan (A) *Euphorbia helioscopia* (B) *Cannabis sativa*



Plate 2: Microscopic study of Pollen of Invasive plants of Lesser Himalaya Pakistan
(A) Optical Microscopic observation (B) Scanning electron microscopic analysis

CHAPTER: 3

Results & Discussion

3. Key Findings of Invasive Plants

Himalaya are globally very important plant diversity hotspots while currently facing rapid loss in biodiversity by wider spread of alien plants. The Lesser Himalayan regions have always been encroached by invasive plants. In this region wide range of climatic zones are available due to great variation in attitude. These conditions supports the luxuriant growth of rich and diversified vegetation. In this regard to best of our knowledge based on literature studies, a very few studies have been conducted to document invasive plants of this region. Therefore the present research study has been carried out to investigate the diversity and distribution of invasive plants with special emphasis on two important para meters i.e. pollen and seed morphology of invasive plants using detailed studies by LM and SEM techniques in order to understand the qualitative and quantitative characters with respect to invasiveness behavior of alien pant diversity. In total of 52 invasive plant species belonging 31 families were compiled and presented separately in two sections.

Section 1: Palynological Findings of Invasive Plants

Qualitative characters include pollen type, pollen shape, and exine sculpturing while quantitative characters include polar dimeter, equatorial diameter, P/E ratio, pore size, exine thickness, colpi number, colpi size and pollen fertility.

Section 2: Seed Morphology of Invasive Plants

Macro-morphological characters include shape of seed, color of seed, length of seed, width of seed and Hilum shape while micro-morphological characters includes position of hilum, surface ornamentation.

Table 2 presents the detailed information of alien invasive plant species with inventory including botanical name followed by family, common name/English name, global distribution, nativity, flowering period, flower color, habit, habitat, life form and purpose of introduction.

The most dominant family in term of invasiveness was Asteraceae (11 species) followed by Fabaceae (04 species), Amaranthaceae, Verbenaceae (03 species each) (Figure 43). Similarly Vikrant et al., (2012) carried out a study regarding the alien invasive plants of Indian Himalayan State of Himachal Pradesh and he also reported that family Asteraceae as a dominant family followed by Poaceae. Studies reported from Europe (Lambdon et al., 2008) and China (Weber et al., 2008; Wu et al., 2010) also reported similar patterns of family dominancy in alien floras as observed in this study. Invasive plants in this region were introduced for different purposes and data analysis revealed that 29 species were introduced or established unintentionally, 13 species as ornamental, 1 species as food, 1 as fodder, 5 as plantation, 3 for medicinal purposes (Figure 46). These results are in accordance with the findings of Reichard and White 2001, Mack and Erneberg 2002. Data shows that the life form of invasive species includes perennials of about 27 while 21 species were annual life form (Figure 44).

It is also noted that the dominant life form was herbaceous i.e. 31 species followed by shrub (11) and trees (8), whereas the grasses and under shrubs were represented by only 1 species each i.e. *Sorghum halepense* and *Cassia occidentalis* (Fig 45).

Table 2: Checklist of Alien Invasive Plants with Distribution Pattern in Lesser Himalaya

S. No.	Taxa	Family	Common English Name	Global Distribution	Nativity	Flowering Period	Flower Color	Habit	Habitat	Life Form	Purpose of Introduction
1.	<i>Achyranthes aspera</i> L.	Amaranthaceae	Prickly chaff flower, Devil's horsewhip	Asian Himalayan, Afghanistan, Pakistan, India, Nepal, Bhutan, China	South-East Asia and Africa	July-December	Greenish-white often tinged with purple-red	Perennial	Cultivated Fields, Grasslands, Forest, Disturbed Areas, Waste Lands	Herb	Unintentional
2.	<i>Ageratum conyzoides</i> L.	Asteraceae	Goat weed	South America, Africa, the Indian Sub-continent, south-eastern Asia, southern USA	South America	Throughout the year	Violet	Annual	Disturbed areas, Roadsides	Herb	Ornamental
3.	<i>Ailanthus altissima</i> (Mill.) Swingle	Simaroubaceae	Tree of Heaven	Tropical Asia, Japan, Northern Africa, Central and Southern Europe, Indian Sub-continent	Asia	June-July	White or Greenish Yellow	Perennial	Disturbed Sites, Waste Areas, Road Sides, Railways	Tree	Plantation
4.	<i>Anagallis arvensis</i> L.	Primulaceae	Scarlet Pimpernel	South America, Tropics, Subtropics, Temperate Countries	North Africa	March-September	Blue	Annual	Crops, Parks, Lawns, roadsides, disturbed sites, waste areas	Herb	Unintentional
5.	<i>Argemone ochroleuca</i> Sweet, Brit. Fl. Gard.	Papaveraceae	Mexican prickly poppy	Eurasia, North Africa, North and South America, New Zealand, Australia	Mexico	March-May	White/ Creamish White, Becomes Yellowish Upon Fading	Annual	Roadsides, Waste Areas, Disturbed Sites, Gardens	Herb	Unintentional

6.	<i>Azadirachta indica</i> A.Juss.	Meliaceae	Indian lilac, Neem	America, Caribbean, East Asia, Australia, India and Southern Africa	India & China	February - June	White	Perennial	Shrub Lands, Grasslands, Coastal Sites, Disturbed natural vegetation	Tree	Medicinal
7.	<i>Bidens pilosa</i> L.	Asteraceae	Beggar's Ticks	Tropical Pakistan, India, Malaysia, Philippine Africa	South & Central America	March -May	White & Yellow	Annual	Gardens, Parks, Crops, Roadsides, Disturbed Sites, Waste Areas	Herb	Unintentional
8.	<i>Brachychiton acerifolius</i> (A.Cunn. ex G.Don) F.Muell.	Malvaceae	Australian Flame Tree	Asia, Australia, Southern Africa	Eastern Australia	March -June	Red	Perennial	Gardens	Tree	Ornamental
9.	<i>Broussonetia papyrifera</i> (L.) L'Hér. ex Vent.	Moraceae	Paper Mulberry	Temperate Regions of Both Hemispheres in The Old World	S.E. Asia	March- May	Pale Green, Red	Perennial	Roadsides	Tree	Plantation
10.	<i>Bryophyllum pinnatum</i> (Lam.) Oken	Crassulaceae	Cathedral Bells, Air Plant	Warmer Regions of The Temperate Zone to The Tropical Zone All Around the World	Madagascar and southern Africa	January- February	green striated red	Perennial	Gardens, Roadsides, Waste Areas	Herb	Ornamental
11.	<i>Calotropis procera</i> (Aiton) Dryand.	Apocynaceae	Apple of Sodom	Afghanistan, Nepal, France, Italy, China	northern and tropical Africa, Western Asia, South Asia	Throughout the year	Purplish inside white outside	Perennial	Disturbed Sites, Roadsides, Waste Areas	Shrub	Unintentional
12.	<i>Cannabis sativa</i> L.	Cannabaceae	Hemp	Europe, Northern and Central Asia	Central Asia	April- September	Whitish to Yellowish- Green	Perennial	Waste Lands, Road Sides	Herb	Unintentional

13.	<i>Callistemon citrinus</i> (Curtis) Skeels .	Myrtaceae	Lemon Bottle brush	Asia, China, India	E. & SE. Australia	Feb-April	Red	Perennial	Gardens, Parks	Shrub	Ornamental
14.	<i>Carthamus oxyacantha</i> M.Bieb.	Asteraceae	Wild Safflower	SW America, Warm Temperate, Tropical and Subtropical Regions	Caucasus to Central Asia	March to June	Yellow	Annual	Waste Areas, Agriculture Fields, Road Sides	Herb	Unintentional
15.	<i>Cassia occidentalis</i> L.	Fabaceae	Coffee Senna, Negro Coffee	Northern Mexico, Eastern Asia, Australia, Western Asia, Indian Sub-Continent, Tropical Southern America	Tropical America	Summer and autumn	Yellow	Annual	Roadsides, Wastelands, Disturbed Sites, Grass Lands	Under Shrub	Unintentional
16.	<i>Chenopodium ambrosioides</i> L.	Amaranthaceae	Mexican-tea	Mexico, Central America, Brazil, The Caribbean, Africa, Western Asia, Indian Sub-Continent	Tropical America	April to June	Yellowish Green	Annual/ Perennial	Gardens, Roadsides,	Herb	Unintentional
17.	<i>Commelina benghalensis</i> L.	Commelinaceae	Tropical Spiderwort	India, Sri-Lanka, Thailand, South Africa, Philippines, Myanmar, China and Pakistan	Tropical Asia & Africa	Throughout the year	Blue	Annual	Disturbed Sites, Road Sides, Agriculture Fields, Gardens	Herb	Unintentional
18.	<i>Convolvulus arvensis</i> L.	Convolvulaceae	Creeping Jenny/ Field Bindweed	Warm Temperate to Tropical Areas of the World	Europe	Throughout the year	White	Annual/ Perennial	Agriculture Fields, Gardens, Road Sides, waste lands	Herb	Unintentional
19.	<i>Conyza canadensis</i> L.	Asteraceae	Horse weed	North America, Pakistan, India, Central Asia and North Africa	North America	May-October	White	Annual	River Banks, Road sides, Fields	herb	Unintentional

20.	<i>Datura innoxia</i> Mill.	Asteraceae	Downy Thorn Apple	Mexico Central America The Caribbean, Africa, India And In South-Eastern Asia, Pacific Islands	Central & South America	May-October	White	Perennial	Road Sides, Waste Lands	Herb	Medicinal	
21.	<i>Digera muricata</i> (L.) Mart.	Amaranthaceae	False Amaranth	China, Taiwan, Germany, Mexico, Newzeland	Eastern Africa	June to October	Purple	Annual	Disturbed Areas, Wasteland	Herb	Unintentional	
22.	<i>Dodonaea viscosa</i> (Linn.) Jacq.	Spindaceae	Hopbush	Europe, N. Africa, S. and W-Asia	Australia, India	Jan-March	Greenish Yellow	Perennial	Disturbed Areas, Hillsides	Shrub	Unintentional	
23.	<i>Eucalyptus camaldulensis</i> Dehnh	Myrtaceae	Red River Gum	East Asia, Vietnam, China,, Thailand, Indonesia	India, Central, Nepal,	Australia	March-May	White	Perennial	Roadside, Forests	Tree	Plantation
24.	<i>Rosa alba</i> L.	Roseaceae	White Rose	Throughout world	Eurpoe, North America	March-June	White	Perennial	Gardens,	Shrub	Ornamental	
25.	<i>Jasminum humile</i> L.	Oleaceae	Yellow Jasmin	Morocco, Yemen, Victoria, Colombia	Srilanka, Finland, China	E. Asia -	March-June	Yellow	Perennial	Gardens, 1500 - 3000 metres in Himalayas	Shrub	Ornamental
26.	<i>Justicia adhatoda</i> L.	Acanthaceae	Malabar nut, Adhatoda	Southern Europe, South Australia, Southern Russia, Asia minor, North America	Indian sub continent, Sri Lanka	November-April (plains); July-October (hills)	White	Perennial	River Banks, at elevations from sea level to 1,300m	Shrub	Ornamental	
27.	<i>Lantana urticoides</i> Hayek	Verbenaceae	Texas lantana/ calico bush	Tropical Regions of World, Central and South America	USA/ Mexico	April - October	Yellow, red orange	Perennial	Woodland, Wastelands	Shrub	Ornamental	
28.	<i>Lantana camara</i> L.	Verbenaceae	Common Lantana	Europe, North Africa, West Central Asia	South America	Throughout The Year	Orange or Yellow, Turning To Red or Scarlet Later	Perennial	Roadsides, Waste Lands, Grasslands	Shrub	Ornamental	

29.	<i>Malvastrum coromandelianum</i> (L.) Garcke	Malvaceae	Three Lobe False Mallow	Mexico, Central America, Caribbean and Tropical South America, South-Eastern Asia, Oceania	North & South America	March -June	Cream/ Pale yellow	Annual/ Perennial	Disturbed Areas, Roadsides,	Herb	Unintentional
30.	<i>Melilotus indicus</i> (L.) All.	Fabaceae	Indian Sweet-Clover	Tropical And Sub-Tropical Southern and Eastern USA	North Africa, Europe, Asia	March- August	Pale yellow	Annual	Agriculture Fields	Herb	Unintentional
31.	<i>Nasturtium officinale</i> R.Br.	Brassicaceae	Watercress	Tropical Africa, China, Afghanistan	Europe	March- July	Milky White	Perennial	Steady and Flowing Waters, Shores, Swamps	Herb	Unintentional
32.	<i>Oxalis corniculata</i> L.	Oxalidaceae	Yellow Sorrel	China, Denmark, New Mexico, New York, New Zealand, Europe, Turkey	Europe	March - December	Pale Yellow	Annual/ Perennial	Waste Lands, Garden Weed	Herb	Unintentional
33.	<i>Parthenium hysterophorus</i> L.	Asteraceae	Congress Grass	Tropics regions of Europe, Africa, Asia, Australia, and Americas	Central America	October- March	white	Annual	Waste Lands, Disturbed Areas	herb	Unintentional
34.	<i>Prosopis juliflora</i> (Sw.) DC.	Fabaceae	Ironwood/Mesquite	East Asia, USA, China, Hawaii, Africa, New Zealand,	South America	March- June	Light Yellow	Perennial	Wastelands	Tree	Plantation
35.	<i>Quisqualis indica</i> L.	Combretaceae	Rangoon creeper	Tropical America, Japan, West and South Africa, Indonesia	Tropical Africa	March- August	Pink- Red	Perennial	Hillsides, Riversides, Roadsides,	Shrub	Ornamental
36.	<i>Ranunculus muricatus</i> L.	Ranunculaceae	Sharp Buttercup	Atlantic and S. Europe, W. & S. W. Asia, Crimea, Caucasus, S. Siberia, Pakistan, India	Southern Europe	March-April	Yellow	Annual	Gardens, Roadsides, Disturbed Areas	Herb	Unintentional

37.	<i>Ranunculus arvensis</i> L.	Ranunculaceae	Corn Buttercup	Siberia, Western and S. W. Asia to India and the Himalaya	Europe	March-April	Yellow-greenish yellow	Annual	Gardens, Roadsides, Disturbed Sites	Herb	Unintentional	
38.	<i>Ricinus communis</i> L.	Euphorbiaceae	Castor-Oil Plant	Africa, America, Asia, Europe, Benin	Africa	Almost throughout the year	Yellow	Perennial	Roadsides, railways, Wastelands Pastures, Gardens	Shrub	Food	
39.	<i>Robinia pseudoacacia</i> L.	Fabaceae	black locust	Malaysia, Argentina, Mexico, Queensland, Palestine	India, Iraq, Eastern USA	March- April	calyx reddish-purple, petals are white	Perennial	Roadsides, Disturbed Sites, Waste Lands, Gardens	Tree	Plantation	
40.	<i>Solanum surattense</i> Burm. f.	Solanaceae	Wild Eggplant	Brazil, Colombia, Mexico, Sri Lanka	North Africa, S &S.E Asia	May- November	Purple	Perennial	Disturbed Areas	Herb	Unintentional	
41.	<i>Sapium sebiferum</i> (L.) Roxb.	Euphorbiaceae	Chinese Tallow Tree, Popcorn Tree	Arabian Peninsula, Northern Africa, Ukraine, Sub-Continent	China/ Taiwan	January- February	Yellow	Perennial	Riverside, Wet Forests, Grassland	Tree	Ornamental	
42.	<i>Sesamum indicum</i> L.	Pedaliaceae	Sesame	India, Sri Lanka, Africa, Sudan, Australia	Asia or Tropical Africa	June-October	Pinkish purple	Annual	Cultivated as crop	Herb	Unintentional	
43.	<i>Silybum marianum</i> (L.) Gaertn.	Asteraceae	Milk Thistle	Asia, twain, china, Korea	Japan, India, Africa/Europe	March- June	Purple	Annual	Waste Lands, Road Sides, Agriculture Fields,	Herb	Unintentional	
44.	<i>Sonchus oleraceus</i> L.	Asteraceae	Sow Thistle	Uganda, Brazil, Zimbabwe	Argentina, Mexica,	Europe	March -April	Yellow	Annual	Waste Lands, Agriculture Fields	Herb	Unintentional
45.	<i>Sorghum halepense</i> (L.) Pers.	Poaceae	Johnson Grass	Bolivia, Brazil, Argentina	Paraguay, Argentina	Europe	May-October	Cream to Buff-Yellow	Perennial	Dry Open Habitats	Grass	Fodder

46.	<i>Taraxacum campyloides</i> G.E.Haglund	Asteraceae	Common Dandelion	America, Pakistan, India, China	Europe and Western Asia	Feb-April	Yellow	Annual	Roadsides, Gardens, Agriculture Fields	Herb	Unintentional
47.	<i>Tecoma stans</i> (L.) Juss. ex Kunth	Bignoniaceae	Yellow Bells	S. America, W. Indies, Africa, Australia	Tropical America	September - December	Yellow	Perennial	Roadsides, waterways, Grass land, Waste Areas	Shrub	Ornamental
48.	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Puncture Vine	USA, Greece, Cyprus, Spain, Australia	France, India, Portugal, Africa	August-November	Yellow	Annual	Grasslands, Dunes	Herb	Unintentional
49.	<i>Tropaeolum majus</i> L.	Tropaeolaceae	Garden Nasturtium	South Europe, Pakistan	Africa, Hawaii, & Europe	Feb-April	Orange	Annual	Gardens, Parks,	Herb	Ornamental
50.	<i>Verbena officinalis</i> L.	Verbenaceae	Common Verbena	Tropical Subtropical America, Easter Island, SE. Canada	USA/ Mexico	June-December	Pale Pink/Purplish	Perennial	Waste Lands, agriculture Fields	Herb	Medicinal
51.	<i>Verbesina encelioides</i> (Cav.) Benth. & Hook.f. ex A.Gray	Asteraceae	Golden Crownbeard	North Siberia, Europe, Iran, Afghanistan, Pakistan	Africa, India, Maxico	March-June	Yellow	Annual	Woodlands, Grasslands, Roadsides, Disturbed Areas	Herb	Unintentional
52.	<i>Xanthium strumarium</i> L.	Asteraceae	Common Cocklebur	Throughout the world	America	October onwards	Yllo	Annual	Agriculture Fields, Roadsides, Disturbed Areas, Waste Areas	Herb	Unintentional

**Section I: Qualitative and Quantitative Morphological
Characteristics of Pollen**

3.1 Palynological Findings of Invasive Plants

3.1.1 *Achyranthes aspera* L.

Pollen morphological characters of invasive *Achyranthes aspera* were examined under SEM and LM. Monad and pantoporate pollen without spines were observed. The shape in the equatorial view was sub-prolate and circular in the polar view. Exine ornamentation was psilate. The equatorial diameter was 11.10 μm polar diameter was 12.80 μm , exine thickness was 1.85 μm , Pollen fertility estimation was noted 81.2% and sterility 18.8%. P/E index was 1.15 (Plate 3, Table 3). In comparison to our findings the pollen grains of *Achyranthes aspera* were studied by Hussain et al., 2018 and they reported Psilate to scabrate exine. Variation in morphology pollen grains and high fertility pollen rate results in successful invasion of this species in Lesser Himalaya. So far morphological features of pollen grains of *Achyranthes aspera* have not been analyzed in terms of their invasiveness role. Pollen viability, variation in morphology and germination have a greater impact on expansion properties (Lechowicz et al., 2021). SEM and LM study of pollen of invasive weeds like *Achyranthes aspera* is useful not only for their taxonomic study and to identify the invasive plant species but also to understand the invasive behavior.

3.1.2 *Ageratum conyzoides* L.

The current research findings on *Ageratum conyzoides* described the tricolporate pollen with prolate-spheroidal shape while exine ornamentation was echinate. These results are similar to previous work done by Younis et al., 2021. Maximum equatorial diameter is 19.6 μm and maximum polar diameter was 20.6 μm . The exine thickness was 2.8 μm . the mean of colpi length and width was 4.50 μm and 6.55 μm respectively. Mesocolpium was 11.7 μm . P/E ratio was 1.05. The earlier findings of Zafar et al., (2007) stated that in *Ageratum conyzoides* pollen of circular shaped in both views with exine thickness of 1.2 μm was observed whereas outcomes of our research elaborate pollen with 2.8 μm thickness.

The high thickness of pollen in invasive species increase viability of pollen thus such plants have high rate of invasiveness. Pollen fertility of 94.1% was calculated.

According to Nazir et al., (2013) rate of pollen fertility is helpful tool that provides the information about the plant growing in the unfavorable conditions or out of their natural range (Plate 4).

Ageratum conyzoides (Asteraceae) is native to Tropical America but now this weed is invasive in many parts of globe. According to Rahman et al., (2008) members of this family are scattered all over the world in the semi-arid tropical regions, subtropical regions and temperate regions of South East Asia, Africa, Madagascar and Central South America. Asteraceae is most dominant family of invasive plants collected from Lesser Himalaya in present investigation. Kong et al., (1999) reported *A. conyzoides* as allelopathic weed. According to a study in India by Dogra et al., (2009) the plant diversity was reduced by 32% in area invaded by *A. conyzoides*.

3.1.3 *Anagallis arvensis* L.

The results of study demonstrate the pollen type was monad, tricolporate, and spines were absent. Shape in equatorial view was spheroidal and circular in polar view. The earlier findings of Khan et al., (2021b) are in accordance with our study. Calculated equatorial diameter was 27.25 μm and polar diameter was 23.25 μm . Mean value of exine thickness, colpi length, colpi width, was 4.55 μm , 7.05 μm , 4.75 μm respectively while maximum length of mesocolpium was 14.50 μm . P/E ratio was noted that i.e. 0.85. Pollen fertility noted was 94.1% and sterility was 5.88%. Verrucate Exine ornamentation was observed while the findings of Moneim et al., (2003) explained the pollen having reticulate ornamentation. The size and shape of pollen grains are two important traits which enhance the dispersal of pollen and ultimately helpful in invasion mechanism. In this study in *Anagallis arvensis* the spherical pollen were investigated. Ackerman (2002) stated that spherical pollen can be dispersed with high velocity and longer distance so there is lower friction force in spherical particles. These characters of pollen play key role in successful invasion of invasive species (Plate 5).

Anagallis arvensis is a known as common annual or biennial weed which mainly found in Southern Europe and extends to Mediterranean part of Britain and Northern Europe (Stace 1997). According to Chevallier (1996) this weed has been cultivated for medicinal purposes but now naturalized in many parts of world. Pollen

morphological studies are carried out as they play an important role in modern taxonomy for the identification and delineation of species not only in native also in invasive species (Sufyan et al., 2018). Number of features like size, shape, surface morphology, and ultrastructure of pollens are of significant value in characterization of pollen grains (Dyakova, 2014) and variations in these features are of key importance in dispersal of invasive species.

3.1.4 *Bidens pilosa* L.

Our demonstration stated that pollen of *Bidens pilosa* was tricolporate with spines and shape in equatorial view was oblate-spheroidal and circular in polar view. Exine ornamentation was echinate. The Palynological studies on five species of family Asteraceae was conducted by Mbagwu et al., in 2009 they also reported echinate pollen surface but with porate pollen aperture in species. The pollen of Asteraceae is characterized by finite spines (Zafar et al., 2007). Echinate pollen have can be dispersal at longer distances as they can stick with bodies of pollinators and play key role in invasion mechanism of invaders. . According to Meo and Khan 2006, spines of family Asteraceae are highly distinctive and serve as an important to delimit the taxa at genus level. The measurements for quantitative characters were also noted, equatorial diameter was 28.5µm, and polar diameter was 27.8. Exine thickness was 4.11 µm, and colpi length was 4.10 µm, colpi width was 2.40µm, spine length was 5.05µm, spine width was 2.75µm. Mesocolpium was 14.7µm, P/E ratio is 0.97. Pollen fertility noted was 97.3% and sterility is 2.67%. The high rate of pollen fertility in *Bidens pilosa* is another factor which stimulates the invasion. Salamah et al., (2019) also examined echinate spines while studying the pollen morphology of eight (08) tribes of Asteraceae from Indonesia, results correlates with present study.

Study of Klimo et al., 2000 indicates that mature pollen size, number of pores, and exine sculpturing are the most attributes which help in correct identification and delimitation of taxa at genus and species level. *Bidens pilosa* belongs to family Asteraceae.

3.1.5 *Brachychiton acerifolius* (A.Cunn. ex G.Don) F.Muell.

Brachychiton acerifolius was introduced as ornamental plant which is commonly known as Australian Flame Tree and belongs to family Malvaceae (cotton

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or mallow family). Palynomorphological characters of this plant was noted using LM and SEM. These characters play significant role at different level of taxonomic hierarchy i.e. generic, subgeneric and interspecific (Santos et al., 2018). Tricolporate and prolate-spheroidal pollen was studied in *Brachychiton acerifolius*. The exine ornamentation was scabrate, verrucate and gemmate. The equatorial and polar diameter was 25.8 μ m and 27.1 μ m. Exine thickness was 4.40 while almost same colpi length and colpi width was observed i.e. 6.3 μ m and 7 μ m. Maximum distance of mesocolpium was 13.1. Polar to equatorial ratio was 1.05 (Plate 7). Yasmeeen et al., in 2010 has proved that P/E is a useful character of systematic importance. The study of Kodela (2000), on pollen morphology of *Brachychiton acerifolius* described colporate, circular, medium size pollen with exine thickness of 1.8 μ m while studying Pollen morphology of some rainforest taxa occurring in the Illawarra region of New South Wales, Australia, which was differs from present findings. These variations in pollen morphology of invasive and native *Brachychiton acerifolius* helps in successful spread and invasion of this species in Lesser Himalaya.

High pollen fertility (95.6 %) was calculated in *Brachychiton acerifolius*. The sufficient pollen availability is an essential process in the reproductive success of invasive plants Bell et al., (2005).

3.1.6 *Broussonetia papyrifera* (L.) L'Hér. ex Vent.

Broussonetia papyrifera was introduced in Pakistan to make it beautiful in 1960's. The extent of biological invasion can be understand with the help of phytosociological studies. As per study of Malik and Hussain in 2007, factors like tremendous growth rate, effective dispersal by birds and strategy of vegetative regeneration played a significant role to its success. Qureshi et al., (2020) carried out a study on invasion impact analysis of *Broussonetia papyrifera* on native vegetation in Pothwar region of Pakistan and found that there was a decrease in diversity indices in invaded areas which indicates that due to Paper mulberry invasion plant communities become less productive. Now paper mulberry is considered as a most problematic plant in northern Pakistan. (Ashraf et al., 2012) and its pollen act as major causative agents in inhalant allergy (Abbas et al., 2012) as this species produces enormous number of

pollen. Therefore appropriate measures are required to control and manage this highly invasive plant.

The plant family to which paper mulberry belongs is the Moraceae. This family includes figs and so many other plants that are good source of food and fiber (Whistler, 2009 and Zerega et al., 2005). In present research study palynomorphological characters were studied using LM and SEM. The pollen type was monad and porate in *Broussonetia papyrifera* while spines were absent. Shape in equatorial view is subprolate and irregular in polar view. Scabrate-verrucate exine ornamentation was observed. The equatorial diameter and polar diameter was 10.75 μ m and 12.75 respectively. The mean exine thickness was 2.00 μ m, while maximum colpi length and colpi width was 3.35 μ m and 5.55 μ m. The calculated P/E ratio was 1.18 in *Broussonetia papyrifera*. Pollen fertility noted was 89.1% and sterility is 10.8%. The earlier study by Kim and Zavada (1993) found dissimilarity that *B. papyrifera* had diporate pollen with scabrate sculpturing. Pollen morphological characters such as surface sculpturing, pollen size, shape and outline are useful in distinguishing the taxa (Teleb and Salah-El-din 2014). This highly reproductive invasive plant possess wide range of adaptations like large number of pollen production, variations in pollen morphological characters and numerous seed production that determine its success in various environments (Plate 6).

3.1.7 *Bryophyllum pinnatum* (Lam.) Oken

The family to which *Bryophyllum pinnatum* belongs is Crassulaceae that is morphologically diverse and systematically complex angiospermic family consist of 35 genera and 1500 species (Berger 1930) which are distributed all over the world. Members of family Crassulaceae are characterized by follicle fruit, succulent habit, numerous and very small seeds. Crassulaceae is a eurypalynous family at the generic level. According to Sarwar, 2002 this family is represented by 9 genera and 32 species. *Bryophyllum pinnatum* is predominant in South Africa and introduced in Pakistan and other parts of world as an ornamental plant. Tricolpate pollen with prolate-spheroidal shape were examined in present study in *Bryophyllum pinnatum* while exine sculpturing was psilate.

In case of *Bryophyllum pinnatum* the previous findings of Qaiser et al., (2015) on the pollen morphology of 28 species of family Crassulaceae that pollen grains were prolate spheroidal shaped pollen as similar in our study but tricolporate with sub psilate exine ornamentation. Equatorial diameter of *Bryophyllum pinnatum* was 7.67 μ m and polar diameter was 7.75 μ m. According to Ullah et al., (2018b), polar size measured in terms of polar and equatorial diameter is valuable for correct identification of species. 0.88 μ m exine thickness was calculated while mesocolpium was 0.67 μ m, P/E ratio was 1.01. Pollen fertility was also noted i.e. 95.6% and sterility 4.34%. The diversity in pollen morphological characters using LM and SEM provides evidence about variations in pollen type, exine sculpturing and pollen apertures. Furthermore, variations in pollen grains characters give this invasive species the ability to produce seeds at different times and in high quantities that could contribute to the invasiveness of this species (Plate 7).

3.1.8 *Cannabis sativa* L.

Single collected invasive alien plant species from area under study of family Cannabaceae is *Cannabis sativa* which is dealt as most aggressive weed of study area. Family Cannabaceae is generally called Hemp family. Hashim et al., (2003), studied 36 problematic weeds from Abbottabad district, Pakistan, out of which 16 were invasive weeds and results indicates that *Cannabis sativa* was introduced unintentionally and later on became aggressive by replacing or suppressing native vegetation.

Pollen grain size, exine ornamentation and number of pores are known as most distinctive features which are helpful in successful invasion. The findings of our study demonstrate that in *C. sativa* the pollen grains were monad, tricolpate and without spines. Prolate-spheroidal shape in equatorial view while angular in polar view was noted while exine sculpturing was reticulate. Equatorial diameter was 18.3 μ m and 19.4 μ m polar diameter was calculated. In this invasive plant species exine thickness was 2.60 μ m, and colpi length and colpi width was 2.60 μ m and 1.90 μ m respectively while mesocolpium was 11.0 μ m. The spheroidal shaped pollens were also reported in this invasive species by Shinwari et al., (2015) are in agreement with our findings. Whereas our results are not in agreement in case of this invasive species with previous work done by Sufyan et al., (2018). The high pollen fertility rate (92.1) was noted in this invasive species. Pollen viability or sterility have been affected by various environmental factors like temperature, salinity, light intensity and substrate humidity (Khatun and Flowers *Morpho-Palynological and Seed Diversity of Invasive Plants in Lesser Himalaya Pakistan*

1995), these factors favors high pollen production which ultimately favors the spread of this species (Plate 8).

3.1.9 *Carthamus oxyacantha* M.Bieb.

Carthamus oxyacantha is very common, troublesome weed in crop fields. Its history of invasion is not exactly known but it may be transported with wheat seeds 25-30 years back (Khan et al., 2011). Invasive weeds are one of the greatest threats to indigenous flora because they reduce the abundance and diversity of local flora and fauna and also shows adverse effects on ecosystem processes.

The pollen grains of *Carthamus oxyacantha* were examined and demonstrated using light microscopy and scanning electron microscopy and monad, radically symmetrical, echinate, micro perforate, large sized, angular in polar view, oblate-spheroidal and tricolporate grains were examined. The findings are similar to the work done by Gul et al., (2021). Aperture orientation is bulged, polar region small to medium, spine irregular with very short broad bases and lacunas was absent.

The observed equatorial and polar diameter was 26.3 μ m and 25 μ m respectively. Colpi length 2.61 μ m, colpi width 2.78 μ m, spine length 1.92 μ m and width 0.4 μ m and mesocolpium length was 21.7 μ m. P/E ratio was calculated 0.95, while exine thickness was 3.18 μ m. The pollen morphology of 6 *Carthamus* species found Turkey was examined by Bülbül et al., (2013), and results are in accordance with the present findings. According to Gul et al., (2021), the pollen morphology has a valuable importance in understanding of taxonomy of different plants groups and play essential role in correct identification of Asteraceous flora at species, generic and tribe levels. Pollen viability estimations shows that 86.6 % pollen are viable. The high pollen viability and variations in pollen morphology are the main reasons for spread of *C. oxyacantha* in studied region (Plate 9).

3.1.10 *Cassia occidentalis* L.

Both qualitative and quantitative characters of *Cassia occidentalis* were observed i.e. equatorial and polar diameter numbers of pores and colpi, exine sculpturing and thickness, pollen shape and P/E ratio. Pollen type was monad and tricolporate. Shape observed in equatorial view was prolate-spheroidal and spheroidal

in polar view and triangular in general view while their sculpturing was examined psilate with smooth surface. Similar findings in terms of shape of pollen and exine sculpturing were studied by Basarkar in 2017 but pollen were tricolpate. Reddy et al., (2016) carried out a study on pollen morphology 10 species of *Cassia* L. belong to Nalgonda District, Telangana State, South India and found tricolporate and prolate to spheroidal shaped pollen which correlates with current study.

Quantitative findings shows that maximum equatorial diameter, polar diameter, exine thickness was 36.9 μ m, 41.2 μ m and 3.50 μ m respectively. While colpi length was 2.45 μ m, colpi width was 3.4 μ m. P/E ratio was calculated for *Cassia occidentalis* i.e. 1.11. Pollen fertility noted were 96.9% and sterility was 3.1%. In flowering plants pollen fertility is vital in fruit and seed production (Rigamoto and Tyagi, 2002). According to Nnamani and Onu (2014) palynological features have proven valuable at all taxonomic levels as structure of pollen wall is usually is not affected by environmental factors and pollens also remain viable for long periods and high rate of pollen viability facilitate the spread and establishment of *Cassia occidentalis* (Plate 10).

Among the conservationists during the past two decades, alien invasive plants are becoming the major concern as these plants have caused serious economic and ecological damage throughout the world (Das and Duarah, 2013). *Cassia occidentalis* belongs to family Fabaceae, native to South America but now distributed in many parts of world including Pakistan. It is cultivated for medicinal purposes but invaded in disturbed areas and replaces the local biodiversity. *Cassia occidentalis* is considered as an invasive species with moderate impact on ecology. The native flora is greatly affected by this due to high allelopathic activity. *Cassia occidentalis* is common weed scattered in Lesser Himalaya Pakistan. So understanding the pollen morphological characters are necessary as these traits are essential mediators for the establishment of invasive species.

3.1.11 *Callistemon citrinus* (Curtis) Skeels

Callistemon citrinus is an invasive plant in the family Myrtaceae and endemic to Eastern Australia. The family Myrtaceae comprises of 140 genera and 3,400 species and mainly distributed in the tropics and subtropics (Rahman and Zaman 2015). *Callistemon citrinus* is mainly cultivated as ornamental plant but it has great medicinal

value. In present study morpho-palynological features of *Callistemon citrinus* were observed and findings shows that dispersal unit was monad and in equatorial view pollen were spheroidal in shape while in polar view shape of pollen was oblique. Pollens were tricolporate with colporus aperture as far as exine ornamentation is concerned it was Psilate with maximum thickness of 1.80 μ m. Equatorial diameter and polar diameter was also calculated and it was 17.85 μ m and 18.03 μ m respectively. Maximum Colpi length and width was 6.12 μ m and 3.45 μ m with mean mesocolpium length of 23.4 μ m. Polar to equatorial ratio was calculated that was 1.01. In *Callistemon citrinus* pollen fertility was 90.5%. Sharanya et al., (2014) carried research study on pollen morphology of *Callistemon citrinus* and investigations of study reveals that triporate pollen with smooth exine were observed which was different from our research. *Callistemon* flowers produce large amounts of readily accessible pollen as pollen fertility estimation shows and attract a wide range of pollinators. This feature play vital role in spread of this invasive species.

3.1.12 *Chenopodium ambrosioides* L.

Chenopodium ambrosioides is commonly known as Mexican tea, native to Tropical America but now it is widely distributed Mexico, Central America, Brazil, The Caribbean, Africa, Western Asia and Indian Sub-Continent. As an invasive plant species *Chenopodium ambrosioides* has strong allelopathic effects on surrounding plants so due to its allelopathic activity it replaces the native flora quickly. Hu et al., in 2012 studied the allelopathic effects of this plant on *Vicia faba* root tip cells, the study shows that volatile oil from *C. ambrosioides* act on surrounding plants by volatilization.

In present study pollen morphology features were examined by LM and SEM and Monad, small sized, spheroidal, and pantoporate grains was found in *C. ambrosioides*. Some other researchers on this family also reported pantoporate aperture pollen (Nowicke, 1975; Nowicke and Skvarla, 1979). Micro- perforate echinate exine sculpture has been observed. The micro echinate scabrate, exine sculpturing with sunken aperture orientation was reported by Nazish et al., (2019) that is not in accordance with present findings.

In this study, the polar and equatorial diameter of 20.0 μm and 19.6 μm was measured respectively. Exine thickness was 2.22 μm while 4.6 μm colpi length was noted. Colpi width was 5.8 μm with sunken orientation. The calculated P/E ratio and pollen fertility was 1.02 and 92.8% respectively. For the diversity of plant pollen fertility status is very critical. High rate of pollen fertility confirms the stability, rigorous reproduction process of pollen and establishment of *Chenopodium ambrosioides* in the study area (Plate 11).

3.1.13 *Commelina benghalensis* L.

In plant systematics palynomorphological characters are considered as a valuable tool. The single species of *Commelina* was collected from Lesser Himalayas Pakistan. *C. benghalensis* have shown medium size tricolporate pollens with sub prolate shape and generally free (monad unit). Pollen shape is known as useful in identification species and it is defined as ratio between length of polar axis and equatorial axis (P/E ratio).

The equatorial and polar diameter was 24.5 μm and 30.8 μm respectively. Psilate exine ornamentation with 0.90 μm exine thickness was noted while colpi length, width and mesocolpium of 1.00 μm , 1.55 μm , and 12.6 μm was measured. P/E ratio was 1.25. Pollen fertility noted was 91.9% and sterility is 8.1%. To draw evolutionary trends among the related taxa, pollen morphology has provided a useful phylogenetic information (Maw, 2020). The present results shows variations from the findings of Salamma et al., (2019) in which they observed prolate shaped pollen with microehinate exine in *C. benghalensis* while aperture was monosulcate, length of sulcus was 21-22 μm and width was 3-4.5 μm while thickness of exine was 1.75-2 μm (Plate 12).

Commelina benghalensis is problematic invasive plant of pastures and crop fields and according to Webster et al., (2005) it is listed as one of the world's worst weeds which affected 25 crops in 29 countries. Its reproductive flexibility is considered as a key to its invasiveness. Walker and Evenson (1985) found that it as fast growing and a high-volume seed producer. A study by Budd et al., (1979) indicates that *C. benghalensis* has ability to regenerate from stem fragments. Furthermore it shows high degree of tolerance to glyphosate (Culpepper et al., 2004). Mentioned characteristics

reveals that when *C. benghalensis* established, it becomes difficult to control in agronomic systems.

3.1.14 *Conyza canadensis* (L.) Cronquist

Conyza Canadensis is an annual plant native to North and Central America. This weed spreads rapidly by high number of wind dispersed seeds. According to Djurdjević et al., (2011) *Conyza Canadensis* plays a significant role due to the synthesis of secondary phenolic metabolites in the first phases of vegetation succession and the process of soil formation on the barren sandy area. The morphology of pollen was investigated in current study and results indicates that pollen were prolate-spheroidal in shape in equatorial view while circular in polar view and trizonocolporate type with echinate exine ornamentation. But tricolporate, oblate-spheroidal pollens with reticulate scabrate exine ornamentation was observed in *Conyza Canadensis* by Khan et al., (2021). The equatorial and polar diameter was 21.2µm and 23.3µm respectively. Exine thickness was 4.7µm, mesocolpium was 10.3µm, P/E ratio was 1.09. Spines length was 2.75µm and width 1.30(µm. Pollen fertility were noted 84.5% and sterility 15.5%. Khan et al., (2021) calculated 83.33% pollen fertility in this species which is almost similar to the present study. The reproductive traits associated with invasiveness of *Conyza* species were studied by Hao et al., (2009) and results indicates that autonomous seed and pollen production, rapid germination rate and high dispersal capability contributed significantly to the invasiveness of the species (Plate 13).

3.1.15 *Convolvulus arvensis* L.

Convolvulus arvensis is placed in morning glory family (Convolvulaceae), this species is native to Europe. The plants of this family are mostly found in tropical and temperate regions of world and widely cultivated as ornamental (Rajurkar et al., 2011). Worldwide *Convolvulus L.* from this family has about 250 species (Perveen et al., 1989). According to Telleria and Daners (2003), palynological study of this family has unique taxonomic significance. The pollen features provide fruitful taxonomic solutions at both generic and specific level. This plant species is mostly found in tropical and temperate regions of world but now widely distribution in Pakistan.

The oblate-spheroidal (in equatorial view) and circular (in polar view) tricolpate pollen with echinate ornamentation was studied in *Convolvulus arvensis* in the present study. The equatorial diameter was 13.9 μm while polar diameter of 13.85 μm was calculated. Exine thickness of 7.87 μm has been observed and polar to equatorial ratio was 0.99 for the selected species. The length of colpi was 4.83 μm which was greater than colpi width (0.55 μm) and 0.33 μm measurement of mesocolpium was calculated. Pollen fertility was calculated in percentage which was 92.9%. Zafar et al., (2006) studied the pollen morphology taxonomic significance of some weeds of district Rawalpindi Pakistan and reported monad, tricolporate pollen with circular shape in polar view with smooth surface that similar to current findings but shape of pollen in equatorial view was prolate (Plate 14). The shape and size of pollen are two important characters that enhance the dispersal (Jackson and Lyford 1999). Spherical particles have higher velocity and can be dispersed at longer distances as these particles faces less forces of friction than those with deviation from sphericity. So spherical shaped pollen and higher pollen fertility in *Convolvulus arvensis* plays critical role in successful invasiveness as reported in present study. Euopalynous nature of family Convolvulaceae was described by Ashfaq et al., (2018).

3.1.16 *Datura innoxia* Mill.

Palynological data is very important is not only for taxonomists but also for related applied and pure sciences and it could have taxonomic value as the supporting evidence to the morphological and phylogenetic studies. Morpho-palynology of pollen grains of *Datura innoxia* was carried out by LM and SEM and findings reveals that grains were large, circular, tricolpate and prolate-spheroidal in shape with regulate striate exine ornamentation. Apterure ornamentation was brevicolpus. Pollen morphology of some genera of family Solanaceae was studied by Rajurkar and Tidke (2019) and findings reveals that pollen was prolate-spheroidal, sub-oblate to oblate spheroidal that is in agreement with our study (Plate 15).

The value of polar diameter (42.4 μm) was greater the equatorial diameter (41.6 μm). The mean length and width of colpi and mesocolpium was calculated i.e. 4.1 μm , 3.7 μm and 17.9 μm respectively in *Datura innoxia*. Exine thickness of 2.75 μm was noted while polar to equatorial ratio in this invasive plant was 1.01. Percentage of pollen fertility was 82.9%. Pollen fertility is as aid in the recognition of variations within the

species and at generic level (Ahmad et al., 2010). Ashfaq et al., (2020) investigated the pollen morphology of 13 species of family Solanaceae and confirmed it as eurypalynous family. The variations in pollen characters of this highly toxic invasive plant are of significant important in its invasiveness.

Dartura innoxia (Downy thorn apple) is native to U.S.A and Mexico. This plant species is toxic to animals and human. According to Dupin and Smith (2018) there are 10 species of *Dartura innoxia* in its native region which occur naturally in South Western USA, Mexico and other parts of America. Due to numerous seed production this species though to spread solely. The seeds are distributed in large areas by the ants and some special birds that are resistance to the chemicals produced by *Datura L.* (Maslo and Šarić 2019).

3.1.17 *Digera muricata* (L.) Mart.

In current study pollen of *Digera muricata* was examined qualitatively and quantitatively as these characters can be active at species level for identification purpose. Monad, tricolpate pollen with spheroidal shape with psilate ornamentation was observed in *Digera muricata*. These results correlates with research study by Hussain et al., (2018), while studying the palynological feature and comparative foliar epidermal using light and scanning electron microscopy of 17 species of Amaranthaceae for taxonomic significance.

For *Digera muricata* in current study calculated polar diameter was 33.0 μm which was greater equatorial diameter (32.0 μm), exine thickness was 3.90 μm , pore length and width was 0.40 μm and 0.50 μm respectively. The calculated measurement of mesocolpium was 3.95 μm . P/E ratio was 1.03. Pollen fertility was noted 92.9% and sterility 5.05%. Pollen fertility provides a handsome information about the regeneration of plant species through sexual reproduction to ensure the species survival (Reijieli and Tyagi, 2002). Hence help in successful invasion of alien plants like *Digera muricata* in introduced region. The reproductive characters of *Digera muricata* like shape of pollen and high rate of pollen fertility enable it to expand its distribution range and become widespread (Plate 16).

According to Ricciardi et al., (2017), alien species are directly involved in habitat destruction and could be serious threat to biodiversity and ecosystem services. Simberloff (2005) stated that higher competitive ability, producing large number of seeds with high dispersal ability, vegetative reproduction, and suppression of native flora by allelochemicals help alien plants to establish in new habitats.

3.2.18 *Eucalyptus camaldulensis* Dehnh

Eucalyptus camaldulensis belongs to family Myrtaceae, highly adaptable tree as it has ability to tolerate extreme conditions such as drought and soil salinity, ability to reproduce at young age, coupled with high-volume seed and pollen production, and rapid growth. Above mentioned characteristics declared it as invasive in a number of countries. Khan et al., (2011) listed *Eucalyptus camaldulensis* as invasive in Pakistan and in Jamaica by Iabin, (2015). Qualitative characters and quantitative characters of pollens of *Eucalyptus camaldulensis* were analyzed by light and scanning electron microscopy. Analysis shows that pollen were without spines and pollen type was monad, pantoporate. Shape in equatorial view was oblate and triangular in polar view. Psilate-perforate exine ornamentation was observed (Plate 17).

Equatorial diameter and polar diameter of this invasive plant was 20.25 μ m 27.25 μ m respectively. Calculated exine thickness, colpi length, colpi width and mesocolpium was 4.60 μ m, 6.70 μ m, 5.15 μ m, 13.15 μ m. 1.34 polar to equatorial ratio was noted. Shubharani et al., (2013) observed colporate, prolate, oblate spheroidal in some species belongs to family Myrtaceae these results are in disagreement with present findings. So variations in pollen morphology, pollen production and pollen viability are the factors related to different evolutionary adaptations and successful implantation of the invasive plants. Pollen fertility (90.4%) in *Eucalyptus camaldulensis* was calculated. The pollen viability is key factor for invasive species dispersal, fitness and survival (Impe et al., 2020).

3.1.19 *Jasminum humile* L.

In *J. humile* the pollen type was monad and tricolpate with prolate-spheroidal shape and exine ornamentation was reticulate. Polar diameter (40.1 μ m) was more than equatorial diameter (38.4 μ m). Tricolpate pollen with prolate shape was also reported

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by Akhtar et al., (2021), these results correlates with our findings. The size of exine thickness was 2.40 μm and length and width of colpi was 2.30 μm and 4.70 μm . P/E ratio was 1.04 (Plate 18). The colpus length can be used to find out the pollen variation among the large germplasm. Denk and Tekleva (2006) suggested it as a good palynological marker for phylogenetic studies. The rate of pollen viability was 93.1% in *Jasminum humile*. Pollen viability comprises various aspects of pollen performance such as fertilization ability, germination ability and sustainability (Dafni and Firmage 2000). Thus facilitating the invasion of introduced plants.

Jasminum humile L. belongs to family Oleaceae, species of this family are mostly distributed in the tropical regions of the world. There are many variants of *J. humie* which are dispersed the temperate and subtropical regions of started from Abbottabab to the upper parts of Kohistan (Akhtar et al., 2021)

3.1.20 *Justicia adhatoda* L.

Justicia adhatoda belongs to family Acanthaceae which is considered as geographically widespread, taxonomically diverse and morphologically and ecologically variable lineages of flowering plants. This family comprises more 5000 species worldwide, therefore it is considered ad most diverse families of angiosperm (Erin et al., 2022). In Pakistan this family is represented by 18 genera and 60 specific and intraspecific taxa out of which 44 are native (Malik and Ghafoor, 1988). In present study family acanthaceae is represented by *Justicia adhatoda* L. this plant was introduced as ornamental plant. Pollen type monad, dicolporate was observed in *Justicia adhatoda*. Shape in equatorial view was sub-prolate and circular in polar view. Exine ornamentation was scabrate. As far as quantitative characters as concerned the equatorial diameter was 48.9 μm while polar diameter was 36.6 μm and exine thickness was 20.2 μm was noted. Colpi length was 3.95 μm , colpi width was 2.70 μm , pore length is 0.40 μm , pore width was 0.50 μm , mesocolpium was 3.95 μm . P/E ratio was 1.25. Pollen fertility noted was 91.1% and sterility is 8.9% (Plate 19). The pollen grains of *Justicia adhatoda* were described by Perveen and Qaiser (2010) as prolate to perprolate, heterocolpate with reticulate ornamentation and Raza et al., (2020) also reported reticulate to coarsely reticulate ornamentation. The pollen and seed characters of some selected plant species of family Acanthaceae in Yemen were studied by Al-Hakimi et al., in 2017, they reported prolate, subprolate and spheroidal while the *Morpho-Palynological and Seed Diversity of Invasive Plants in Lesser Himalaya Pakistan*

apertures were varied from dicolporate, tricolporate, porate to colpate with reticulate exine ornamentation. The variations in exine ornamentation involve in dispersal of pollen grains in invasive and native species, furthermore size of pollen and shape also affects the dispersal of pollen grains and invasion process in plants species (Arredondo-Nunez et al., 2011).

3.1.21 *Lantana camara* L.

The pollen characters like aperture type, pollen shape, size, pollen wall architecture and polarity are phylogenetically useful at higher taxonomic levels (Hickey and Wolfe, 1975). In current palynological study monad lobate, medium, prolate and tricolporate pollen was observed in *L. camara* while perforate exine sculpturing and sunken aperture was noted. According to Perveen and Qaiser (2010), pollen shape is one of the significant characters for delimiting the taxa in both native and invasive species. The occurrence of prolate and tricolporate pollen were also mentioned in *L. camara* by Jalal et al., (20210. But they noticed psilate ornamentation that is contrary to present investigations.

The quantitative characters of pollen are primarily used for the determination of size of pollen but it is also used to find out the shape of pollen by finding the ratio between them. Observed equatorial length was 22.6µm and polar length was 32.3µm. Colpi length was 2.2µm and colpi width of 1.1 µm was calculated and mesocolpium distance 18.4 µm were measured. Exine thickness was 2.5 µm, while P/E ratio was 1.42. Pollen fertility and sterility was 88.5% and 11.4% respectively. Pollen fertility is helpful in assessing the sustainability in the species of plants which grow under unfavorable conditions. A study of Steep et al., (2019) described that introduced species of *Lantana* have high fertile pollen then the native *Lantana* (Plate 20).

Lantana camara is a native plant of USA and it is considered as ten worst weed of the world. This alien invasive pant species is widely distributed in Pakistan and as per studied of Khan et al., (2010) it has invaded in hilly areas of Islamabad, Margalla Hills and Rawal Lake. *Lantana camara* has an ability to covers the open areas quickly so invasion rate is very high. It spread successfully and speedily due to large number of fruit production and ability to grow under wide range of climatic conditions (Dobhal et al., 2010), production of allelochemicals which reduce the growth of other surrounding species (Ambika et al., 2003).

3.1.22 *Malvastrum coromandelianum* (L.) Garcke

Malvastrum coromandelianum L. (Malvaceae) is considered as highly invasive alien weed mainly distributed in Tropics and Subtropics of the globe. Malvaceae family is a cosmopolitan family of herbs, shrubs, climbers and small trees. Morpho palynological studies are of great importance for plant identification and taxonomic classification. In present study pollen morphological features were investigated by LM and SEM. Pollen features qualitatively described as monad, apolar, medium in size, pantoporate. Circular shape in polar view and spheroidal shape in equatorial view was noted. Exine ornamentation was echinate. But Paramjeet Kaur (2018) described inaperturate, oblate spheroidal with reticulate exine with spines in *M. coromandelianum* these findings are slightly differs from our findings. Quantitatively, the polar diameter and equatorial diameter was 35.2 μm and 34.3 μm respectively. The number of pores per pollen varies from 45 to 56. Pore was 0.28 μm long and 0.16 μm wide respectively. P/E ratio was 1.02. Average number of spines per pollen was 33. Spine length was 0.25 μm and width 0.22 μm . Exine thickness was 0.74 μm . Well developed, stainable pollen grains were observed in *M. coromandelianum*. Percentage of fertility and sterility was calculated 92.6% and 7.3% respectively. Pollen stainability is good indicator of male fertility (Plate 21).

3.1.23 *Melilotus indicus* (L.) All.

Europalynous type pollen were described by Khan et al., 2019 in family Fabaceae. Both qualitative and quantitative characters of the pollen can be used for differentiating between taxa at specific level. Pollen type monad, tricolporate and spines was absent. The shape in the equatorial view was sub-prolate and circular in the polar view while exine ornamentation was reticulate. Taia (2004) reported pollen grains of *M. indicus* tricolpate, perprolate, rarely subprolate that contradicts our findings but he reported reticulate sculpturing as we found in present study (Plate 22).

Equatorial diameter was 29.5 μm , polar diameter was 37.6 μm , exine thickness was 2.55 μm , colpi length was 1.80 μm , colpi width 3.10 μm . Mesocolpium is 14.7 μm . P/E ratio was 1.27. Pollen fertility noted was 89.1% and sterility was 10.8%. Reticulate ornamentation was also reported by Lashin (2006) in *M. indicus* that is accordance with

present research findings. The invasive species have prolific seed and pollen production, generalist pollination systems, efficient seed and pollen dispersal mechanisms and persistent seed banks that often have fire, heat, or disturbance triggered germination cues (Russo et al., 2019).

3.1.24 *Oxalis corniculata* L.

The origin of *Oxalis corniculata* L. is not exactly known or obscure but some authors suggested that Mediterranean region is the native range of this species (Lourteig, 1979; Gray and Duretto 2001). Features that make this plant a successful colonizer and persistent weed includes its sticky seed, extended flowering period, short generation period and explosive capsules (Groom et al., 2019). In present study tricolporate pollen with sub prolate to prolate spheroidal shape was observed in *Oxalis corniculata* while scabrate ornamentation was examined. Similar pollen shape in this plant species but with reticulate sculpturing was reported by Khan et al., 2020, while studying the palynology of allergenic and invasive weeds plants for biodiversity in Lakki Marwat Pakistan. Equatorial and polar diameter ranges from 45.4 μ m and 46.4 μ m with exine thickness of 3.35 μ m. Colpi length, width, and length of mesocolpium was 4.25 μ m, 7.95, 15.6 μ m respectively. According to Torres (2000), the size of pollen cannot be stable inherited due to impacts of morphology, physiology and ecology. P/E ratio was noted in *Oxalis corniculata* i.e 1.02 while pollen fertility shown by this plant species was 90.4% with sterility of 8.36%. Pollen viability depends on mass and the nutritional value of pollen per follower. Intraspecific pollen variation is related to the species size in the area and the ecological diversity (Guinet & Ferguson, 1989). The high percentage of fertile pollen in *Oxalis corniculata* is referred as its pollination and distribution success (Plate 23).

3.1.25 *Parthenium hysterophorus* L.

The pollen grains are smallest unit of plants with number of characters of taxonomic and phylogenetic importance. In *Parthenium hysterophorus* pollen monad, tricolporate and echinate were examined. These results are in accordance with the study by Zafar et al., 2007. Shape of pollen in polar and equatorial view was circular to sub-oblately. Spines were also noted. Equatorial diameter was 16.75 μ m and polar diameter

was 15.70 μm , exine thickness was 2.00 μm , colpi length was 3.25 μm , colpi width was 4.55 μm , spine length was 1.20 μm , spine width was 0.55 μm , mesocolpium was 9.30 μm . P/E ratio was 0.93. Pollen fertility noted was 93.1% and sterility was 6.89%. Zafar et al., 2007 calculated 94.8 pollen fertility in *Parthenium hysterophorus* and 15.58 μm and 16.37 μm polar and equatorial which is almost similar to current findings. This weed is highly noxious due to higher pollen production per flower. *P. hysterophorus* have fast growth rate, short life cycle, high reproductive potential and allelopathy, make it successful invader of native habitats (Plate 24).

The native range of *Parthenium hysterophorus* is Mexico, Central and South America. It was accidentally introduced in many countries. In 1980's this weed was first time reported in Pakistan. *Parthenium hysterophorus* L. is known as serious hazard to human health, livestock health, crop production and native biodiversity as this plant contain toxins called sesquiterpene lactones. Main component of sesquiterpene lactones is Parthenin that is lethal to livestock and humans. Other health problems like dermatitis and rhinitis (Shabbir and Bajwa, 2006) is also caused by *Parthenium hysterophorus*. This plant belongs to family Asteraceae and it is europalynous family (Erdtman, 1952). In Pakistan, *P. hysterophorus* is becoming a dominant weed with highest values of relative density and frequency as well as affecting Maize growth (Shabbir & Javaid, 2010). A study by Tower and Rao (1992) indicates that pollen of this invasive plant also have inhibitory effects on fruit set in crops such as tomato, beans, brinjal and capsicum and also reduces grain filling in cereals.

3.1.26 *Prosopis juliflora* (Sw.) DC.

Prosopis juliflora is placed in family Fabaceae, comprises of more than 12,000 species (Cronquist, 1988). As per studies of Burkart 1976 and Silva 1988 the genus *Prosopis* consists of 44 species which are distributed throughout Western Asia, Africa, America, Southwestern United States to Central Chile and Argentina. It is medium tree and introduced around the world. *Prosopis juliflora* is a great source of highly allergenic pollen grains which results in serious allergic reactios and asthma (Hussain et al., 2020).The identifications of pollen is based upon the both light and scanning electron microscopic studies of pollen of *Prosopis juliflora*. Pollen type monad, tricolporate was noted shape in equatorial view was prolate-spheroidal and oblate

spheroidal in polar view. Psilate exine sculpturing was observed and pores were absent in *P. juliflora*. Quantitative Characters were statistically analyzed using SPSS software. Equatorial diameter was 26.4 μm , polar diameter was 30.1 μm , exine thickness was 2.45 μm . The size of colpi was calculated based upon colpi length and width i.e. was 3.65 μm and 6.25 μm respectively while mesocolpium length was 20.2 μm , P/E ratio was 1.14. Pollen fertility noted were 94.2% and sterility was 5.76%. Khan et al., 2021 reported sub prolate shaped, tricolporate pollen in *Prosopis juliflora* and their findings are almost similar to the current study. Ahmad et al. (2019) gives general idea of the LM pollen morphological parameters in *P. juliflora* (tricolporate) similar to our findings, whereas khan et al. (2021) described SEM high resolution structural details elaborated granular colpus and foveolate grains in *P. juliflora*. The variations in pollen morphology in *Prosopis juliflora* make it successful invader in new habitats (Plate 25).

3.1.27 *Quisqualis indica* L.

Quisqualis indica L. belongs to family Combretaceae which is commonly known as mangrove family. It has been widespread in Tropical Africa and Asia and cultivated throughout the Tropics and regarded as emerging environmental weed. According to Christenhuze et al., 2016 family Combretaceae comprises of about 530 species of trees, shrubs and lianas. Pollen type monad, tricolporate and spines were absent. Shape in equatorial view was prolate-spheroidal and circular in polar view. Pores were absent. Exine sculpturing was reticulate and appear as a network like (Plate 26). Equatorial diameter was 21.80 μm , polar diameter was 22.50 μm , exine thickness was 20.05 μm , colpi length was 7.75 μm , colpi width 3.30 μm , mesocolpium was 1.95 μm . P/E ratio was 1.03. Pollen fertility was 97.7% and sterility 2.7%. In *Q. indica* sub oblate shape was presented by Rao et al., 2010. Producing smaller pollen grains with high rate of viability appeared to increase the fertilization ability of pollen in invasive species (Cruzan et al., 1990).

3.1.28 *Ranunculus arvensis* L.

Ranunculus arvensis is placed in family Ranunculaceae that is cosmopolitan in distribution but mainly present in cold and temperate regions of North and South hemisphere, some members are also found in Tropical and Subtropical regions expect

in Montane region (Wiegleb, 2017). According to Du et al., 2018 and Sauque and Thomas, 2003, palynological investigations are of great value in systematics and phylogenetic studies of plant species. Pollen morphological features of *Ranunculus arvensis* was investigated using both light microscopy and scanning electron microscopy in current study. Investigations showed that pollen type monad, tetracolpate, tricolpate, and spines were absent. Shape in equatorial view was oblate-spheroidal and irregular in polar view. Exine ornamentation was scabrate-verrucate but echinate to scabrate surface was observed for this plant species by Ahmad et al., 2018 with tricolpate, spheroidal shape pollen. Pollen size was calculated on the basis of polar and equatorial diameter. Equatorial diameter was 22.70µm, polar diameter was 22.5µm with exine thickness was 3.35µm. Colpi size was calculated on the basis of colpi length and width so colpi length was 2.45 µm and colpi width was 2.55µm, mesocolpium was 14.3µm. P/E index was calculated for *Ranunculus arvensis* 0.99.

Pollen fertility noted was 92.7% and sterility was 7.20%. Qureshi et al., 2019 observed 85% pollen fertility in this plant species that is contrary to present investigations. The quality and quantity of pollen produced by a plant is considered as an important factor of the reproductive success (Dafni and Firmage, 2000).

3.1.29 *Ranunculus muricatus* L.

Ranunculus muricatus also belongs to family Ranunculaceae. Morphopalynological features of some genus of this family was investigated by researchers (Bot et al., 1968) but in Pakistan very little work has been done on the pollen morphology (Perveen & Qaiser, 2006). In present study pollen grains of *Ranunculus muricatus* were measured and demonstrated using LM and SEM. Pollen type monad, tricolporate and spines was absent with spheroidal shape in equatorial view and circular was polar view. Tectum medium regulate and sexine was thicker than nexine. Pores were absent. Qureshi et al., 2019, noticed prolate spheroidal pollen in *Ranunculus muricatus*.

Equatorial diameter was 18.7 µm and polar diameter was 19.0 µm, exine thickness was 3.75 µm, colpi length is 2.35 µm, colpi width 4.85µm, mesocolpium was 13.5µm. P/E ratio was 1.01. In *Ranunculus muricatus* 92.7% pollen fertility was

determined by using technique of Khan & Stace, 1999. Pollen fertility is known as primary characteristics that help in spread and establishment of invasive species.

3.1.30 *Ricinus communis* L.

Ricinus communis (castor bean) belongs to family Euphorbiaceae, this family is heterogeneous palynologically (Rao and Airyshaw 2003). It was originated in Africa and has been introduced in number of countries for oil extraction now distributed worldwide as highly invasive plant species. Tricolporate pollen were observed in *Ricinus communis* while pollen shape and exine sculpturing was prolate spheroidal and reticulate. Tricolporate pollen grains with reticulate exine surface and prolate spheroidal to subprolate was also studied by AL-Hadeethi et al., 2016. Perveen and Qaiser (2005) observed palynological characters of 40 species of Euphorbiaceae and they described it as eurypalynous family. The equatorial and polar diameter was 13.45 μm and 13.95 μm respectively. Exine thickness was 1.05 μm , colpi length was 5.2 μm , colpi width was 6.4 μm , mesocolpium was 1.86 μm , P/E ratio was 1.03. Pollen fertility estimated was 84.5% and sterility 15.5% (Plate 27). According to Nazir et al., 2013, pollen fertility is beneficial in evaluating the stability of plant in particular area. Dicolporate, spheroidal pollen with echinate surface was noticed by Alyas et al., 2020, while studying the some species of family Euphorbiaceae the results differs from present study.

3.1.31 *Rosa alba* L.

Rosa alba is placed in family Rosaceae, this plant is widely cultivated as an ornamental plant in Europe, Asia, North America and Northwest America. Pollen was monad, medium in size, colporate, iso-polar, circular in polar view. Colporus aperture type, prolate-spheroidal shape in equatorial view was observed. Striate pollen sculpturing was examined. Wronska Pilarek & Jagodzinski (2011) observed three types of exine sculpturing i.e. Granular verrucate, striate-psilate and striate in genus *Rosa* that is not agreement with present findings. Equatorial diameter was 56.6 μm , polar diameter was 59.0 μm , exine thickness was 5.35 μm , colpi length was 6.50 μm , colpi width was 7.00 μm , mesocolpium was 19.1 μm . P/E ratio was 1.04. Pollen fertility estimated was 92.3% and sterility was 7.69% (Plate 33). Noor et al. (2004) calculated 98% pollen

fertility in *Rosa alba* in previous studies, our recent studies shows less viability rate during approximately 20 years spans. The highest variation in pollen size and shape is noticed in invasive *Rosa* species that could be a result of genetic and environmental factors (Pavlova et al., 2021).

3.1.32 *Sorghum halepense* (L.) Pers.

Sorghum halepense belongs to family Poaceae. This plant species is cultivated as fodder but it is also extremely invasive weed with worldwide distribution. Qualitative and quantitative characters were observed using LM and SEM in *Sorghum halepense* and monoporate, oblate-spheroidal pollen in equatorial view and triangular in polar view with Psilate-perforate exine sculpturing was found (Plate 28).

Monoporate pollen with psilate to reticulate exine was noticed by Khan et al., 2020 in *Sorghum halepense*. Perveen and Qaiser (2012), also reported monoporate but rarely diporate pollen grains in Poaceae. The present findings shows that equatorial diameter was 24.40 μm and polar diameter was 21.75 μm while exine thickness was 0.52 μm , colpi length was 3.20 μm , colpi width was 1.75 μm , pore length was 0.40 μm , pore width was 0.50. P/E ratio was 0.89. Pollen fertility noted was 94.7%. Harun et al. (2021) indicated that pollen shape, pore size, P/E ratio, pore sculpturing, polar and equatorial diameter are of good taxonomic value and holds a significant position in identification and delimitation of taxa. In this highly invasive species the high percentage of fertile pollen is considered as its pollination and distribution success.

3.1.33 *Taraxacum campylodes* G.E.Haglund

In *Taraxacum campylodes* the monad, tricolporate pollen with pines were observed while shape in equatorial view was sub-prolate and sub oblate in polar view. Echininate exine ornamentation was observed. Nabila et al. (2022) described the pollen morphology of *Taraxacum campylodes* as tricolporate and echinate that correlates with present study. Equatorial diameter (14.85 μm), polar diameter (15.3 μm), exine thickness (2.65 μm), colpi length (4.8 μm), colpi width (2.7 μm), mesocolpium (18.99 μm) were calculated. P/E ratio was 1.31. Estimated pollen fertility and sterility was 91.3% and 8.9% respectively. Spines length was 1.4 μm and width was 1.1 μm

(Plate 29). Previously Karna (2017) elaborates *T. campylodes* grains with pentagonal, polyporate, echinate, and pentahedral features which are dissimilar to present findings.

Taraxacum campylodes is a species within family Asteraceae and it is one of the most wide spread weed of North America, Asia and Europe. According to Funk et al. (2009) this family consists of 12 subfamilies and 43 tribes. It is diverse family in terms of size, the largest in Pakistan, comprising of 650 species in 15 tribes (Bano et al., 2015).

3.1.34 *Tecoma stans* (L.) Juss. ex Kunth

Tecoma stans is considered as aggressive plant invader which reduces the native biodiversity and also alter the natural resources. In Pakistan *Tecoma stans* was introduced from its native region (America) as ornamental plant but due to aggressive nature it become an invasive plant. *Tecoma stans* belongs to family Bignoniaceae. Palynology serve as a tool for reconstruction and past vegetation and environment. Pollen of *Tecoma stans* were examined to study the palynological features by LM and SEM. Pollen studies provides significant knowledge about phylogenetic association and assembling of modern pollen from existing vegetation (Hesse and Blackmore 2013).

In present palynological investigations of *Tecoma stans* monad type, tricolporate pollen (without spines) was found. Shape in equatorial view was prolate-spheroidal and circular in polar view. Tectum sculpture regulate and pores were absent. Equatorial diameter was 24.65 μ m, polar diameter was 18.80 μ m, exine thickness was 1.80 μ m, colpi length was 3.25 μ m, colpi width was 5.60 μ m, mesocolpium was 15.65 μ m. P/E ratio was 1.05. Pollen fertility was calculated 94.2% and sterility 5.76% (Plate 30).

In 2011, Saensouk & Saensouk examined the pollens of some plants of family Bignoniaceae under LM and SEM from Northeastern Thailand. The findings of study showed that pollen grains of selected species of family Bignoniaceae were sub spheroidal or prolate with polar axis 17.5-50 μ m, equatorial diameter 22.5-75 μ m in size, colpate or 3-4colporate, while microreticulate exine sculpturing was observed. Oblong shaped pollen in *Tecoma stans* with pollen fertility 100% was also reported by Noor et al., 2004.

3.1.35 *Tribulus terrestris* L.

Tribulus terrestris is an annual plant in family Zygophyllaceae widely distributed in semiarid and arid regions of the world (Semerdjieva and Yankova-Tsvetkova, 2017). Khalik (2012) described this family as palynologically heterogeneous. The qualitative and quantitative characters were noted and monad, medium, pantoporate and oblate-spheroidal pollen grains were found. Colpi orientation was sunken and tapering at end. Exine sculpturing was reticulate (Plate 31). Polar diameter was 36.3 μm while the equatorial diameter was 38.5 μm . P/E ratio was 0.94. Pore length and width was 1.18 μm and 1.3 μm respectively. Exine thickness of 4.6 μm was observed. Pollen fertility and sterility was 91.4% and 8.6% respectively. Oblate-spheroidal, tricolporate and pentaporate pollen with 0.97 P/E ratio was observed in this plant by Ahmad et al., 2020, the findings differs to current investigations in *Tribulus terrestris*. Emer et al., (2015) reported that high amount of pollen and nectar production, bigger and colorful flowers plays an important role in integration of alien species into native communities.

3.1.36 *Tropaeolum majus* L.

Tropaeolum majus belongs to family Tropaeolaceae, this family consist of herbaceous vines, after climbing by means of twining leaf petioles. Tropaeolaceae is distributed in Central and South America. *Tropaeolum majus* is introduced as an ornamental plant in Pakistan. Both qualitative and quantitative pollen morphology characters of this plant species were studied and results shows that pollen grains were monad, tricolporate with prolate-spheroidal shape in equatorial view and angular in polar view. Tectum regulate and sexine is thicker than nexine. Measurements of equatorial diameter was 20.0 μm polar diameters 22.5 μm , exine thickness was 2.65 μm , colpi length was 3.70 μm , colpi width 7.00 μm , mesocolpium was 17.0 μm . P/E ratio was 1.12 (Plate 32). Pollen fertility and sterility were noted 92.07% and 7.92% respectively. Al-Mashhadani et al., (2018) studied pollen grains of this invasive species as tricolporate, semispheroidal in equatorial view, triangular in polar view with microreticulate exine sculpturing which are disagreement with our findings. The variation in pollen size and shape in introduced species may result from the pollination and habitat conditions. The published reports shown that the change in grain size can influence

pollen germination, pollen tube growth and fertilization (Giovannini et al., 2017). These changes in pollen morphology increase the chances of pollen viability and pollen germination rates (Ejmond et al., 2011) thus help in establishment and spread of invasive species.

3.1.37 *Verbena officinalis* L.

The family Verbenaceae is mainly distributed in the Tropics and Subtropics of the Southern hemisphere. According to Jafri & Ghafoor, 1974 in Pakistan family Verbenaceae is represented by 17 genera and 35 species. Palynological characteristics are helpful to cater the systematic position of species with its respective family. In current investigations the pollen grains of *Verbena officinalis* were found monad, tricolporate without spines. The shape in equatorial view was oblate-spheroidal and triangular in polar view. Psilate-perforate exine ornamentation was observed (Plate 33). Perveen and Qaiser (2007) observed oblate-spheroidal in this plant species which in agreement with our results. Pollen equatorial diameter was 28.2 μm , polar diameter 25.7 μm , exine thickness was 3.45 μm , colpi length was 5.30 μm , colpi width was 5.80 μm , mesocolpium was 13.8 μm . P/E ratio was 0.91. The calculated pollen fertility in *V. officinalis* was 86.5% while Zafar et al. (2006) calculated 96.11 % pollen fertility. Munsif et al., (2007) conducted comparative pollen study on of genera *Lantana*, *Verbena* and *Vitex* of family Verbenaceae from Pakistan and observed pollen grains of *Verbena officinalis* tricolporate as well as tetracolporate with granular surface which are in disagreement with present investigations. The high amount of pollen in invasive species attracts the potential pollinators from other native plants resulting in reduced seed set and fitness among other plants. The variations in pollen size and pollen fertility shown by introduced plants in new habitat plays significant role in establishment of introduced plants. The variations in pollen characters is also positively linked with ecological conditions and changes in soil mineral nutritive elements (Katiyar et al., 2012).

3.1.38 *Verbesina encelioides* (Cav.) Benth. & Hook.f. ex A.Gray

Verbesina encelioides is placed in family Asteraceae, pollen grains of this family shows a broad variety of shapes, sizes, apertures and sculpturing and exine

ornamentation. The importance of palynological characteristics of this family has been long recognized to understand phylogenetic and taxonomic relationships (Blackmore et al. 2010). Pollen morphological analysis reveals that pollen grains were monad, tricolporate, prolate-spheroidal in equatorial view and circular in polar view without spines. Echininate exine sculpturing was examined. The grains mean equatorial and polar diameter was 20.5 and 21.9 respectively. Colpi length was 3.60 μm , width was 4.90 μm , length and width of spines was 7.35 μm and 2.35 μm while mesocolpium distance 14.0 μm were calculated. The exine sculpturing, number of spines and distance of between colpi and pollen fertility and sterility gives significant information for the documentation of plant species (Plate 34). P/E index were 1.06 and pollen fertility and sterility were noted as 95.5% and 4.47%. Monad pollen grains with prolate spheroidal shape was also observed by Ullah et al., 2021 in *Verbesina encelioides*. Tellería (2017) noted 5.25 μm spine length with 25.64 μm polar diameter and 24.73 μm equatorial diameter, findings were not in accordance with our results. The pollen fertility estimation in *Verbesina encelioides* was 95.5% which indicates that flora of this invasive species is well established in studied area.

Table 3: Qualitative pollen attributes of Invasive species

S. No	Plants Species	Size	Polar View	Equatorial View Shape	Pollen Type	Exine Ornamentation
1.	<i>Achyranthes aspera</i> L.	Small	Circular	Sub-Prolate	Pantoporate	Psilate
2.	<i>Ageratum conyzoides</i> L.	Small	Circular	Prolate-Spheroidal	Tricolporate	Echinate
3.	<i>Anagallis arvensis</i> L.	Small To Medium	Circular	Spheroidal	Tricolporate	Verrucate
4.	<i>Bidens pilosa</i> L.	Medium	Circular	Oblate-Spheroidal	Tricolporate	Echinate
5.	<i>Brachychiton acerifolius</i> (A.Cunn. ex G.Don) F.Muell.	Medium	Circular	Prolate-Spheroidal	Tricolporate	Scabrate, Verrucate, Gemmate
6.	<i>Broussonetia papyrifera</i> (L.) L'Hér. ex Vent.	Small	Irregular	Sub-prolate	Tricolporate	Scabrate, Verrucate
7.	<i>Bryophyllum pinnatum</i> (Lam.) Oken	Medium	Circular	Prolate-Spheroidal	Tricolpate	Psilate
8.	<i>Cannabis sativa</i> L.	Small	Angular	Prolate-Spheroidal	Tricolpate	Reticulate
9.	<i>Carthamus oxyacantha</i> M.Bieb.	Large	Angular	Oblate-spheroidal	Tricolporate	Micro-perforate, Echinate
10.	<i>Cassia occidentalis</i> L.	Medium	Spheroidal	Prolate-Spheroidal	Tricolporate	Psilate
11.	<i>Callistemon citrinus</i> (Curtis) Skeels	Small	Oblique	Spheroidal	Tricolporate	Psilate
12.	<i>Chenopodium ambrosioides</i> L.	Small	Circular	Spheroidal	Pantoporate	Micro-perforate echinate
13.	<i>Commelina benghalensis</i> L.	Medium	Angular	Sub-prolate	Tricolporate	Psilate
14.	<i>Conyza canadensis</i> (L.) Cronquist	Small To Medium	Circular	Prolate-Spheroidal	Trizonocolporate	Echinate
15.	<i>Convolvulus arvensis</i> L.	Small	Circular	Oblate-Spheroidal	Tricolpate	Echinate
16.	<i>Datura innoxia</i> Mill.	Large	Circular	Prolate-Spheroidal	Tricolpate	Rugulate-striate
17.	<i>Digera muricata</i> (L.) Mart.	Medium	Circular	Spheroidal	Tricolpate	Psilate
18.	<i>Eucalyptus camaldulensis</i> Dehnh	Medium	Circular	Oblate	Pantoporate	Psilate-Perforate.
19.	<i>Jasminum humile</i> L.	Small	Circular	Prolate-Spheroidal	Tricolpate	<i>Reticulate</i>

20.	<i>Justicia adhatoda</i> L.	Medium	Circular	Sub-prolate	Dicolporate	Scabrate
21.	<i>Lantana camara</i> L.	Medium	Semi-circular	Prolate	Tricolporate	Perforate
22.	<i>Malvestrum coromandelianum</i> (L.) Garcke	Medium	Circular	Spheroidal	Pantoporate	Echinate
23.	<i>Melilotus indicus</i> (L.) All.	Small To Medium	Circular	Sub-Prolate	Tricolporate	Reticulate
24.	<i>Oxalis corniculata</i> L.	Medium	Sub-Prolate	Prolate-Spheroidal	Tricolporate	Scabrate
25.	<i>Parthenium hysterophorus</i> L.	Small	Circular	Sub-Oblate	Tricolporate	Echinate
26.	<i>Prosopis juliflora</i> (Sw.) DC.	Small	Oblate--Spheroidal	Prolate-Spheroidal	Tricolporate	Psilate
27.	<i>Quisqualis indica</i> L.	Small To Medium	Circular	Prolate-Spheroidal	Tricolporate	Reticulate
28.	<i>Ranunculus arvensis</i> L.	Medium	Irregular	Oblate-Spheroidal	Tetracolpate, Tricolpate	Scabrate, Verrucate
29.	<i>Ranunculus muricatus</i> L.	Small	Circular	Spheroidal	Tricolporate	Micro Reticulate
30.	<i>Ricinus communis</i> L.	Small	Circular	Prolate-Spheroidal	Tricolporate	Reticulate
31.	<i>Rosa alba</i> L.	Medium	Prolate Shape	Prolate-Spheroidal	Colporate	Striate
32.	<i>Sorghum halepense</i> (L.) Pers.	Medium	Triangular	Oblate-Spheroidal	Monoporate	Psilate-Perforate
33.	<i>Taraxacum campylodes</i> G.E.Haglund	Small	Sub-Oblate	Sub-prolate	Tricolporate	Echinate
34.	<i>Tecoma stans</i> (L.) Juss. ex Kunth	Medium	Circular	Prolate-spheroidal	Tricolporate	Psilate
35.	<i>Tribulus terrestris</i> L.	Medium	Circular	Oblate-Spheroidal	Pantoporate	Reticulate
36.	<i>Tropaeolum majus</i> L.	Medium	Angular	Prolate-spheroidal	Tricolporate	Psilate- Perforate
37.	<i>Verbena officinalis</i> L.	Small	Triangular	Oblate-Spheroidal	Tricolporate	Psilate-Perforate
38.	<i>Verbesina encelioides</i> (Cav.) Benth. & Hook.f. ex A.Gray	Medium	Circular	Prolate-spheroidal	Tricolporate	Echinate

Table 4: Quantitative features of Pollen measures of Invasive species

S. No	Plants species	P/E ratio	Exine thickness M(Min-Max)±SE (µm)	Polar diameter M(Min-Max)±SE (µm)	Equatorial diameter M(Min-Max)±SE (µm)	Length of colpi M(Min-Max)±SE (µm)	Width of colpi M(Min-Max)±SE (µm)	Mesocolpium M(Min-Max)±SE (µm)	Fertility (%)	Sterility (%)
1.	<i>Achyranthes aspera</i> L.	1.15	1.85(1.7- 2.1)±0.7	12.8(10.2- 15.5)±0.71	11.10(10.3- 13.50)±0.6	Absent	Absent	Absent	81.2	18.8
2.	<i>Ageratum conyzoides</i> L.	1.05	2.8(1.7-4.5)±0.46	20.6(12.5-22.7)±0.57	19.6(18.2-20.7)±0.40	4.50(3.25-5.25)±0.38	6.55(5.25-7.75)±0.46	11.7(10.7-12.7)±0.32	94.1	5.88
3.	<i>Anagallis arvensis</i> L.	0.85	4.55 (5.25-4)±0.48	23.25 (26.25-21.2)±2.09	27.25 (31.25-23.7)±2.85	7.05 (7.75-6.25)±0.69	4.75 (5.25-4.25)±0.39	14.50 (16.25-13.2)±1.39	94.1	5.88
4.	<i>Bidens pilosa</i> L.	0.97	4.11(2.75-6)±0.72	27.8(26.5- 29.7)±0.68	28.5(26.2-31)±0.78	4.10(1.75-3)±0.29	2.40(1.75-3)±0.21	14.7(12.2-17.0)±0.94	97.3	2.67
5.	<i>Brachychiton acerifolius</i> (A.Cunn. ex G.Don) F.Muell.	1.05	4.40(2.75-6)±0.65	27.1(25.5-29.2)±0.71	25.8(22.7-31.5)±1.49	6.30(5.25-7.25)±0.33	7.00(5.25-8.00)±0.48	13.1(10.2-15.7)±0.87	95.6	4.34
6.	<i>Broussonetia papyrifera</i> (L.) L'Hér. ex Vent.	1.18	2.00 (2.75-1.25)±0.5	12.75 (14.75-9.50)±2.06	10.75 (12.5-9.75)±1.04	3.35 (4-2.75)±0.51	5.55 (7.25-3.00)±1.70	8.05 (10.25-5.50)±2.17	89.1	10.8
7.	<i>Bryophyllum pinnatum</i> (Lam.) Oken	1.01	.88(1.25-.50)±0.11	7.75(11.00-5.25)±0.88	7.67(10.75-5.50)±0.78	5.9(6.50-5.25)±0.20	0.42(.75-.25)±0.08	0.67(1.00-.50)±0.08	95.6	4.34
8.	<i>Cannabis sativa</i> L.	1.06	2.60(1.75-25)±0.60	19.4(17.7-20.5)±1.14	18.3(16.5-20.5)±1.70	2.60(1.75-3.25)±0.60	1.90(1.50-2.25)±0.28	11.0(9.75-12.7)±1.11	92.1	7.89
9.	<i>Carthamus oxyacantha</i> M.Bieb.	0.95	3.18(2.45-3.8)±0.42	25(22.6-26.7) ±1.49	26.3(21.7-30.1)±2.61	2.61(1.35-3.4) ±0.59	2.78(2.15-3.12) ±0.26	21.7(18.5-23.7) ±2.18	86.6	13.3
10.	<i>Cassia occidentalis</i> L.	1.11	3.50(2.75-4.25)±0.25	41.2(37.5-43.2)±0.93	36.9(34.7-39.5)±0.77	2.45(2.00-3.00)±0.18	3.40(2.75-4.25)±0.26	18.3(17.25-20.2)±0.50	96.9	3.03
11.	<i>Callistemon citrinus</i> (Curtis) Skeels	1.01	1.80(1.35-2.55) ±0.23	18.03(16.80-18.6) ±0.34	17.85(15.30-19.65) ±0.8	6.12(1.65-10.20) ±1.4	3.45(1.95-7.20) ±0.97	23.4(22.5-24)±0.27	90.5	5.9
12.	<i>Chenopodium ambrosioides</i> L.	1.02	2.22(1.75-2.85)±0.21	20(19.7-20.4)±0.13	19.6(18.2-20)±0.24	4.6(3.25-5.45)±0.27	5.8(4.75-6.7)±0.31	Absent	92.8	7.2
13.	<i>Commelina benghalensis</i> L.	1.25	0.90(0.25-2)±0.30	30.8(26.2-35.2)±1.82	24.5(20.2-30.2)±1.96	1.00(0.25-1.75)±0.25	1.55(0.50-2.25)±0.31	12.6(10.2-15.2)±0.87	91.9	8.06
14.	<i>Conyza canadensis</i> (L.) Cronquist	1.09	4.7(3.25-6.1) ±1.61	23.3(16.7-30.2) ±3.12	21.2(12.6-27.7) ±2.32	4.33(4.43-9.2) ±1.71	3.41(4.21- 7.73) ±0.81	10.3(11.6-23.2) ±2.81	84.5	15.5
15.	<i>Convolvulus arvensis</i> L.	0.99	7.87(10.7-5.5)±0.8	13.85(15.50-12.00)±0.65	13.9(16.25-11.50)±0.66	4.83(8.00-3.25)±0.74	0.55(0.80-0.25)±0.08	0.33(0.50-0.25)±0.05	92.9	7.05
16.	<i>Datura innoxia</i> Mill.	1.01	2.75(2.2-3.2)±0.12	42.4(34.2-52.2)±3.25	41.6(35.5-48.7)±02.52	4.1(3.50-4.75)±0.13	3.7(2.75-4.50)±0.28	17.9(13.7-22.7)±1.08	82.9	17.1
17.	<i>Digera muricata</i> (L.) Mart.	1.03	3.90(3.25-3.75)±0.26	33.0(30.2-36.2)±1.10	32.0(28.7-35.2)±1.11	Absent	Absent	Absent	92.9	5.05
18.	<i>Eucalyptus camaldulensis</i> Dehnh	1.34	4.60 (5.00-4.25)±0.12	20.25 (23.75-18.7)±1.00	27.25 (31.25-23.7)±1.27	6.70 (8.00-4.75)±0.58	5.15 (5.75-4.50)±0.23	13.15 (16.25-11.0)±0.95	90.4	8.36
19.	<i>Jasminum humile</i> L.	1.04	2.40(2.00-3.00)±0.20	40.1(39.5-40.7)±0.21	38.4(35.7-43.0)±1.21	2.30(1.75-2.75)±0.20	4.70(4.25-5.25)±0.16	16.2(14.7-17.2)±0.47	93.1	6.89
20.	<i>Justicia adhatoda</i> L.	1.25	2.2 (1.97-2.40)	36.6(34.7-40.2)	48.9 (44.7-52.7)	3.95 (2.75-4.75)	2.70 (2.00-3.25)	3.95 (2.75-4.75)	91.1	8.9

21. <i>Lantana camara</i> L.	1.42	2.5(2-3.4)±0.22	32.3(19.5-36.10)±2.78	22.6(18.2-25.7)±1.09	2.2(0.95-2.8)±0.43	1.1(0.7-1.5)±0.18	18.4(14.2-20.2)±1.11	88.5	11.4
22. <i>Malvestrum coromandelianum</i> (L.) Garcke	1.02	0.74(0.5-1.05)±0.35	35.2(30.3-41.2)±1.94	34.3(32.2-36.7)±0.84	0.28(0.12-0.56)±0.07	0.16(0.10-0.23)±0.02	Absent	92.6	7.3
23. <i>Melilotus indicus</i> (L.) All.	1.27	2.55(1.75-300)±0.24	37.6(33.0-42.7)±1.85	29.5(25.2-35.2)±1.64	1.80(0.75-300)±0.39	3.10(2.75-3.75)±0.18	14.7(13.2-16.2)±0.50	89.1	10.8
24. <i>Oxalis corniculata</i> L.	1.02	3.35(3-3.7)±0.12	46.4(43.2-48.2)±0.90	45.4(42.7-47.7)±0.86	4.25(3.75-4.75)±0.17	7.95(7.00-9.75)±0.48	15.6(14.7-16.7)±0.3	90.4	8.36
25. <i>Parthenium hysterophorus</i> L.	0.93	2.00 (2.75-1.2)±0.55	15.70 (17.25-14.50)±1.12	16.75 (17.50-16)±0.93	3.25 (4.00-2.75)±0.46	4.55 (5.50-3.75)±0.77	9.30 (10.25-8.5)±0.71	93.1	6.89
26. <i>Prosopis juliflora</i> (Sw.) DC.	1.14	2.45(2.00-3.00)±0.18	30.1(26.2-35.5)±1.73	26.4(25.2-28.0)±0.44	3.65(2.75-4.75)±0.40	6.25(5.25-7.00)±0.30	20.2(19.7-21.0)±0.23	94.2	5.76
27. <i>Quisqualis indica</i> L.	1.03	2(2.10-1.87)±0.41	22.50(23.50-21.25)±0.43	21.80(22.75-21.25)±0.28	7.75(8.50-7)±0.29	3.30(4.00-2.75)±0.22	1.95(2.75-1.00)± 0.28	97.7	2.27
28. <i>Ranunculus arvensis</i> L.	0.99	3.35(2.25-4.7)±0.53	22.5(25.7-20.7)±0.93	22.70(20.2-24.5)±0.69	2.45(2.00-3.00)±0.18	2.55(2.10-3)±0.18	14.3(12.7-15.5)±0.63	92.7	7.20
29. <i>Ranunculus muricatus</i> L.	1.01	3.75(2.75-4.7)±0.35	19.0(17.7-20.2)±0.49	18.7(17.2-20.5)±0.70	2.35(1.75-3.00)±0.23	4.85(4.25-5.50)±0.23(13.5(12.0-15.2)±0.65	92.7	7.29
30. <i>Ricinus communis</i> L.	1.03	1.05(0.75-1.2)±0.09	13.95(12.0-15.50)±0.63	13.45(12.0-15.50)±0.70	5.2(4.2-6.1)± 0.15	6.4(5.7-7.3)± 0.15	1.86 (1.3-2.4)±0.1	84.5	15.5
31. <i>Rosa alba</i> L.	1.04	5.35(2.75-6.7)±0.69	59.0(53.2-63.5)±1.76	56.6(54.2-63.5)±1.76	6.50(5.25-775)±0.44	7.00(6.00-7.75)±0.37	19.1(17.2-20.5)±0.55	92.3	7.69
32. <i>Sorghum halepense</i> (L.) Pers.	0.89	0.52(8.75-6.25)±.52	21.75(23.75-18.75)±.93	24.40(28.75-21.2)±1.38	3.20(3.50-3.00)±.09	1.75(2.75-1.25)±0.13	1.70(2.50-1.35)±0.44	94.7	4.1
33. <i>Taraxacum campylodes</i> G.E.Haglund	1.31	2.65(2.25-3.0) ±0.12	15.3(14.75-15.75) ±10.1	14.85(14.0-15.75) ±0.3	4.8(4.4-5.4)± 0.29	2.7(2.4-3.1)± 0.2	18.99 (17.10-21.45)±0.8	91.3	8.9
34. <i>Tecoma stans</i> (L.) Juss. ex Kunth	1.05	1.80 (3.00-1)±0.34	18.80 (20.25-18.00)±0.38	24.65 (25.7-23.7)±0.34	3.25 (3.75-2.75)±0.17	5.60 (6.25-5.25)±0.18	15.65 (16.50-14.75)±0.3	94.2	5.76
35. <i>Tribulus terrestris</i> L.	0.94	4.6(3.9-5.2)±0.18	36.3(30.7-42.2)±1.5	38.5(32.5-42.5)±1.33	1.18(0.75-1.85)±0.18	1.3(0.35-2.1)±0.25	Absent	91.4	8.6
36. <i>Tropaeolum majus</i> L.	1.12	2.65(2.00-3.2)±0.23	22.5(21.7-23.7)±0.35	20.0(19.5-20.5)±0.18(3.70(2.75-5.25)±0.42	7.00(5.25-8.00)±0.48	17.0(15.7-18.2)±0.46	92.07	7.92
37. <i>Verbena officinalis</i> L.	0.91	3.45(4.7-6.25) ±0.29	25.7(23.4-21.2) ±1.02	28.2(24.7-31.7) ±1.63	5.30(6.23-9.75) ±0.54	5.80(5.40-7.65) ±0.42	13.8(13.2-15.5) ±1.12	86.5	13.5
38. <i>Verbesina encelioides</i> (Cav.) Benth. & Hook.f. ex A.Gray	1.06	2.70(2.00-3.5)±0.26	21.9(19.7-23.2)±0.6	20.5(19.7-22.2)±0.46	3.60(2.75-4.25)±0.26	4.90(4.50-5.50)±0.20	14.0(12.2-15.2)±0.54	95.5	4.47

Keywords: P=Polar; E=Equatorial; M=Mean; Min=Minimum; Max=Maximum; SE=Standard Error; µm=Micrometer



Figure 5: *Achyranthes aspera*: Aerial parts



Figure 6: *Ageratum conyzoides*: Floral branch



Figure 7: *Anagallis arvensis*: Aerial and floral parts



Figure 8: *Bidens pilosa*: Flower branch



Figure 9: *Broussonetia papyrifera*: Catkin



Figure 10: *Bryophyllum pinnatum*: Leaves



Figure 11: *Cannabis sativa*: Vegetative part



Figure 12: *Carthamus oxyacantha*: Aerial parts with flowers



Figure 13: *Cassia occidentalis*: Flower



Figure 14: *Callistemon citrinus*: Aerial parts



Figure 15: *Chenopodium ambrosioides*: Whole plant



Figure 16: *Commelina benghalensis*: Aerial parts



Figure 17: *Conyza canadensis*: Floral branch



Figure 18: *Convolvulus arvensis*: Floral branch



Figure 19: *Datura innoxia*: Flower and leaves



Figure 20: *Digera muricata*: Floral and leaves parts



Figure 21: *Eucalyptus camaldulensis*: Leaves and anthers with pedicel



Figure 22: *Jasminum humile*: Whole plant



Figure 23: *Justicia adhatoda*: Flowers and vegetative branch



Figure 24: *Lantana camara*: Floral view



Figure 25: *Malvestrum coromandelianum*: Flowers and leaves



Figure 26: *Melilotus indicus*: Floral branch



Figure 27: *Oxalis corniculata*: Aerial parts



Figure 28: *Parthenium hysterophorus*: Floral branch



Figure 29: *Prosopis juliflora*: Inflorescence and vegetative branches



Figure 30: *Quisqualis indica*: Floral branch



Figure 31: *Ranunculus arvensis*: Floral branch



Figure 32: *Ricinus communis*: Floral branch



Figure 33: *Rosa alba*: Flowers with leaves



Figure 34: *Silybum marianum*: Capitulum



Figure 35: *Solanum surattense*: Flower with spiny leaves



Figure 36: *Sorghum halepense*: Spike and vegetative part



Figure 37: *Taraxacum campylodes*: Capitulum



Figure 38: *Tribulus terrestris*: Habitat and floral branch



Figure 39: *Tropaeolum majus*: Flower with leaves



Figure 40: *Verbena officinalis*: Floral branch



Figure 41: *Verbesina encelioides*: Habitat and floral branches



Figure 42: *Xanthium strumarium*: Fruits and leaves

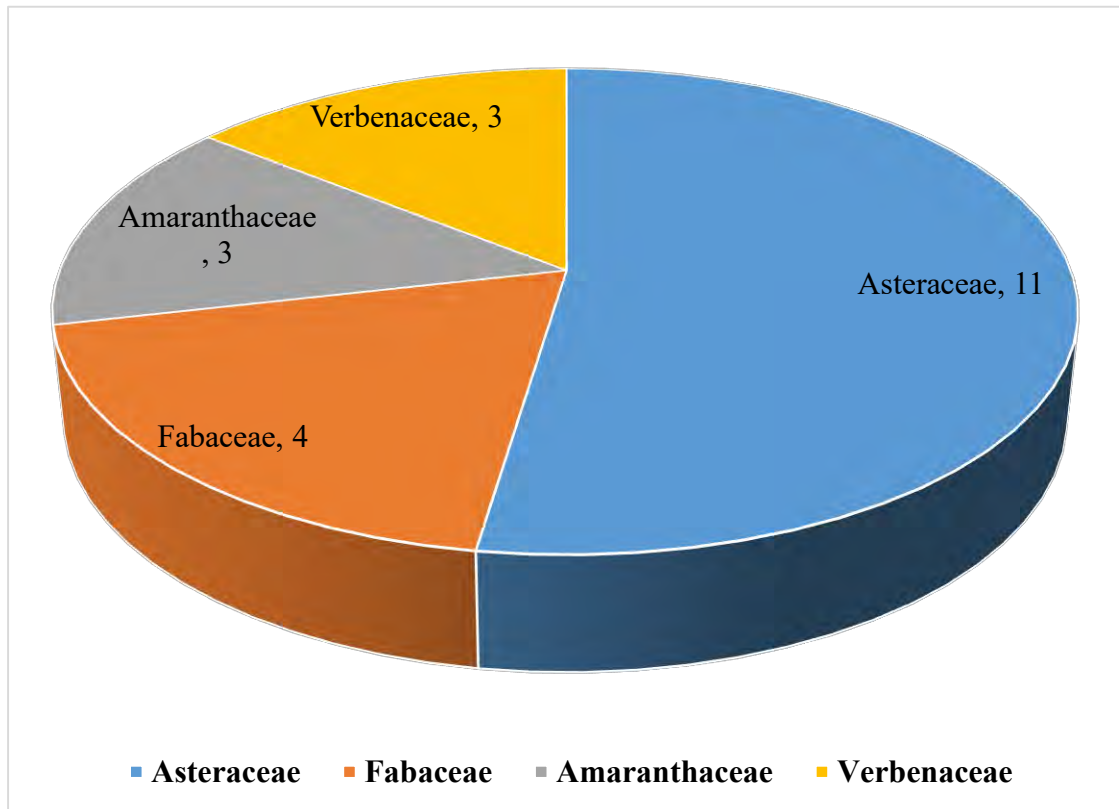


Figure 43. Four dominant families showing percentage of invasive plant species

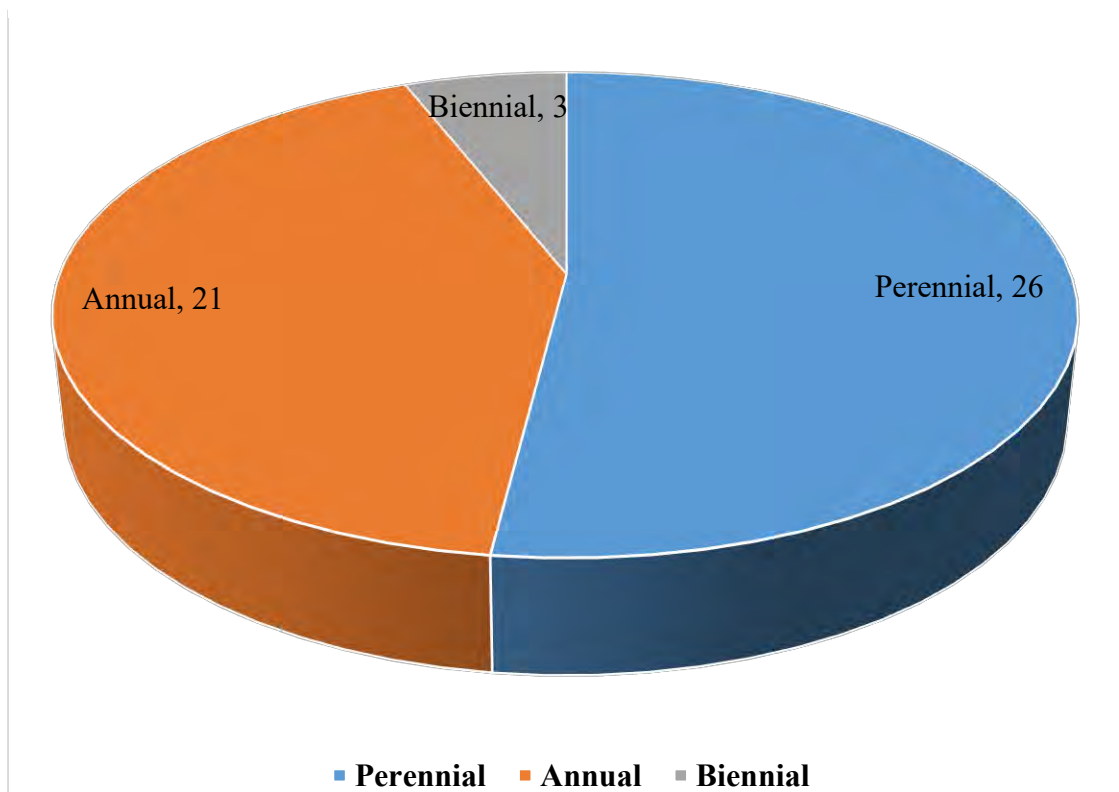


Figure 44. Habits of invasive species in Lesser Himalayas-Pakistani

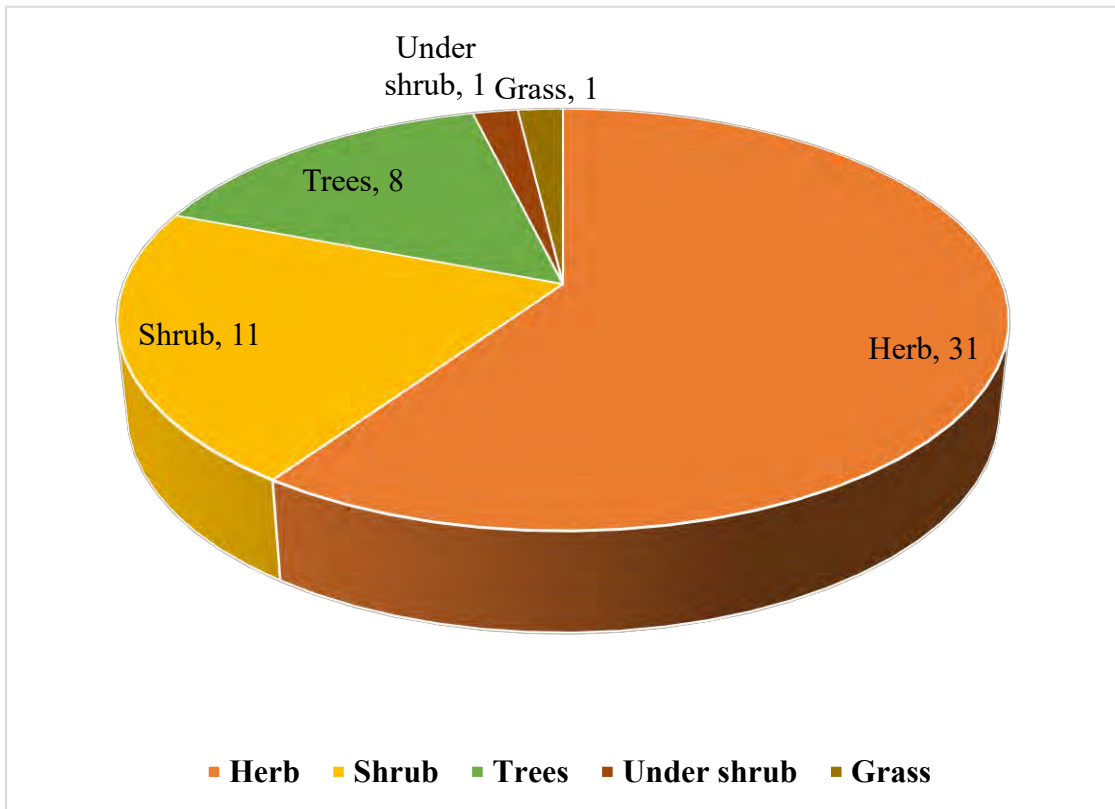


Figure 45. Life forms / status of invasive plant species of Lesser Himalayas Pakistan

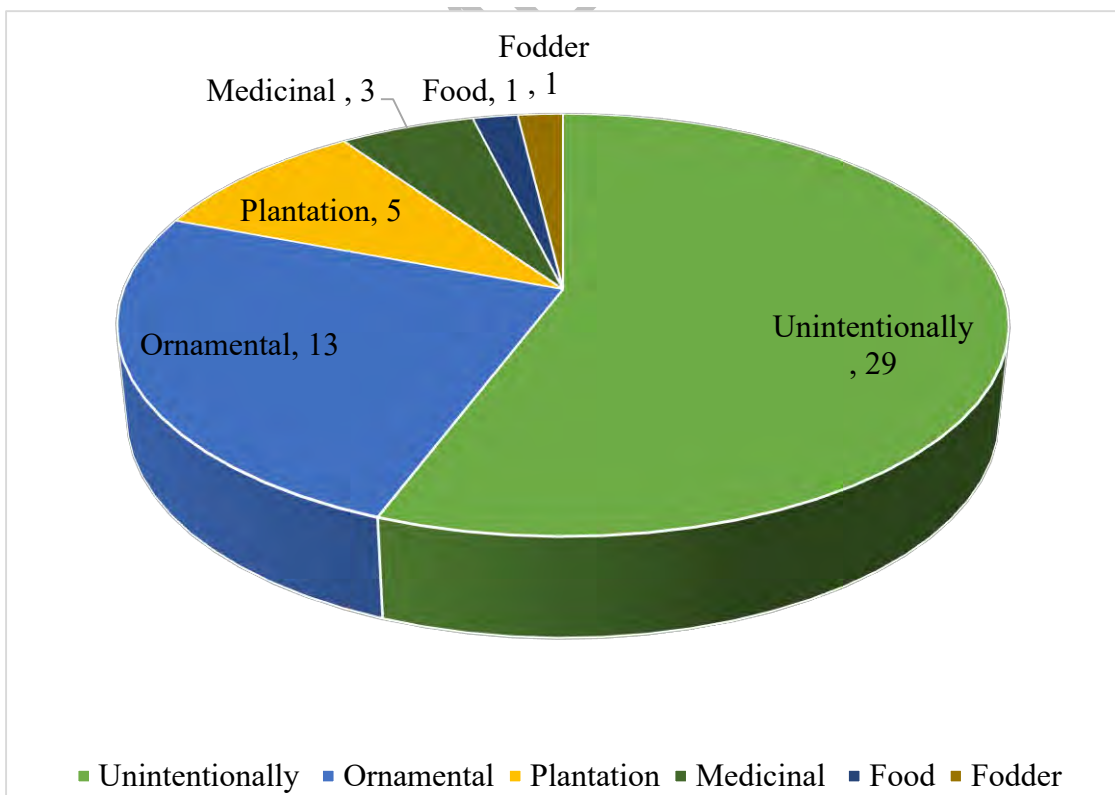


Figure 46. Purpose of introduction of invasive species in Lesser Himalayas-Pakistan

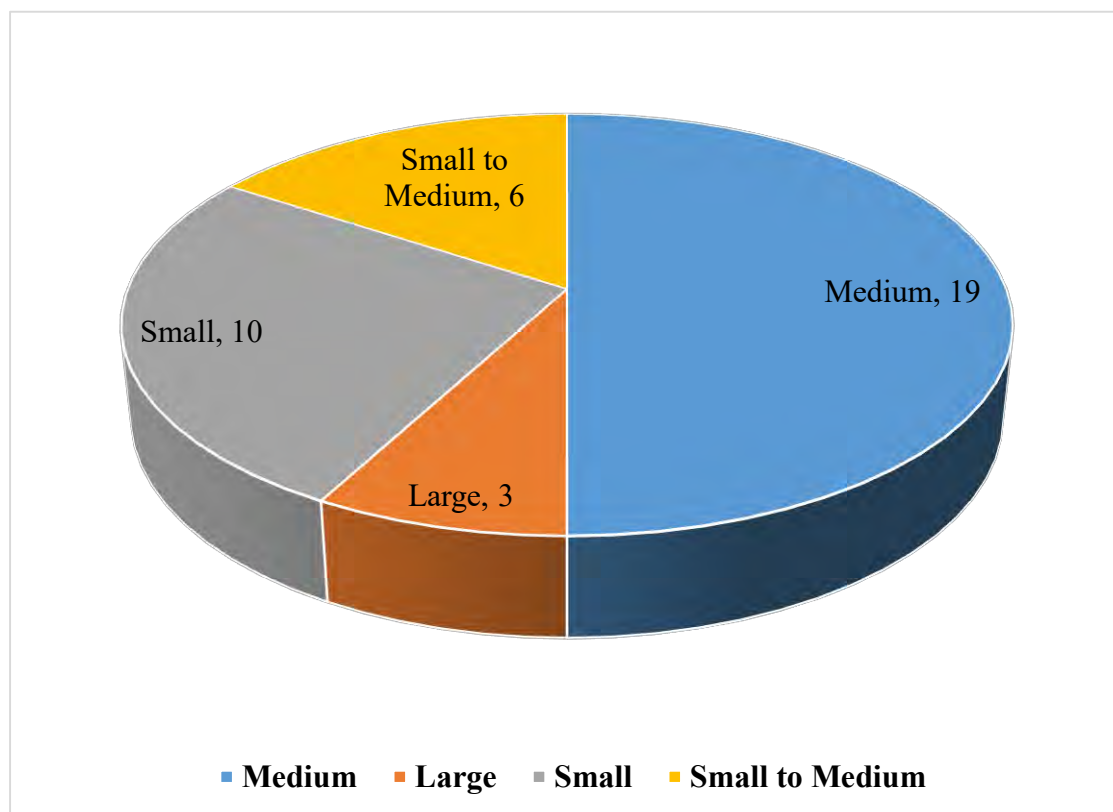


Figure 47. Pollen Size of invasive plants of Lesser Himalayas Pakistan

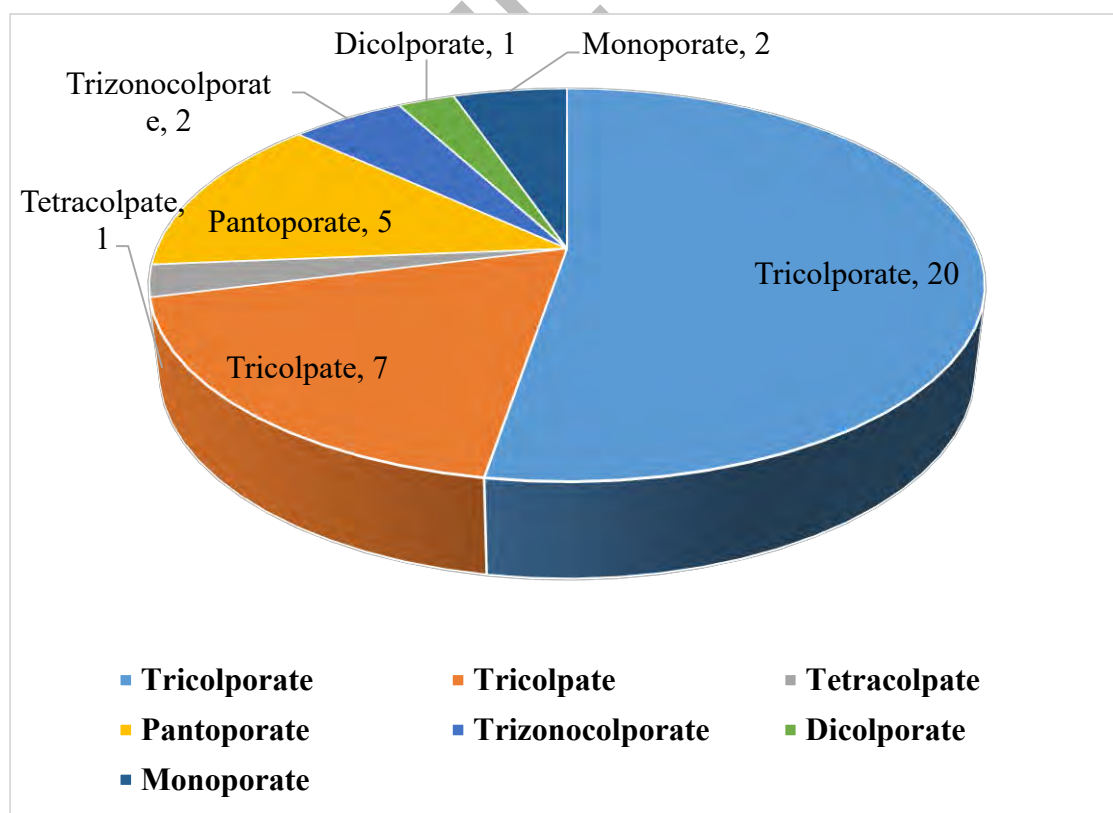


Figure 48. Percentage of Pollen Types of invasive Plant Species of Lesser Himalayas

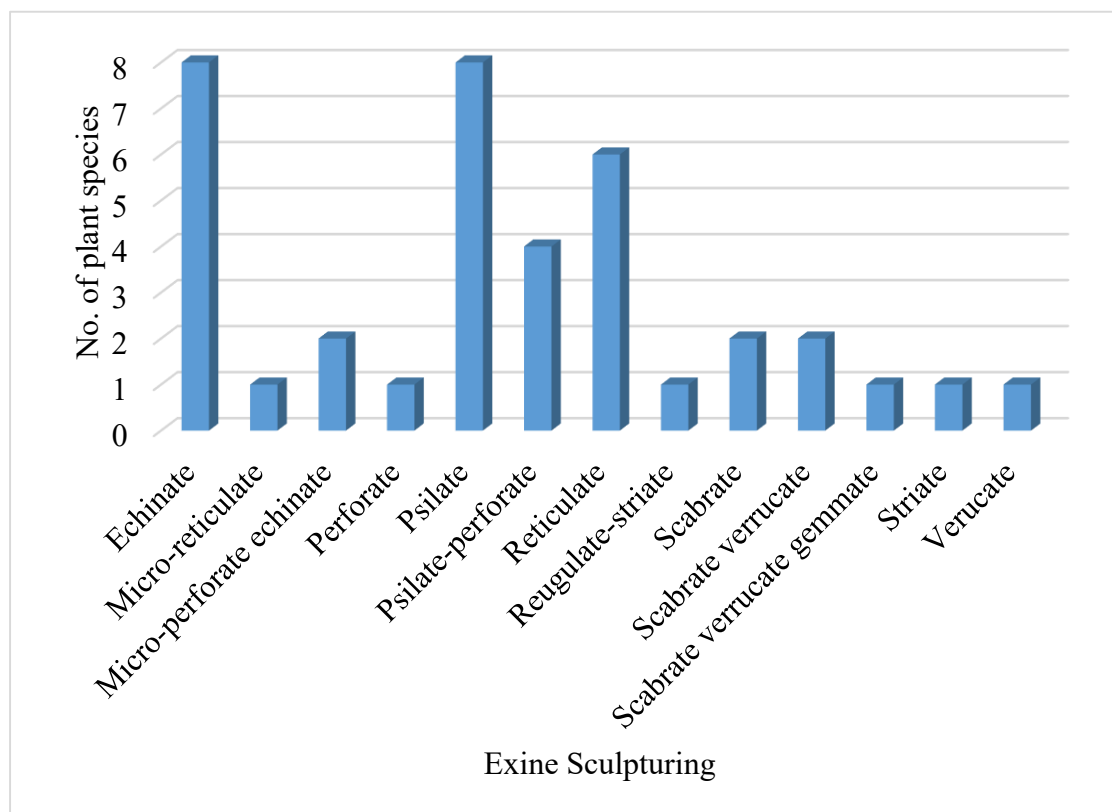


Figure 49. Variation in exine ornamentation of Invasive Plant Species

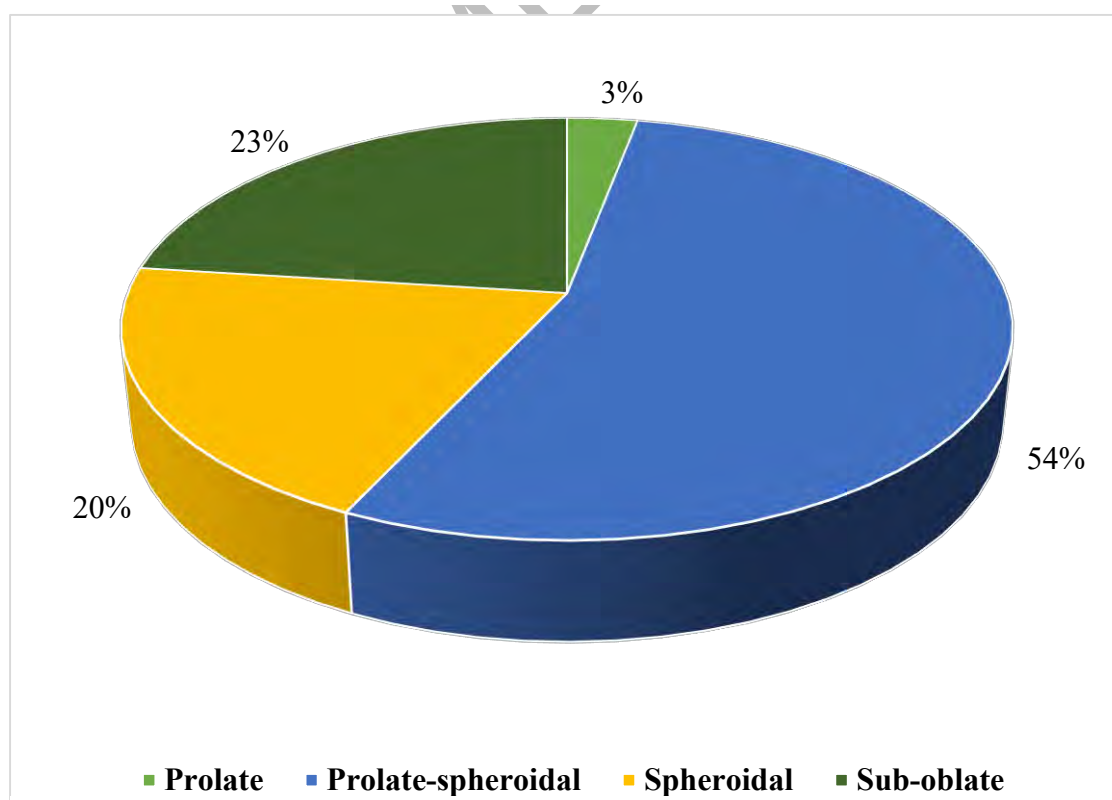


Figure 50. Percentage of pollen shape in equatorial view of invasive plant species

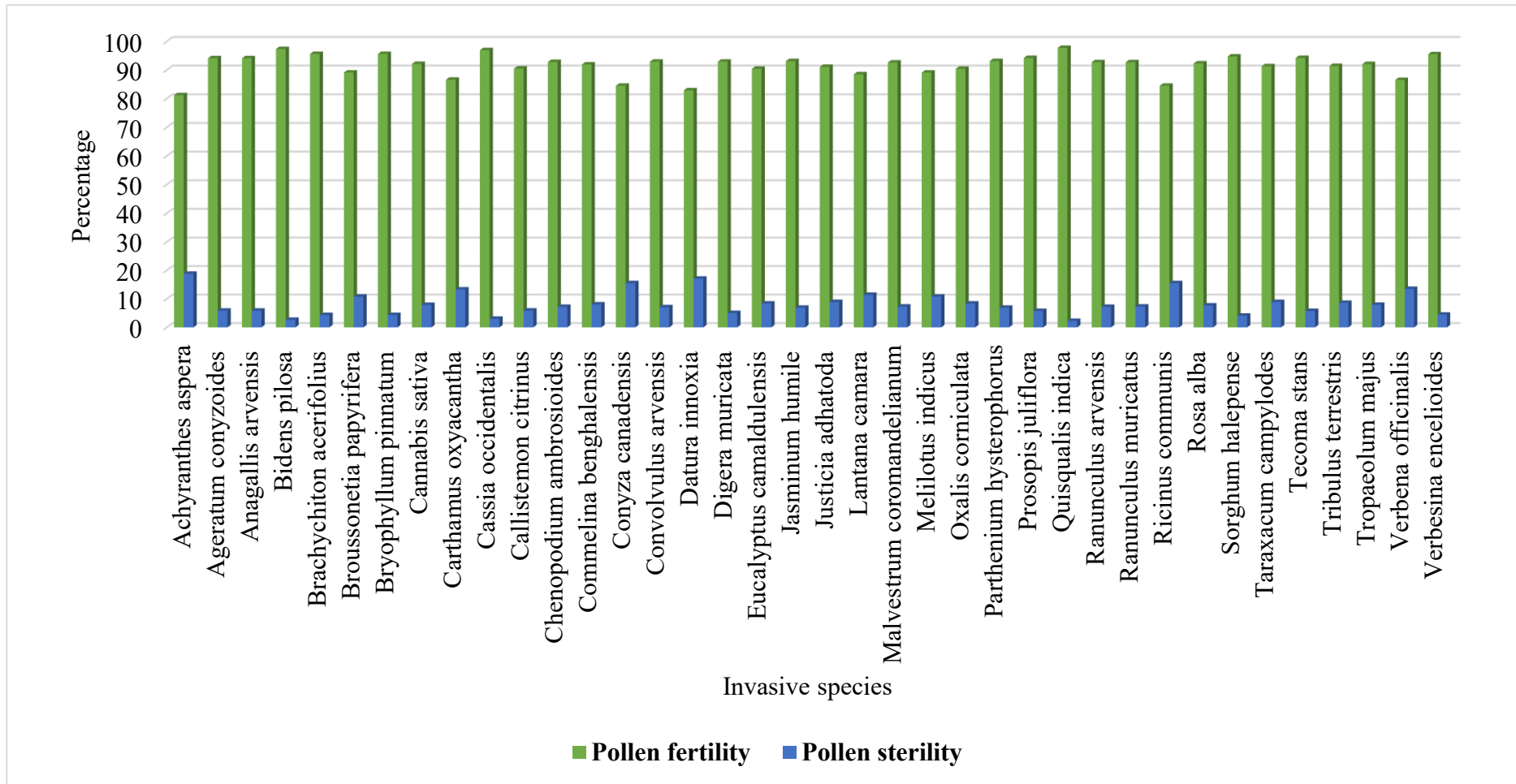


Figure 51. Distribution percentage of fertility and sterility of invasive Plant Species

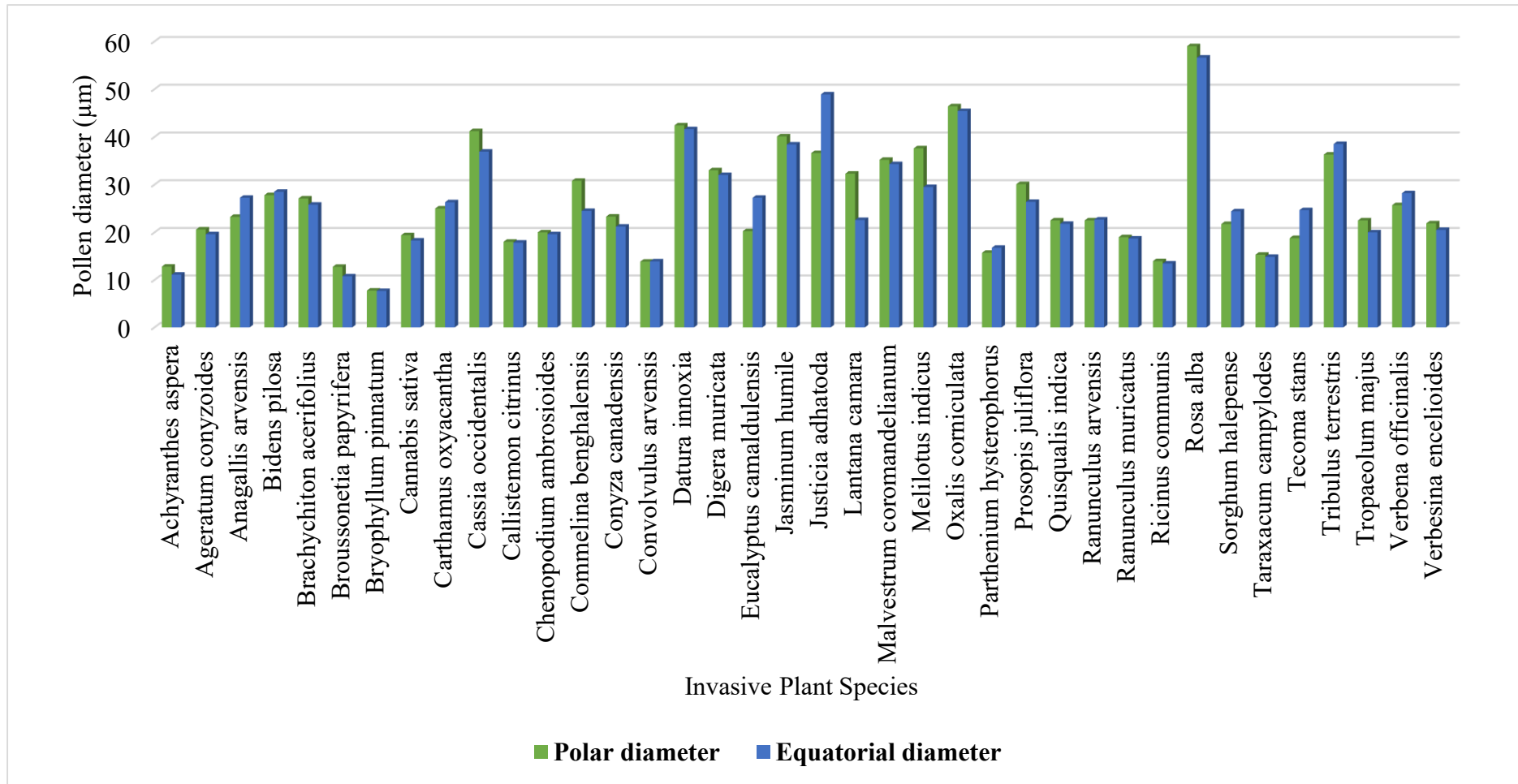


Figure 52. Polar and equatorial diameter comparison of pollen grains of invasive plant species

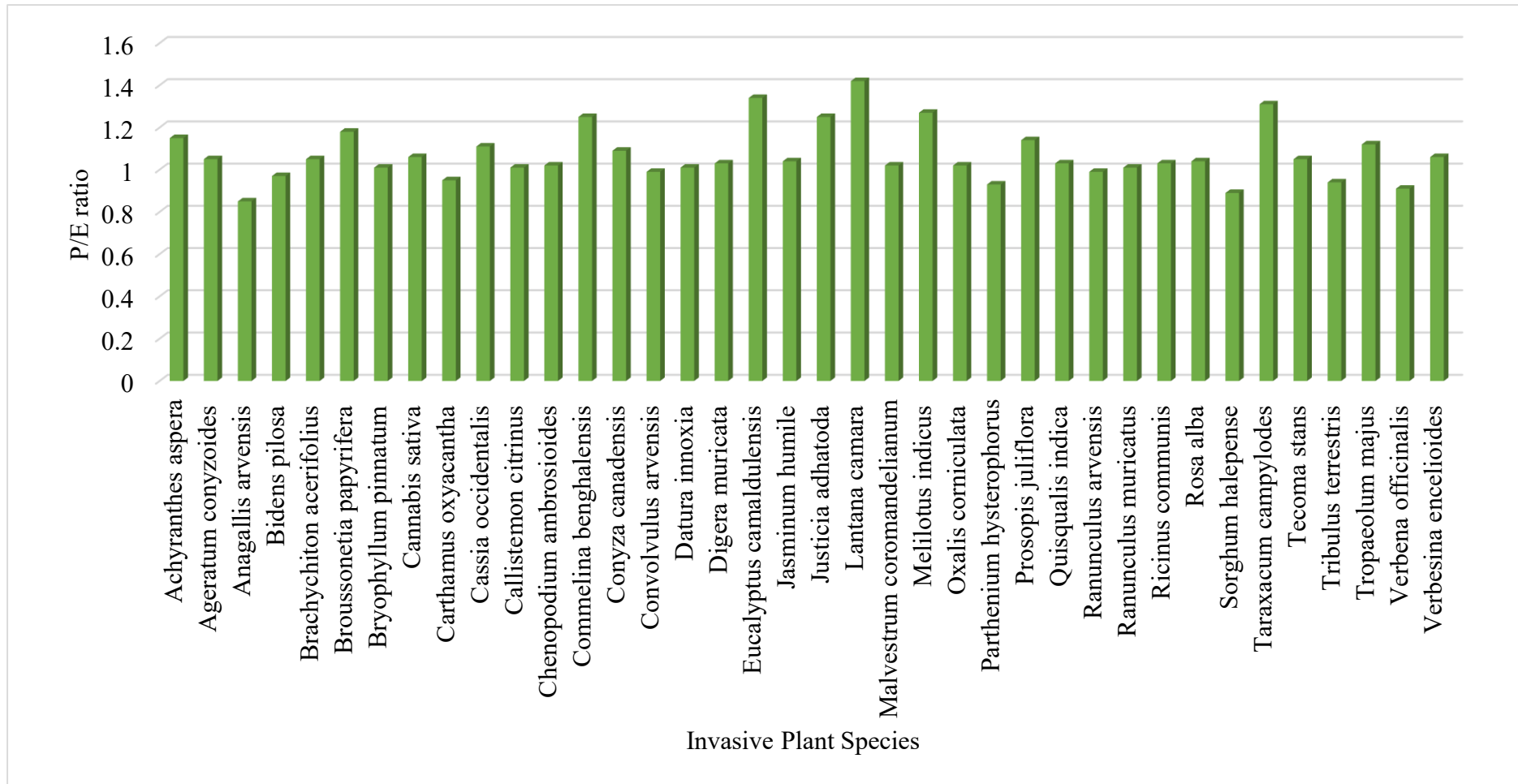


Figure 53. P/E ratio of Invasive Plant Species of Lesser Himalayas Pakistan

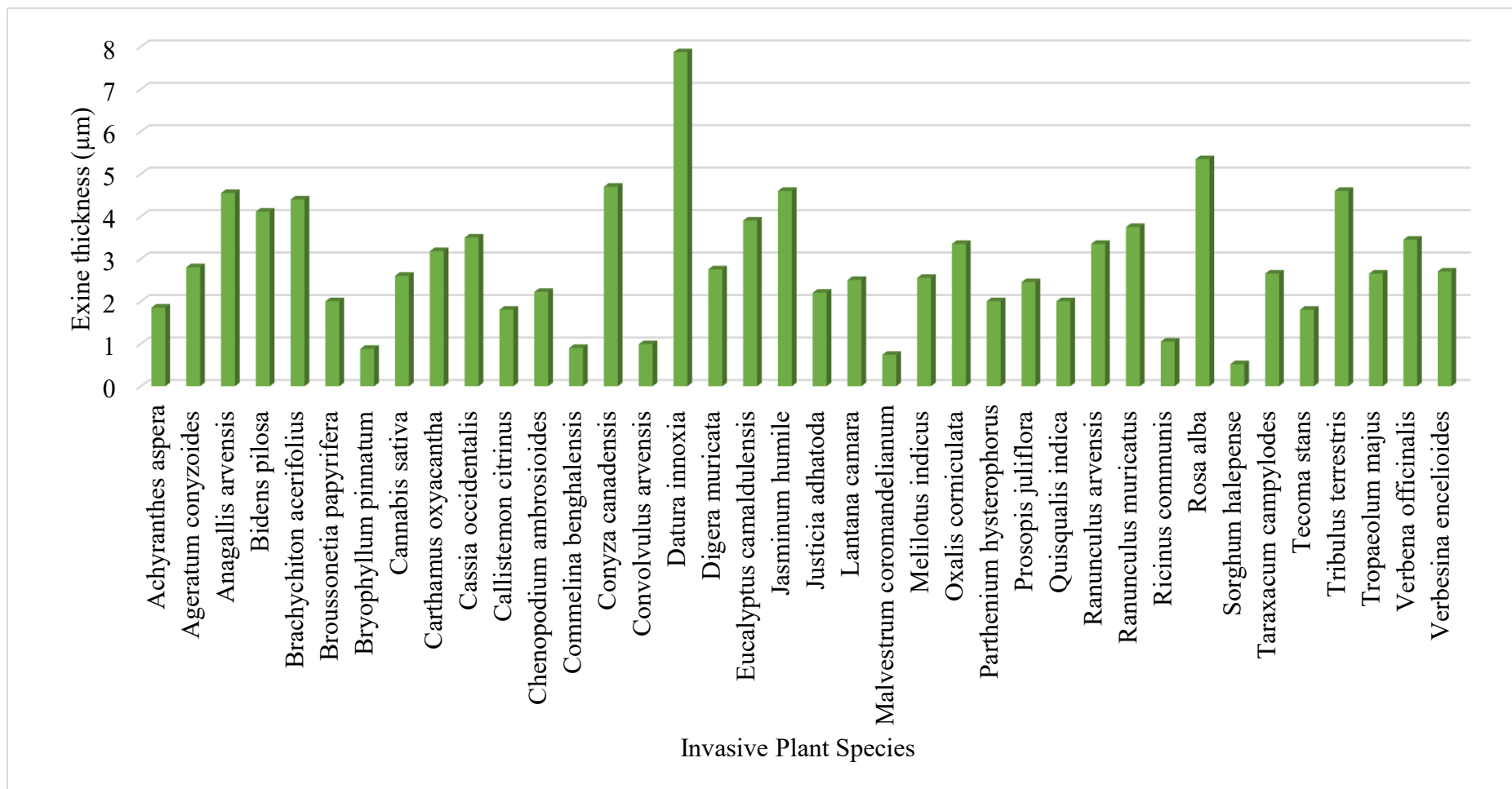


Figure 54. Exine thickness variations among Invasive plant species

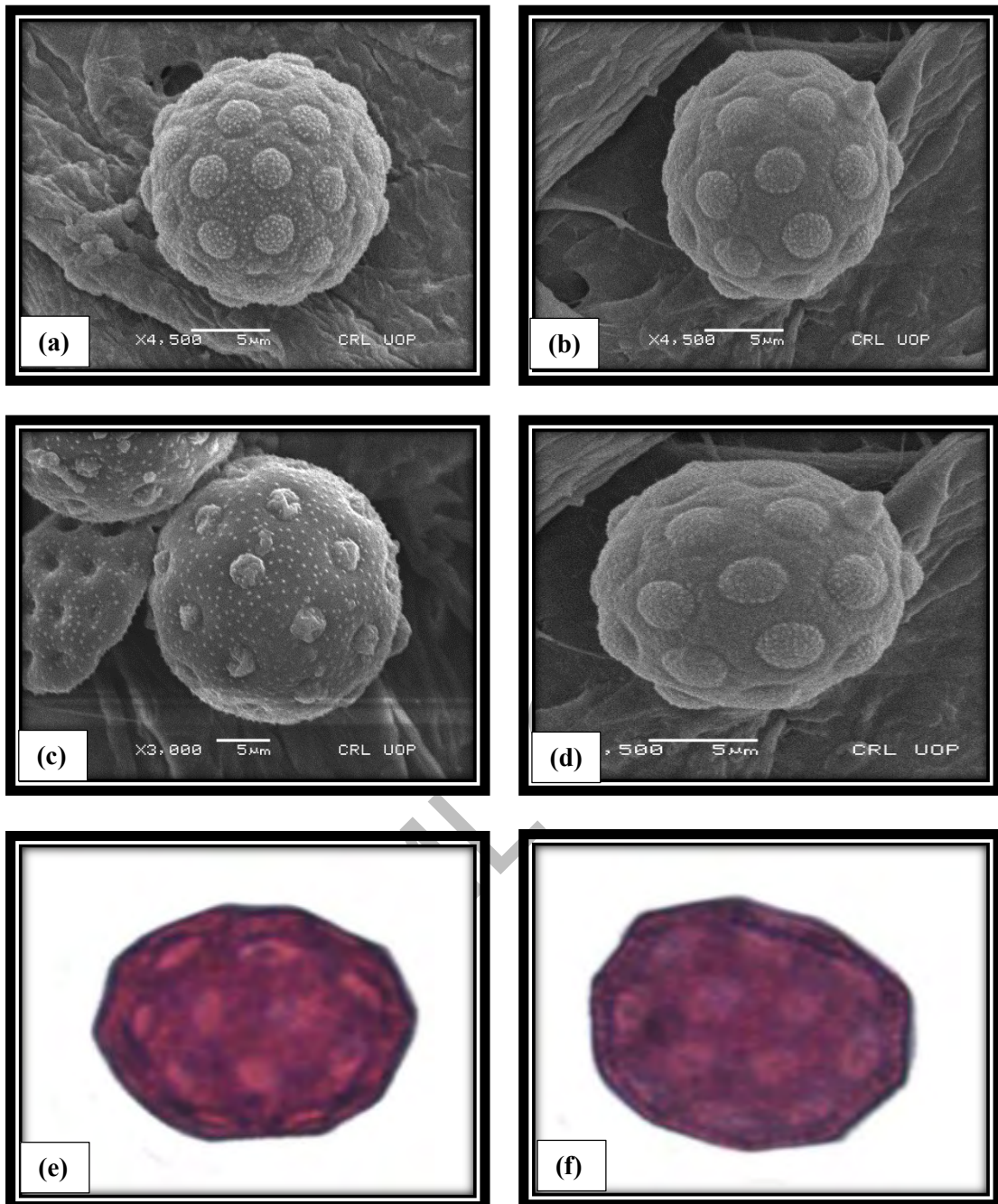


Plate 3. Pollen Microphotographs of *Achyranthus aspera* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

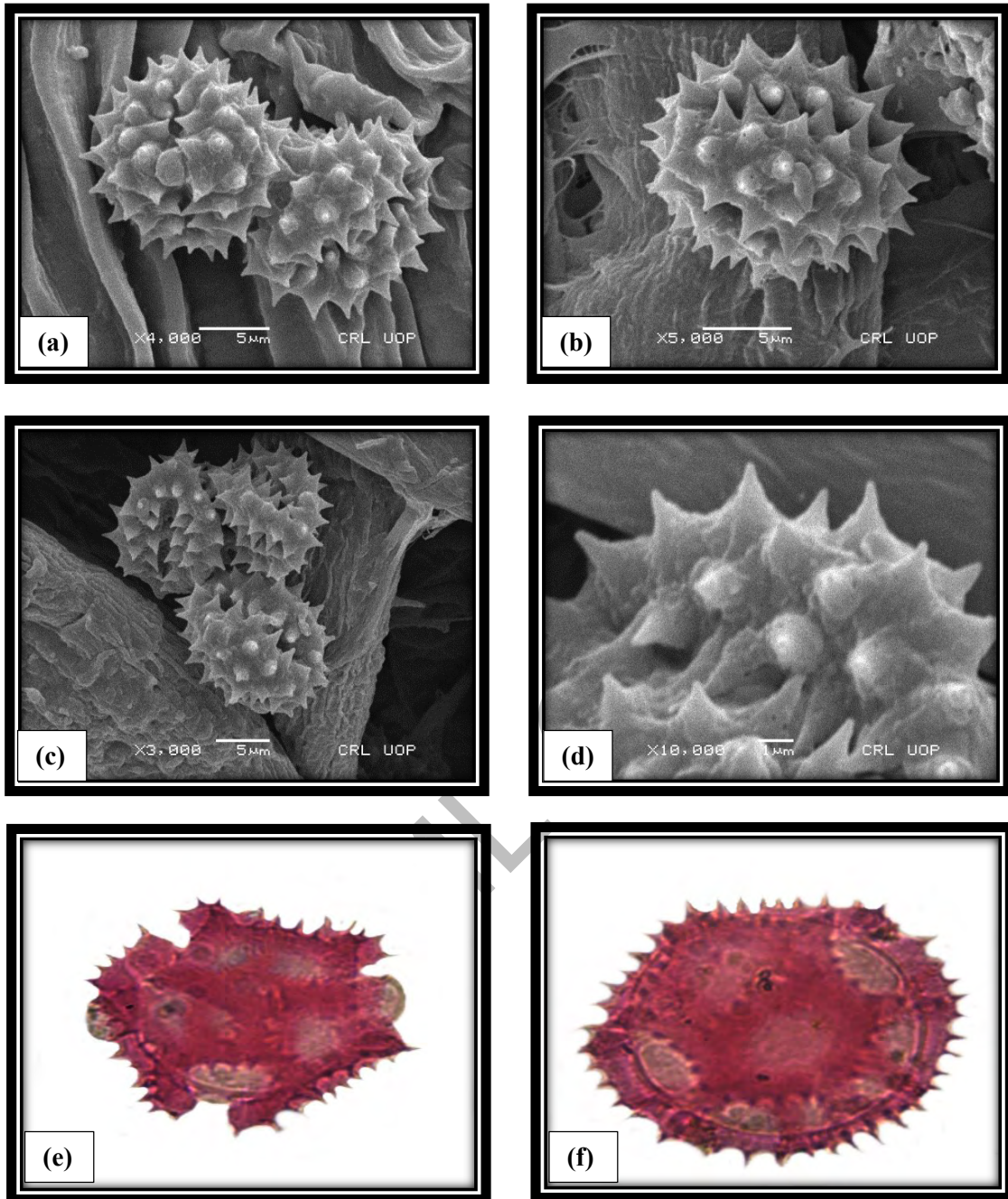


Plate 4. Pollen Microphotographs of *Ageratum conyzoides* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

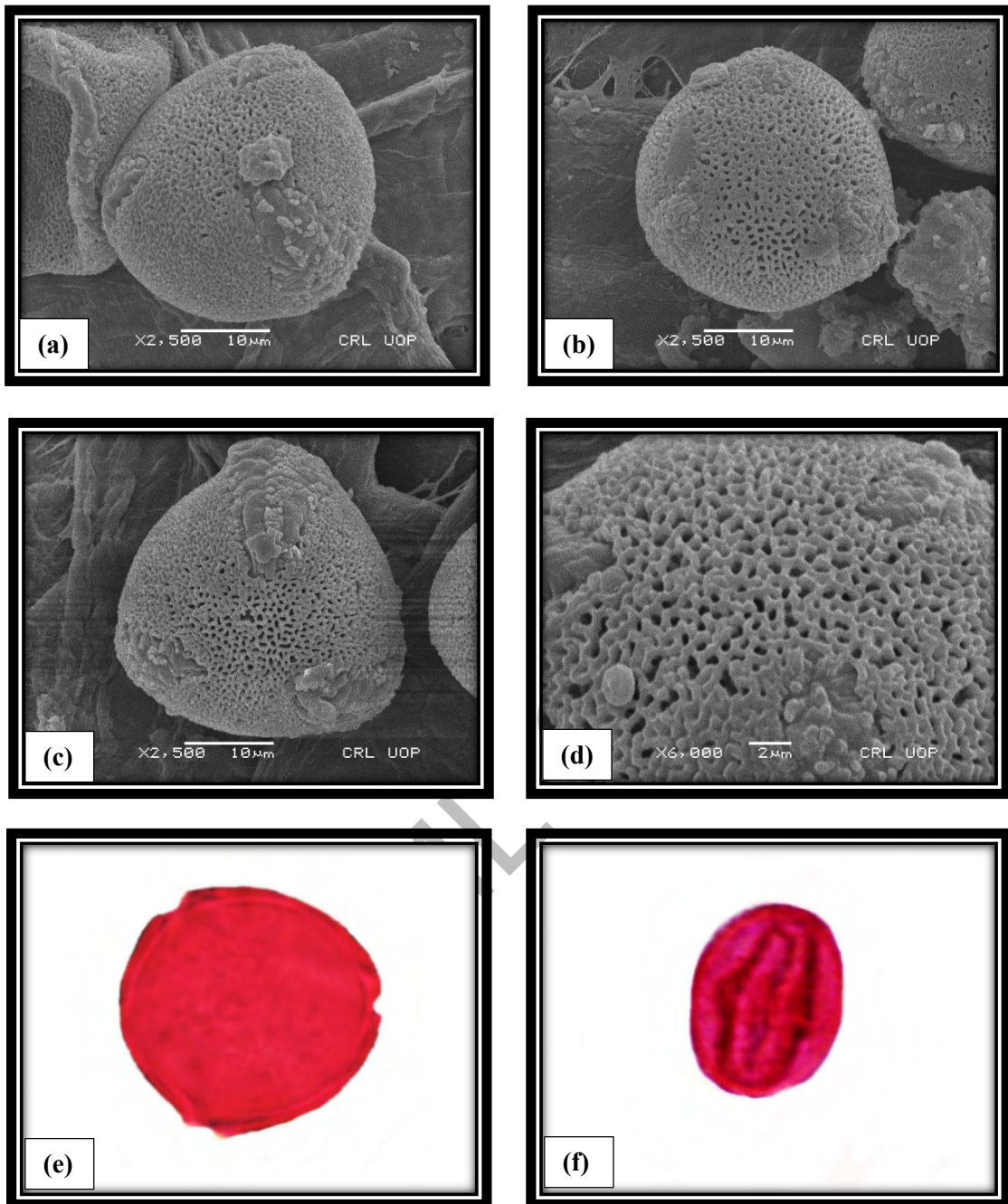


Plate 5. Pollen Microphotographs of *Anagallis arvensis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

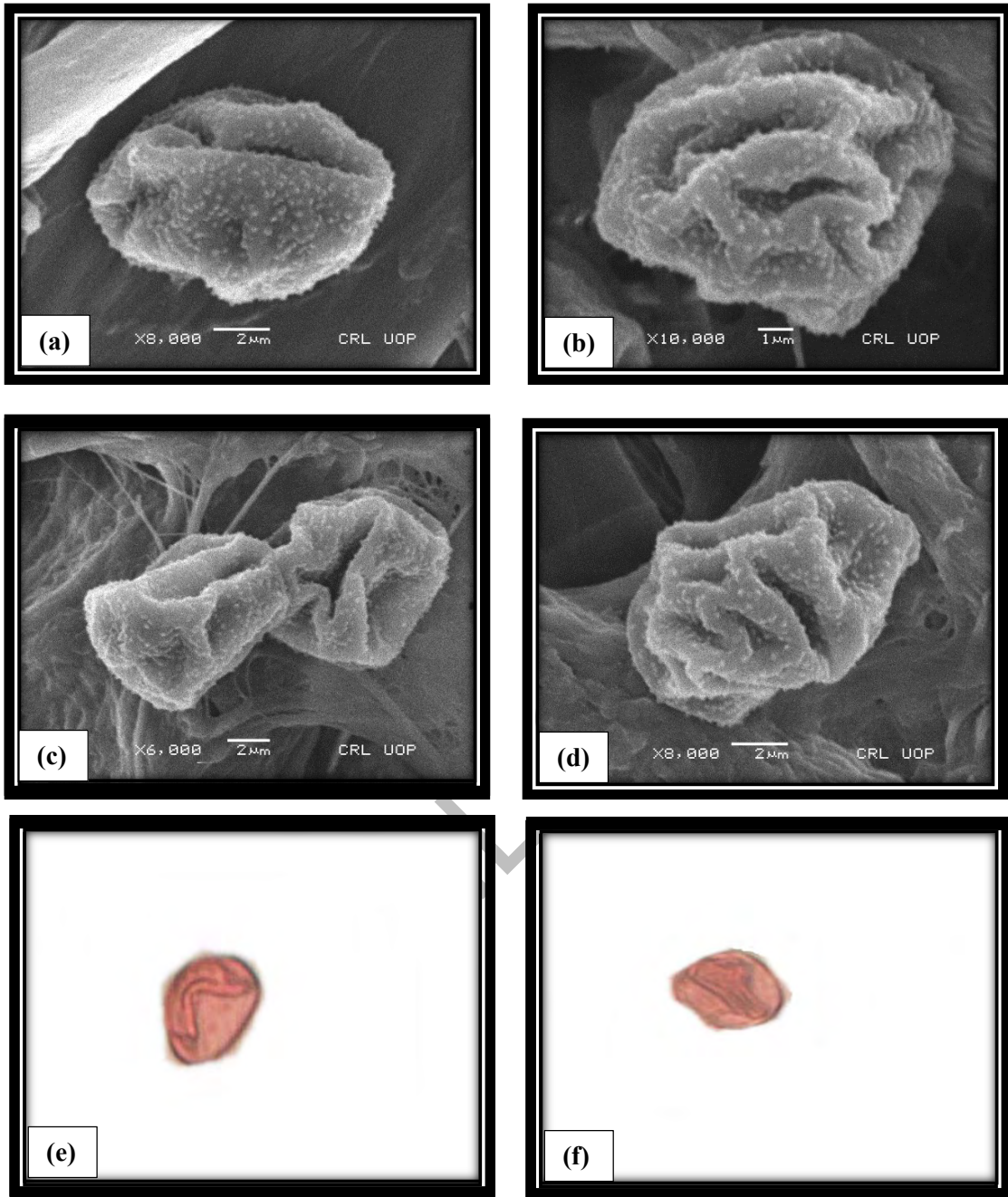


Plate 6. Pollen Microphotographs of *Broussonetia papyrifera* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

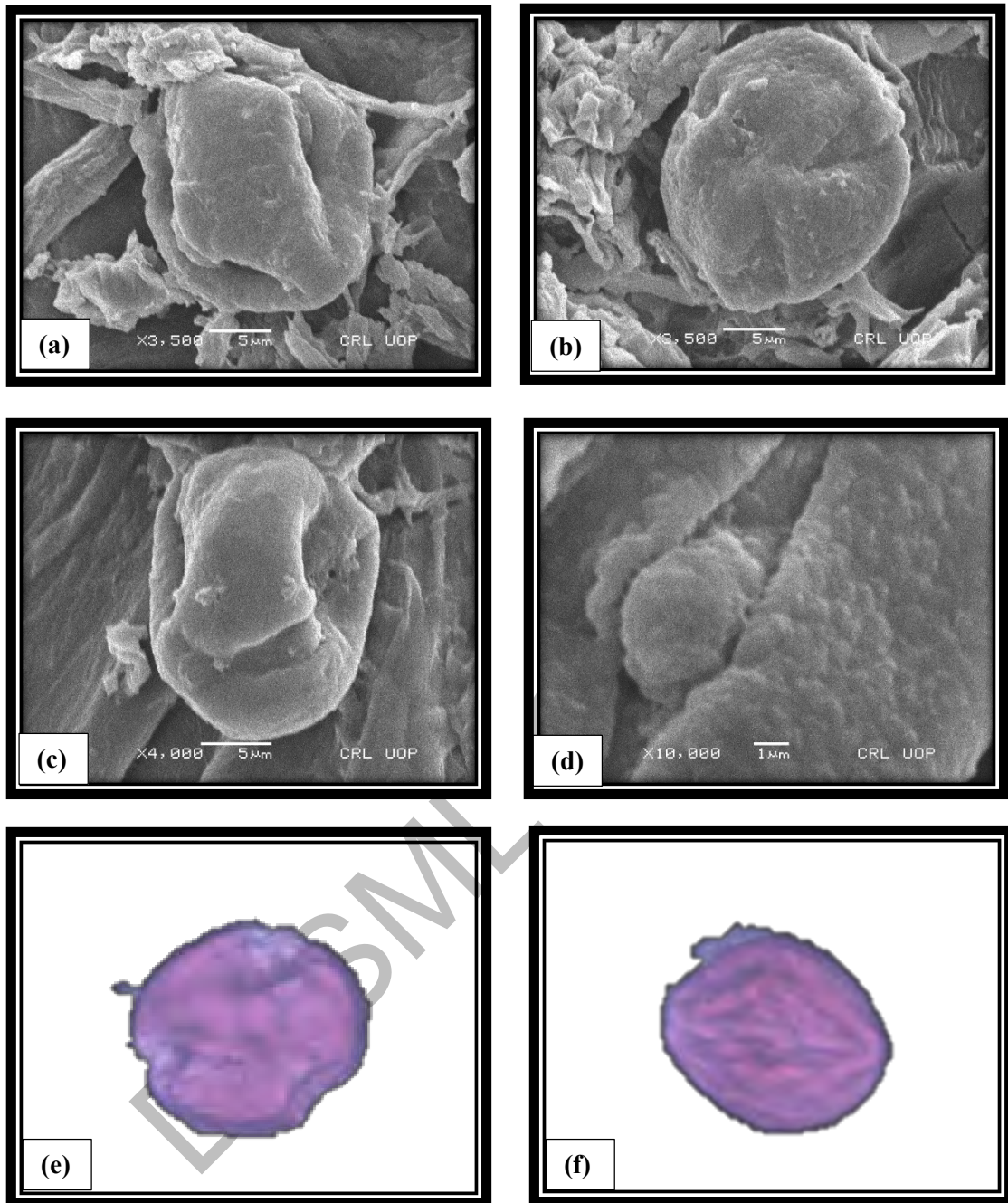


Plate 7. Pollen Microphotographs of *Bryophyllum pinnatum* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

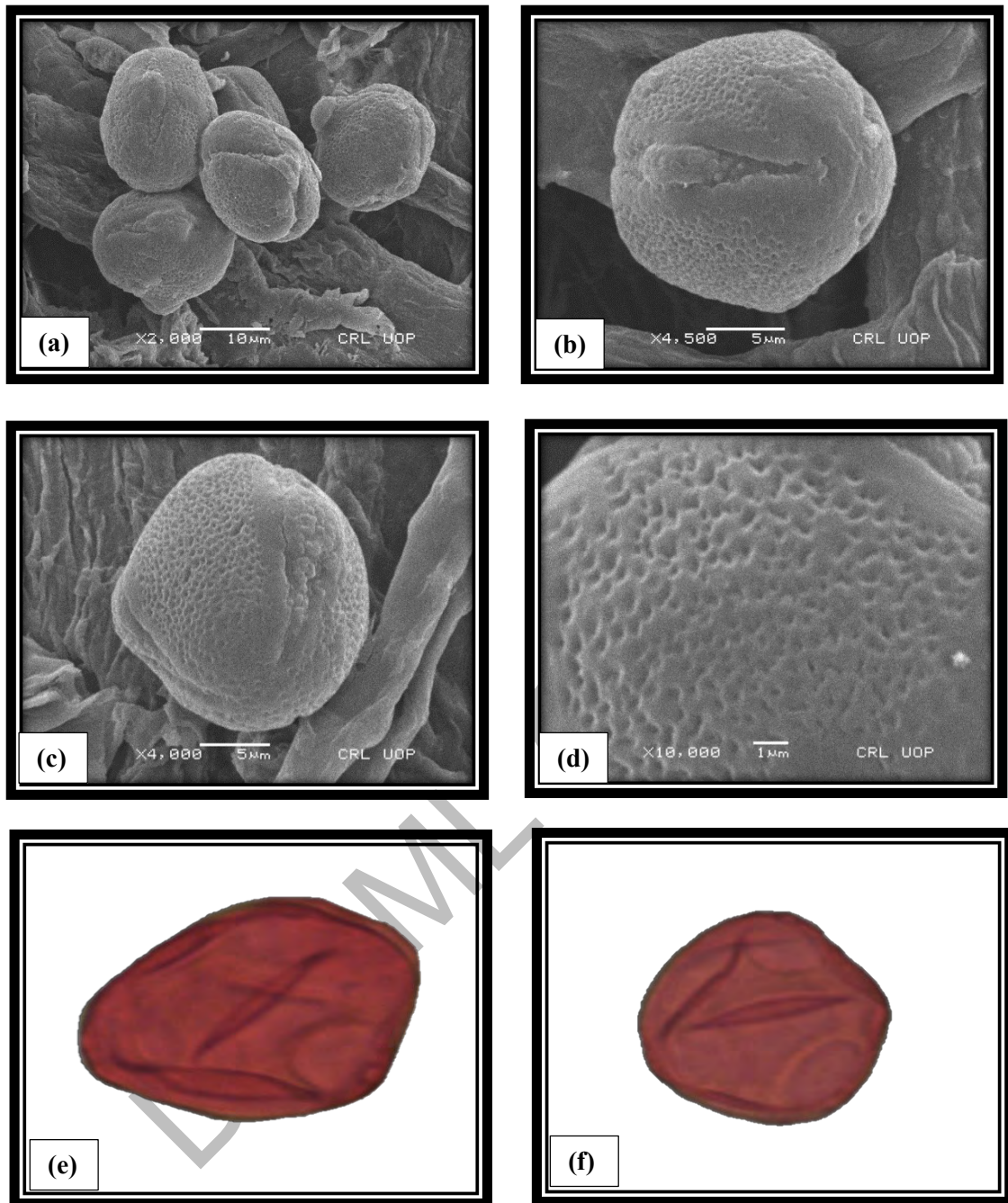


Plate 8. Pollen Microphotographs of *Cannabis sativa* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

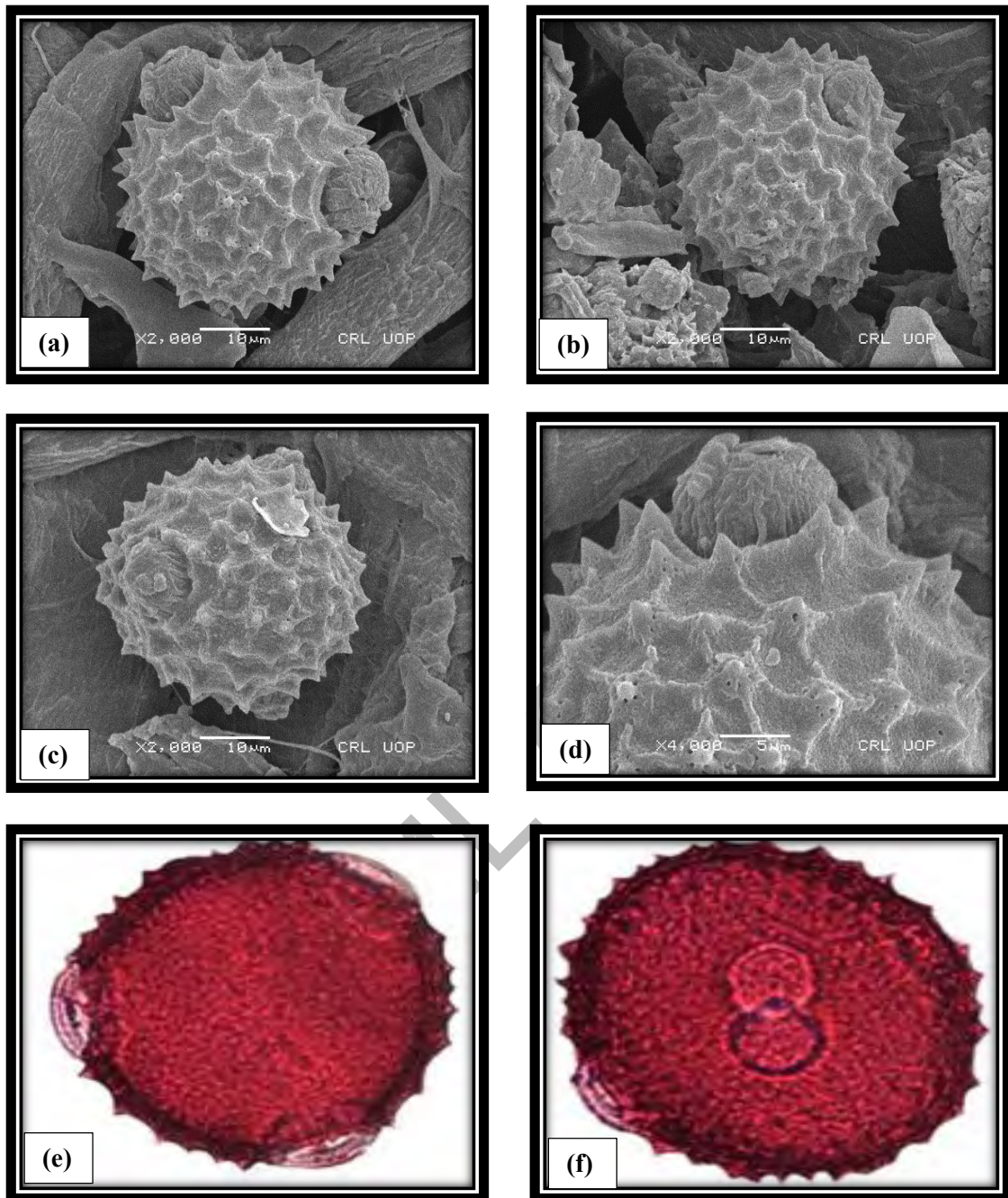


Plate 9. Pollen Microphotographs of *Carthamus oxyacantha* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

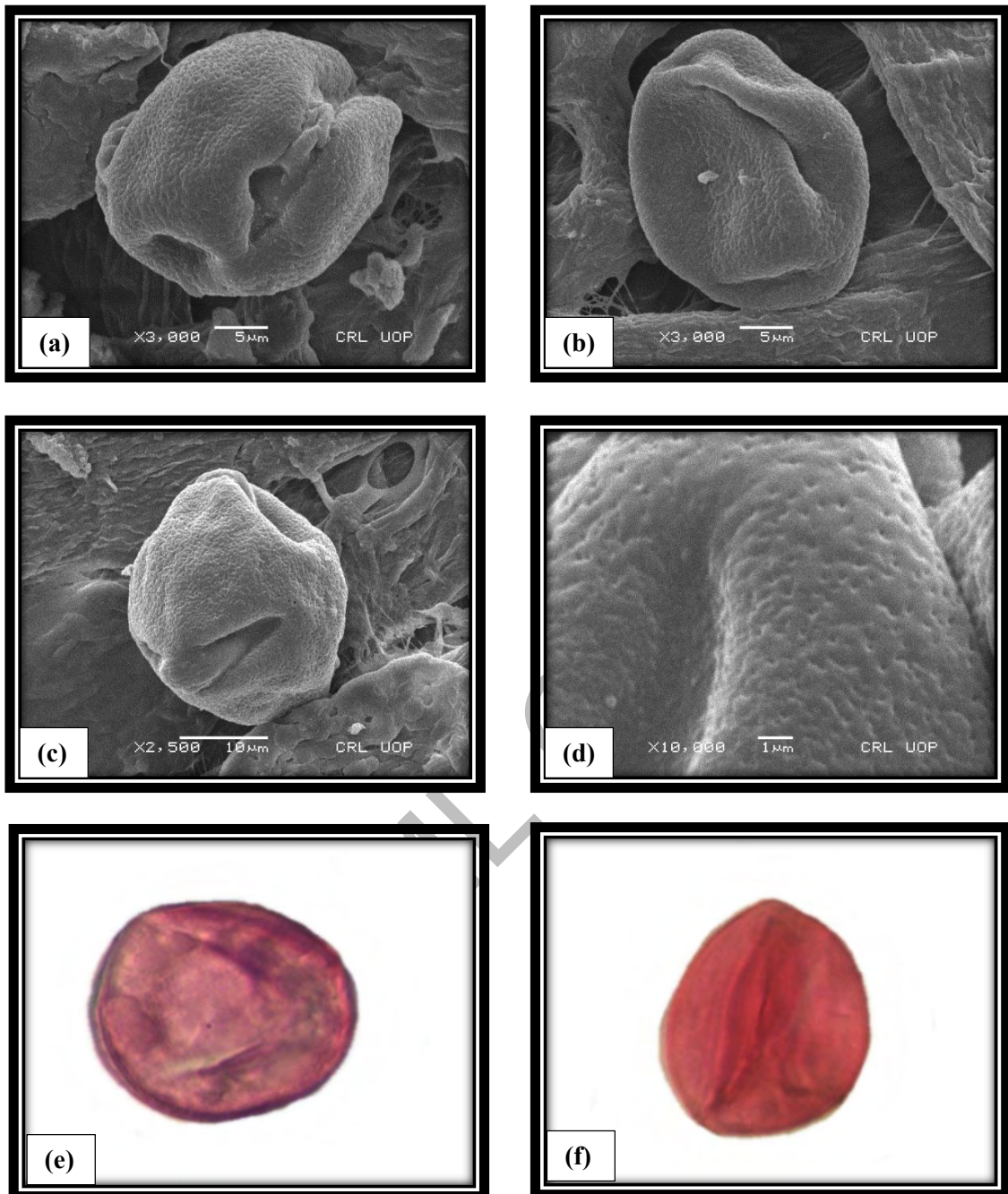


Plate 10. Pollen Microphotographs of *Cassia occidentalis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

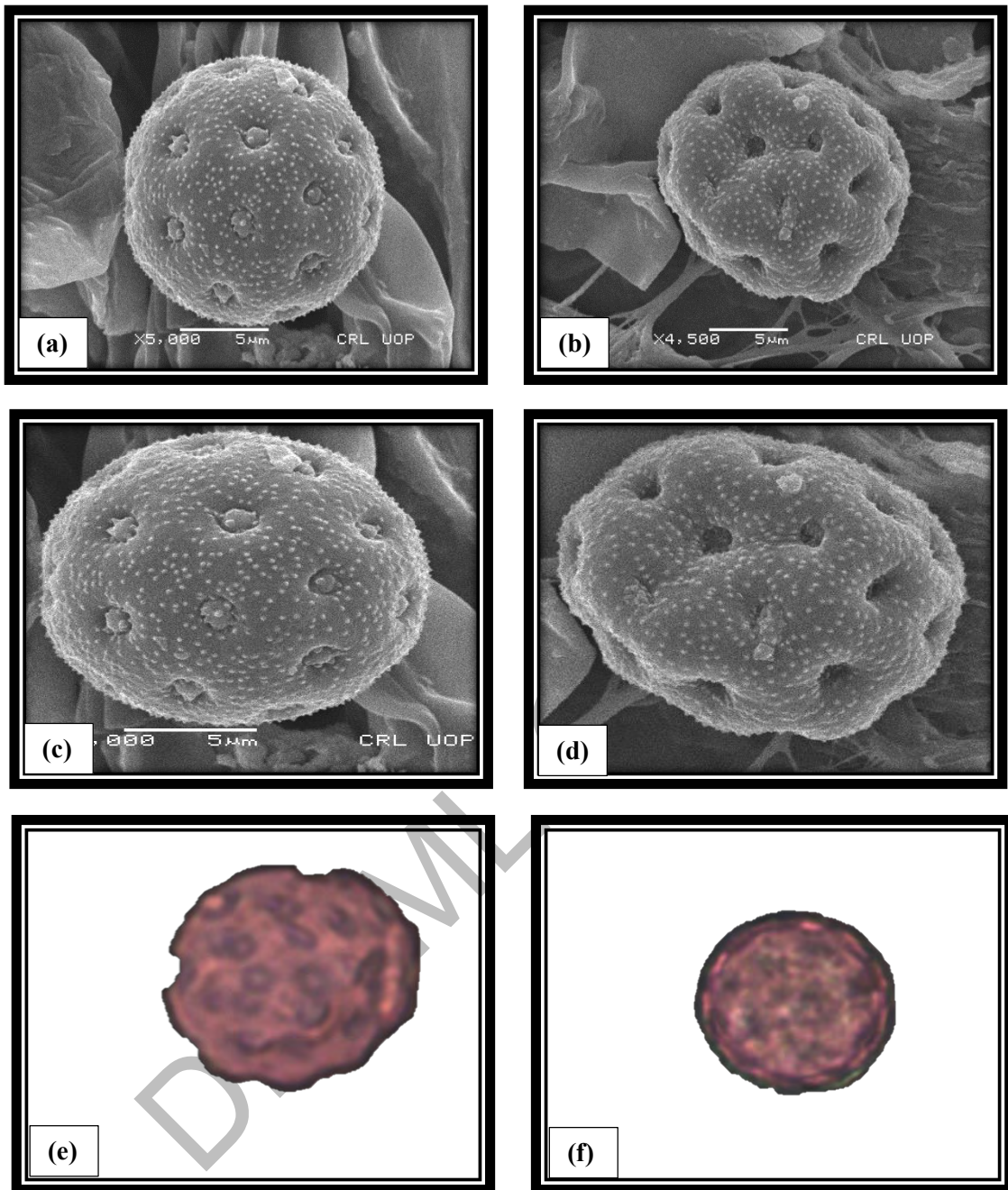


Plate 11. Pollen Microphotographs of *Chenopodium abrosioides* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

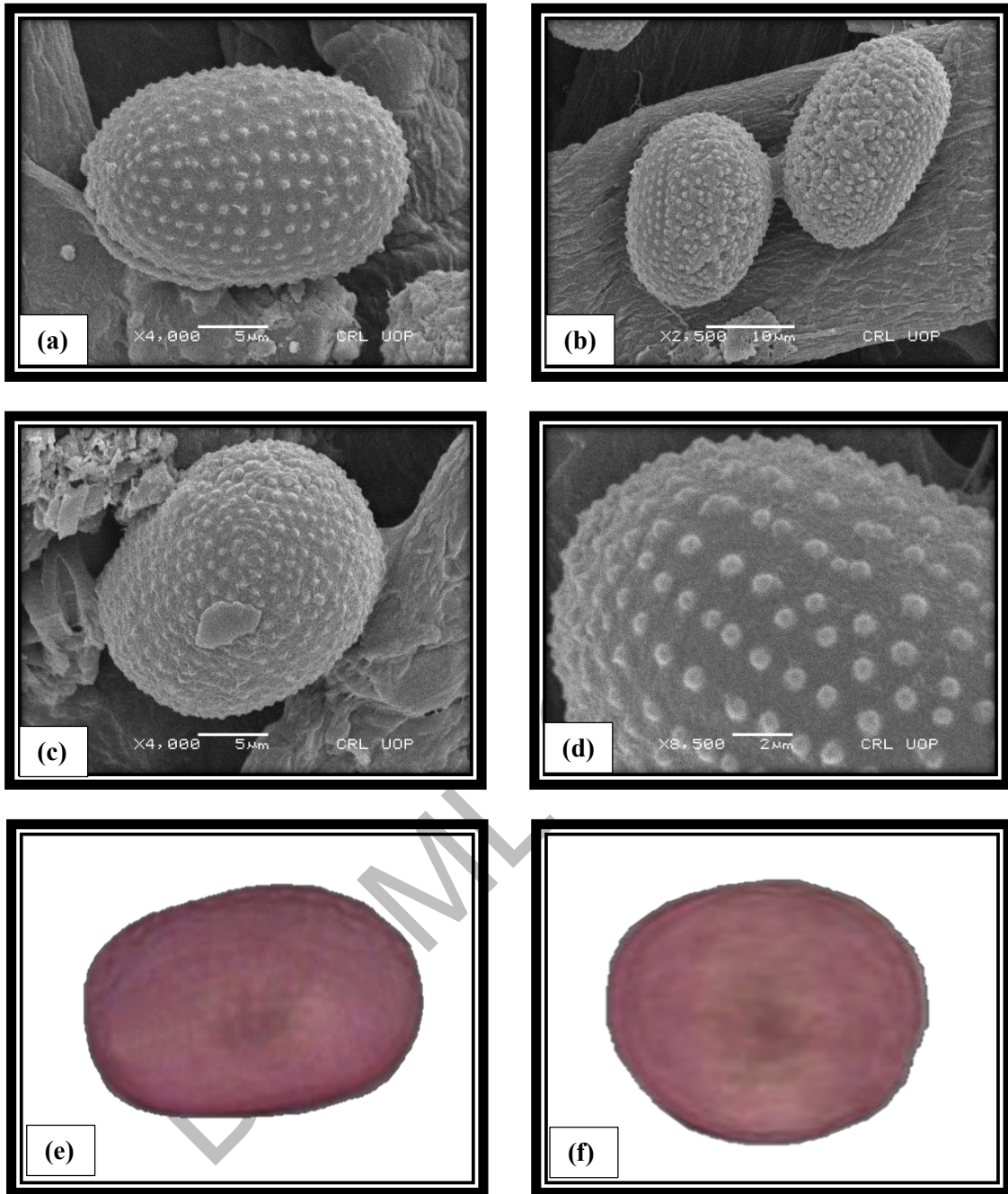


Plate 12. Pollen Microphotographs of *Commelina benghalensis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

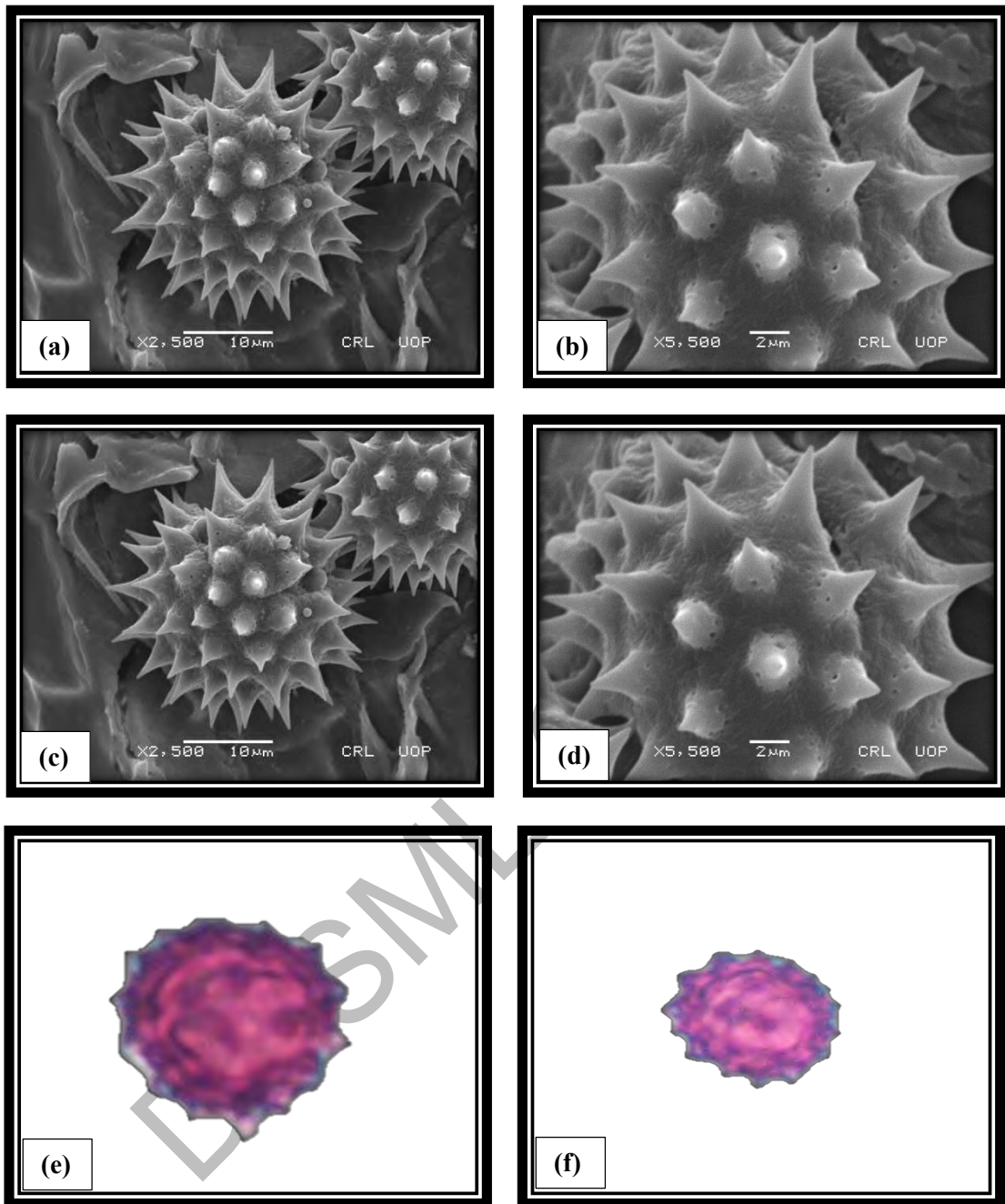


Plate 13. Pollen Microphotographs of *Conyza canadensis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

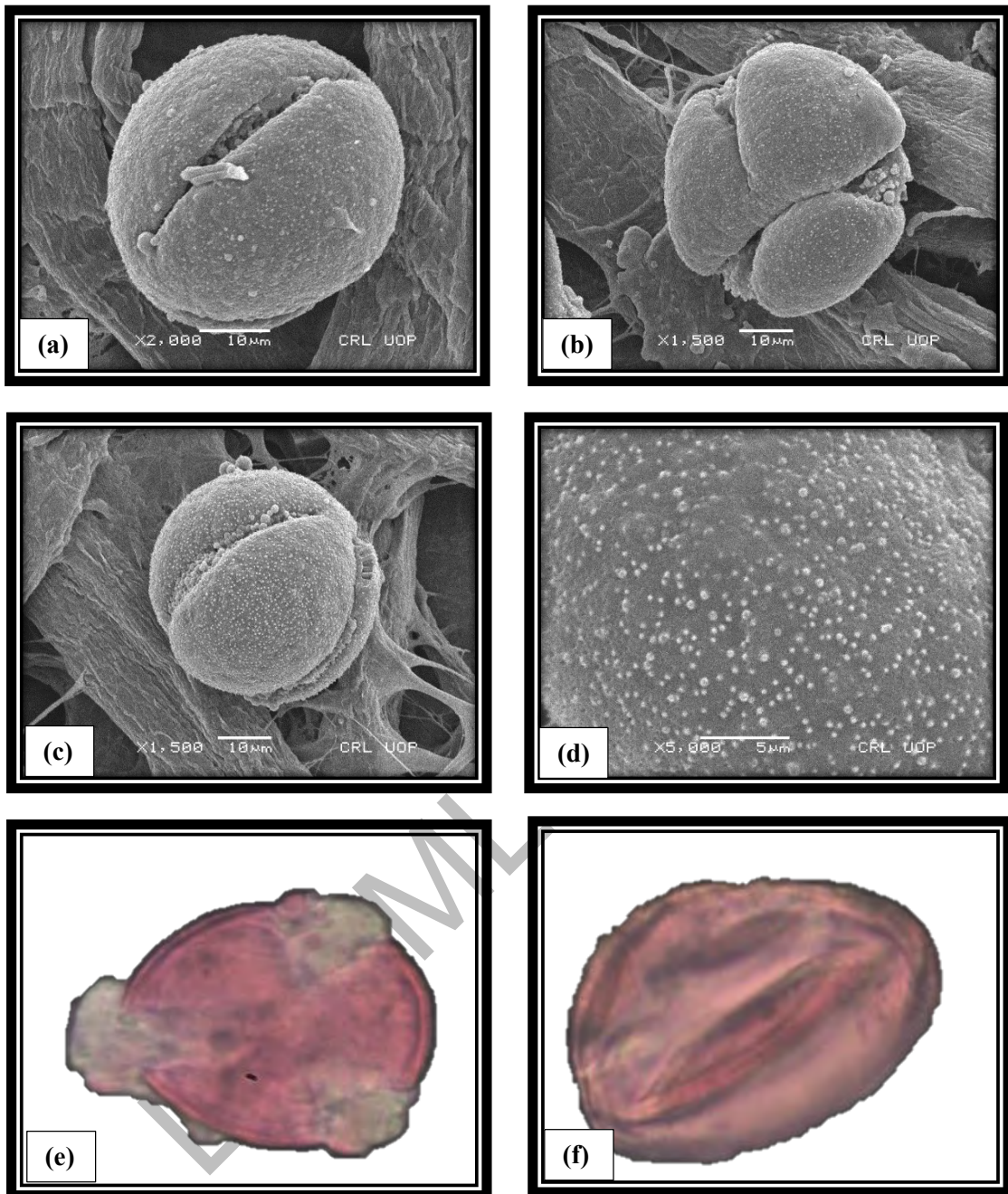


Plate 14. Pollen Microphotographs of *Convolvulus arvensis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

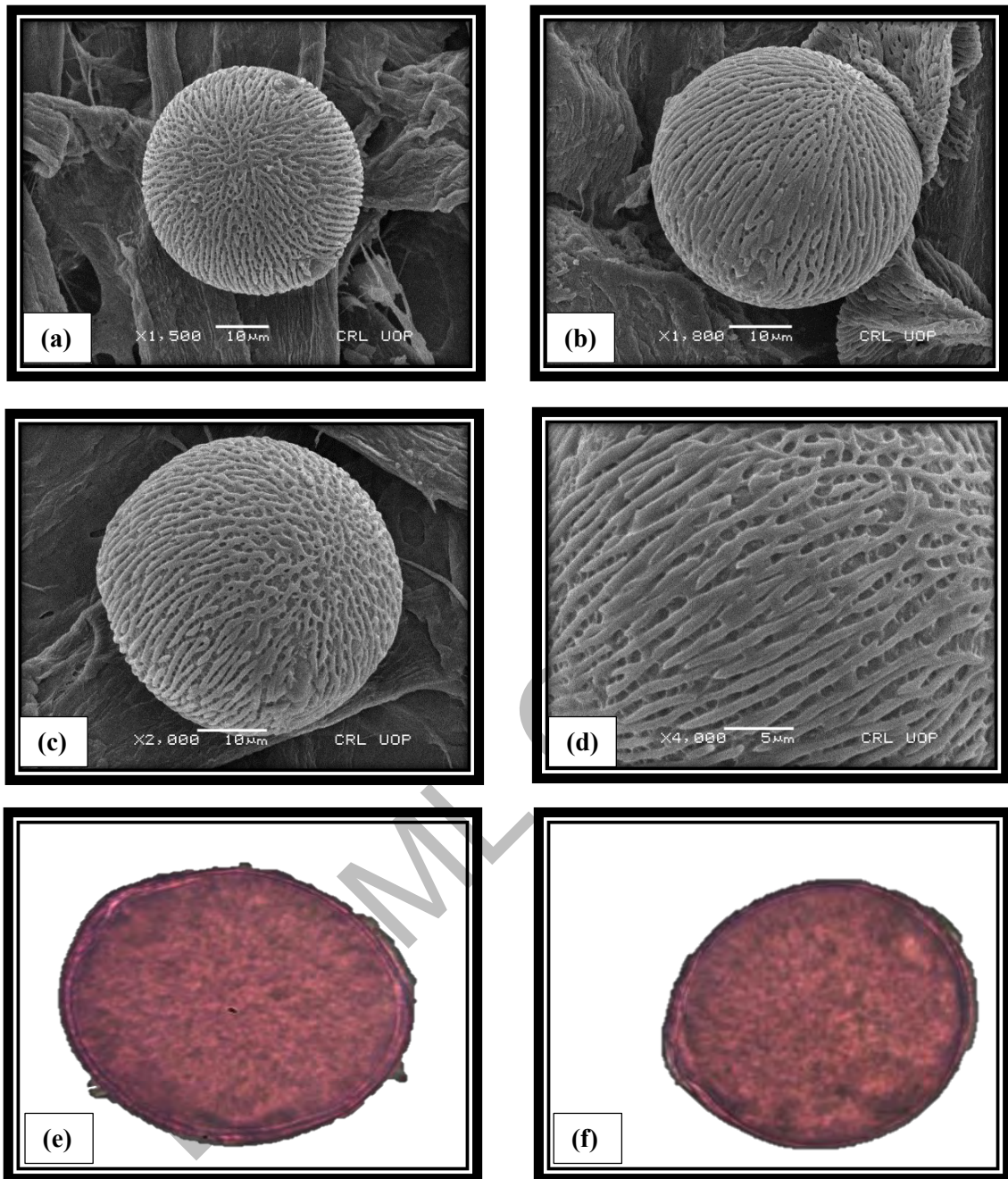


Plate 15. Pollen Microphotographs of *Datura innoxia* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

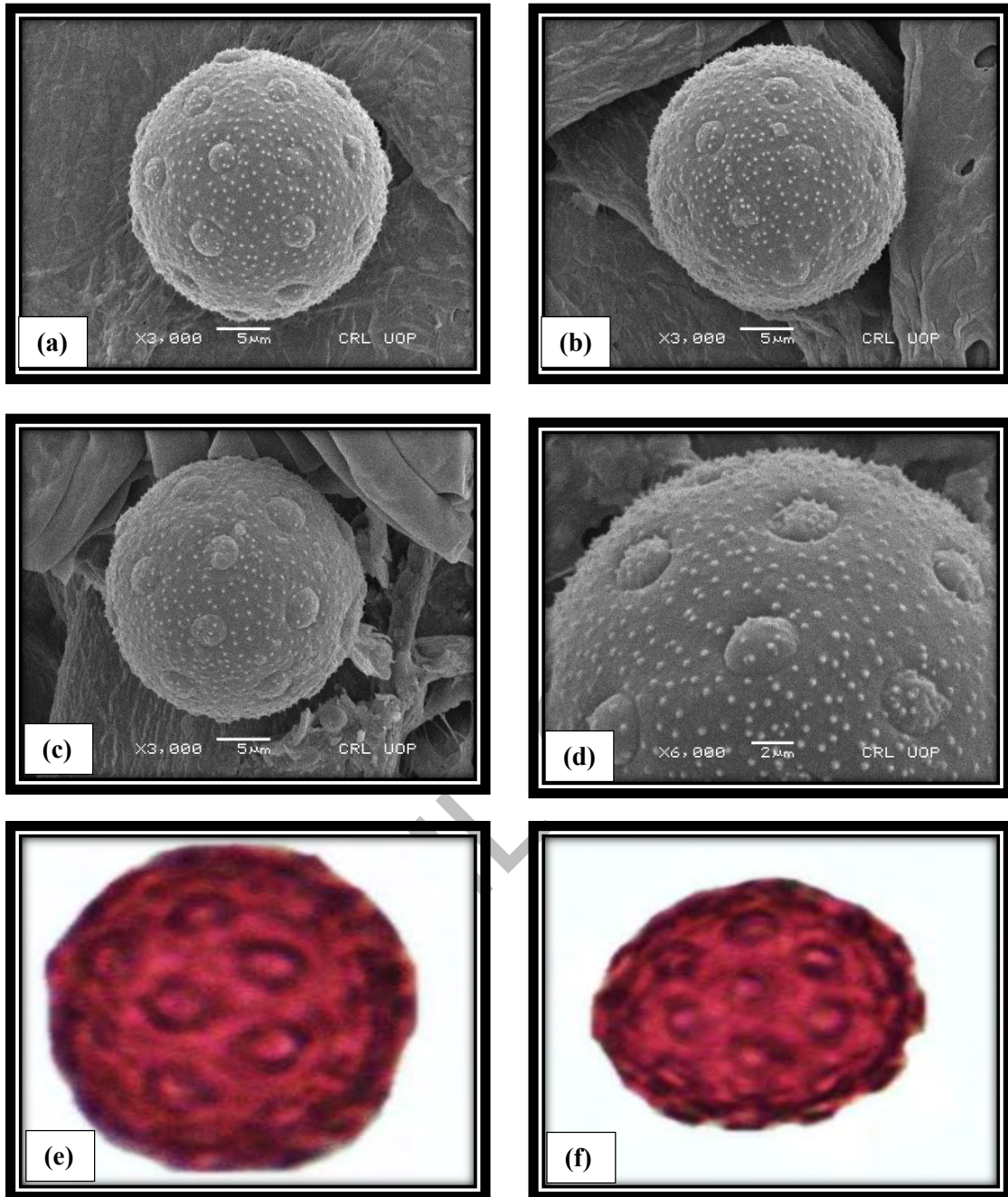


Plate 16. Pollen Microphotographs of *Digera muricata* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

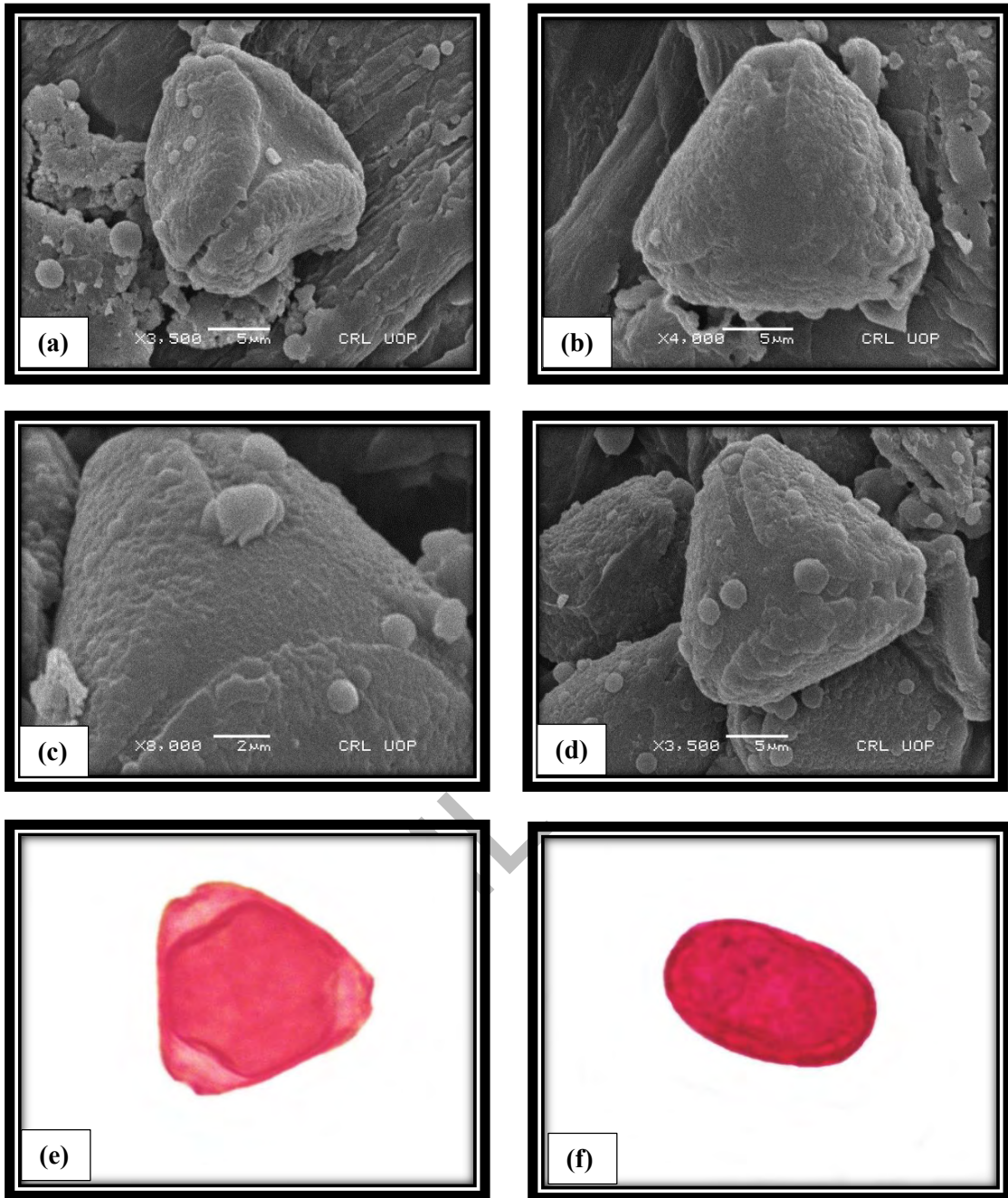


Plate 17. Pollen Microphotographs of *Eucalyptus camaldulensis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

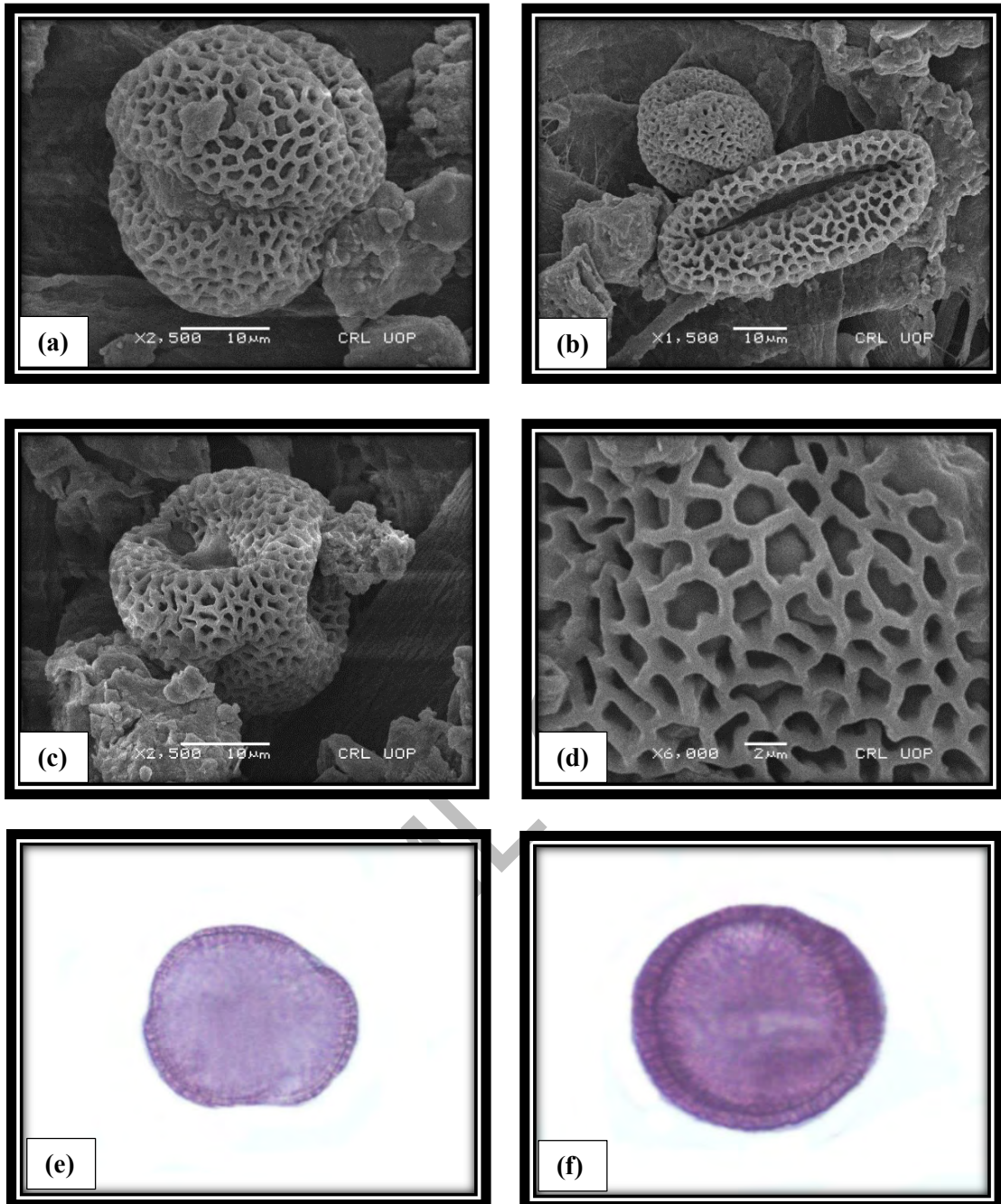


Plate 18. Pollen Microphotographs of *Jasminum humile* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

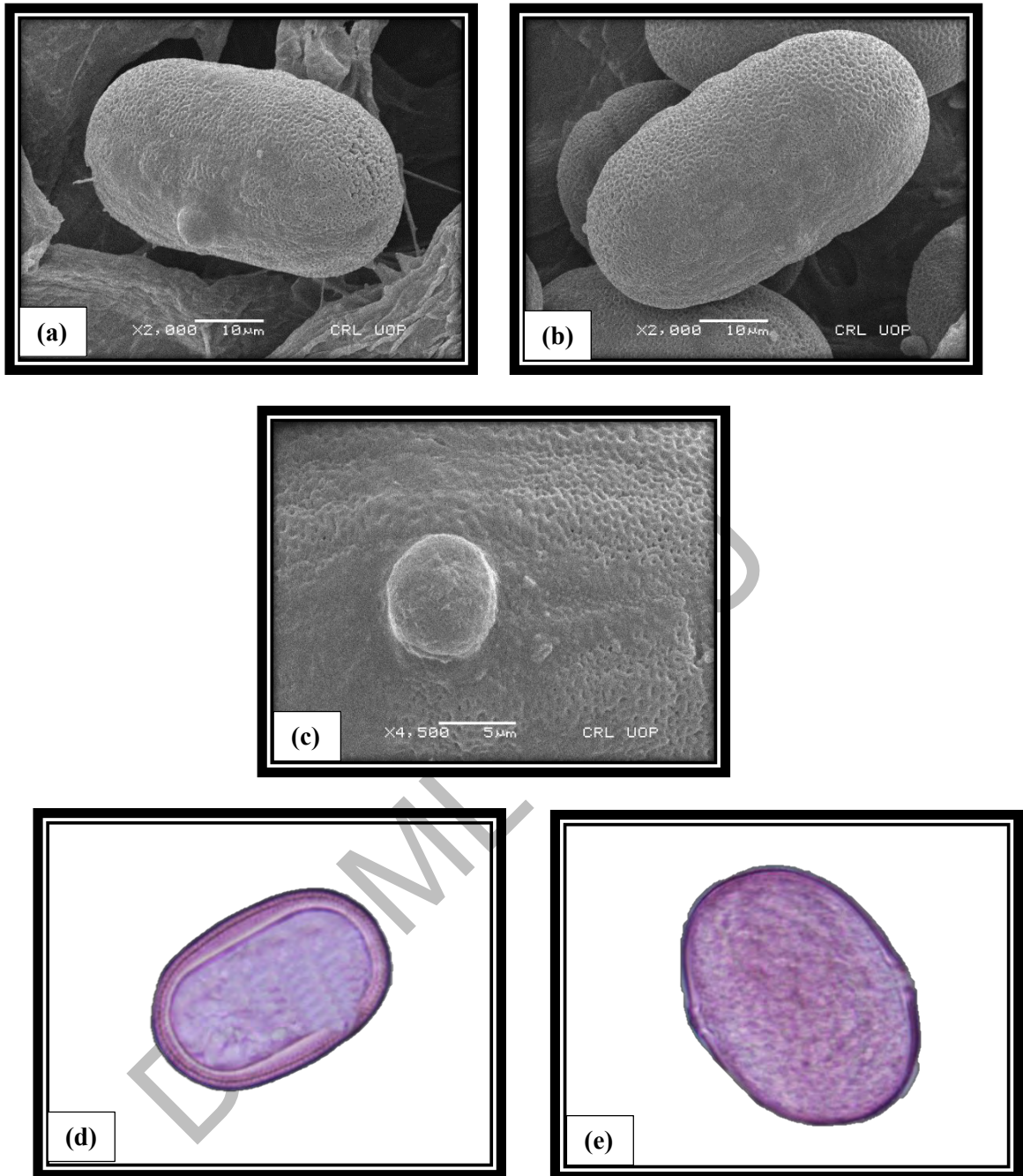


Plate 19. Pollen Microphotographs of *Justicia adhatoda* showing (a, b & c) scanning microscopic imaging (d & e) light microscopic polar and equatorial view (40X)

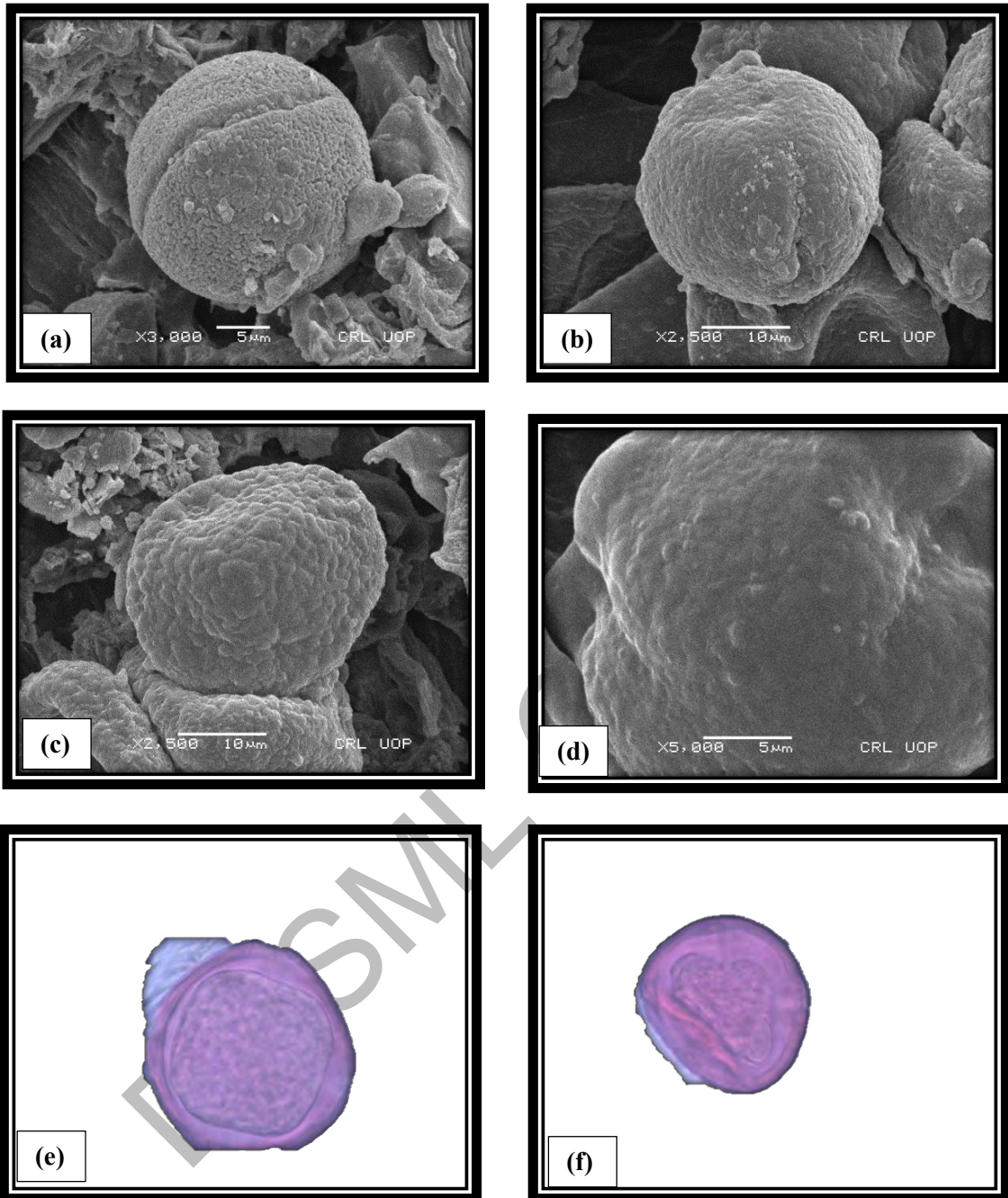


Plate 20. Pollen Microphotographs of *Lantana camara* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

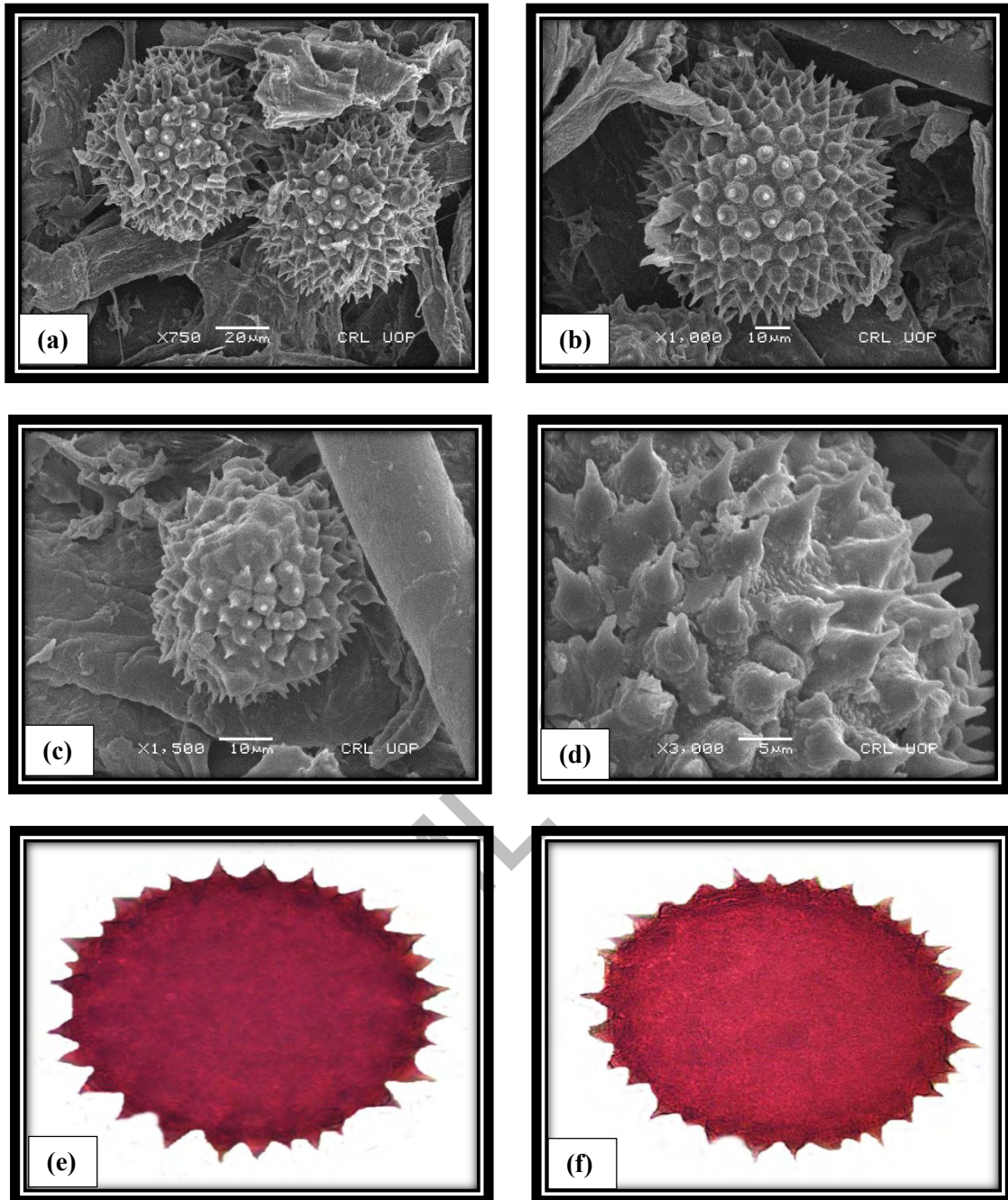


Plate 21. Pollen Microphotographs of *Malvastrum coromandelianum* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

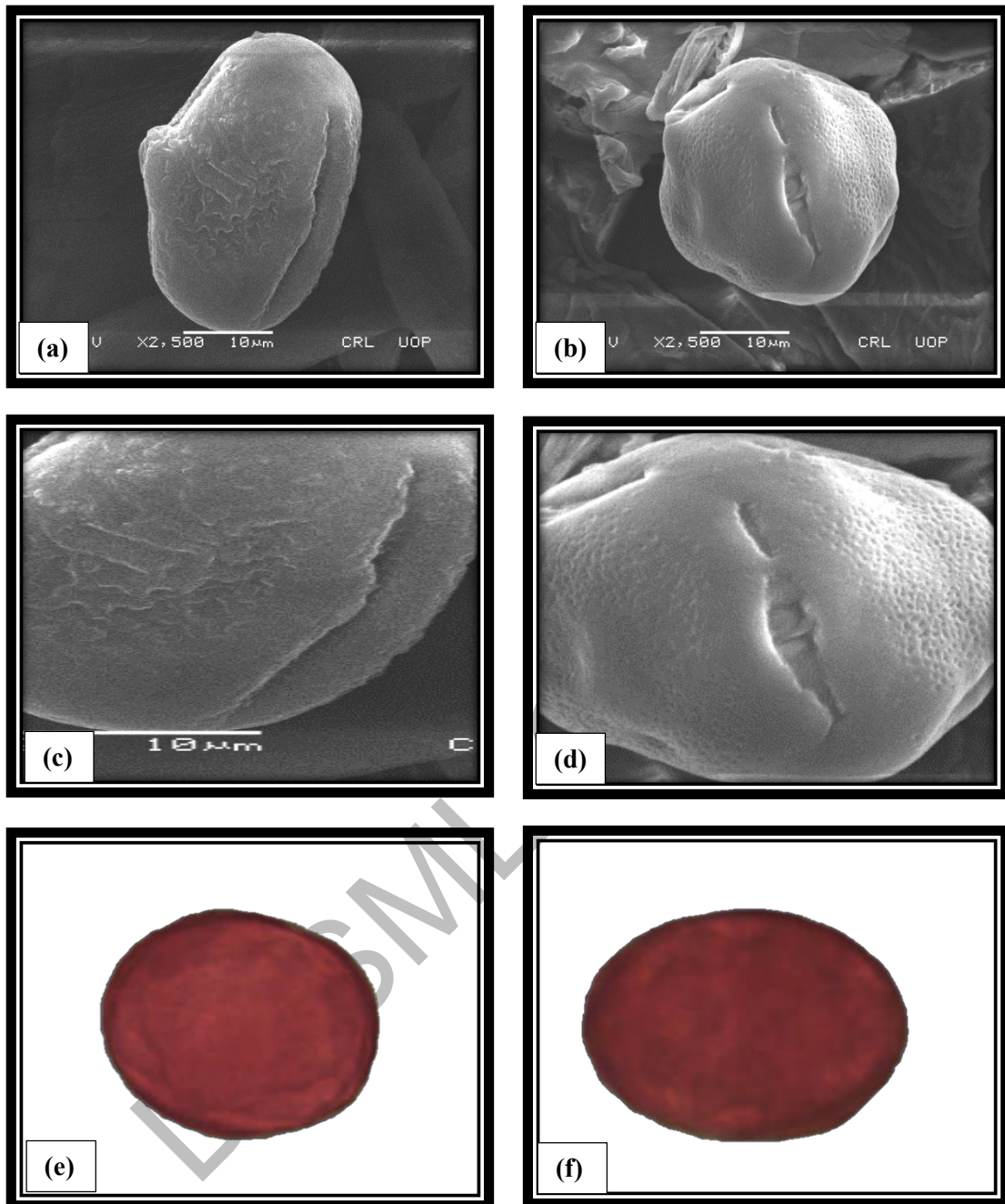


Plate 22. Pollen Microphotographs of *Melilotus indicus* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

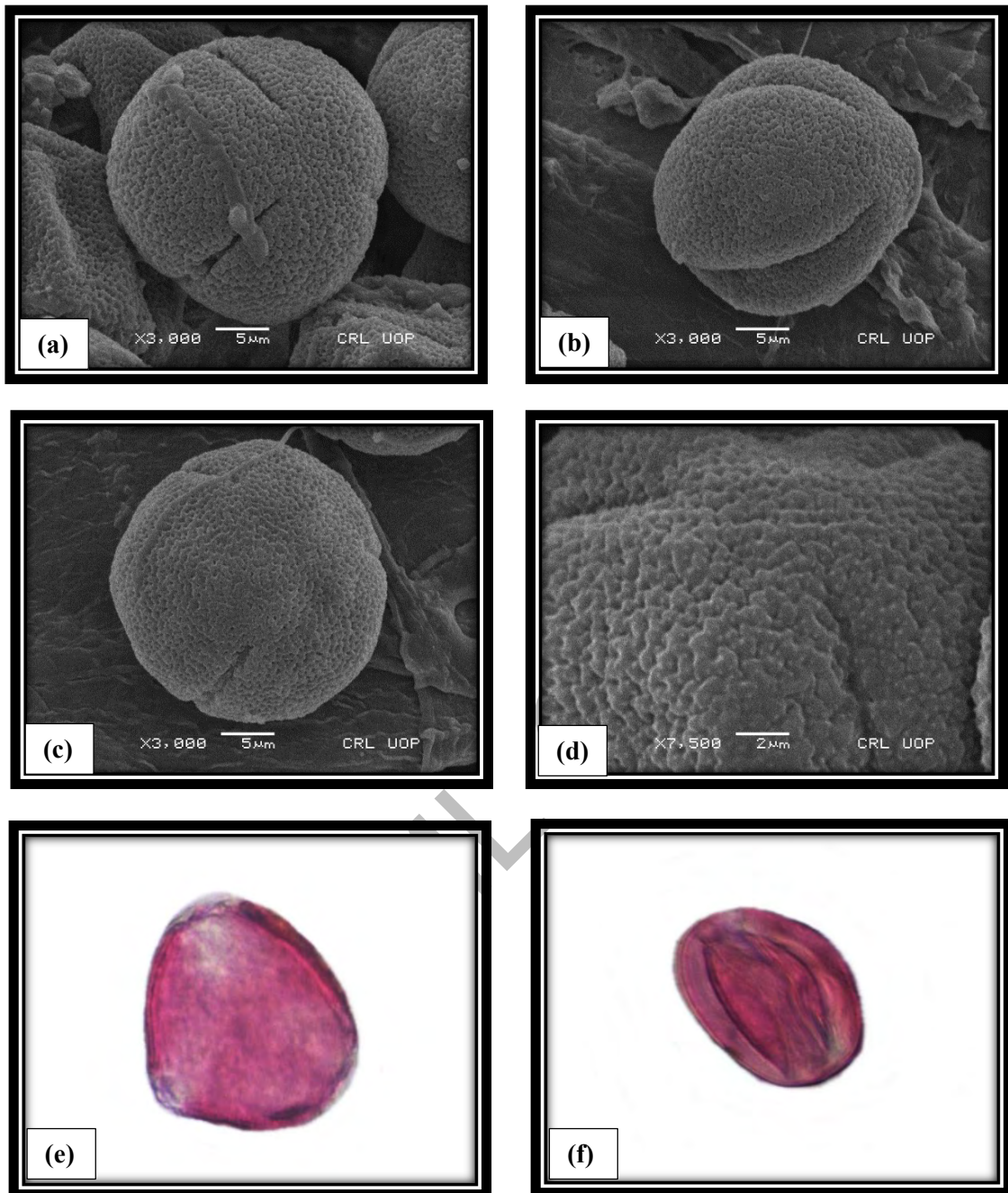


Plate 23. Pollen Microphotographs of *Oxalis corniculata* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

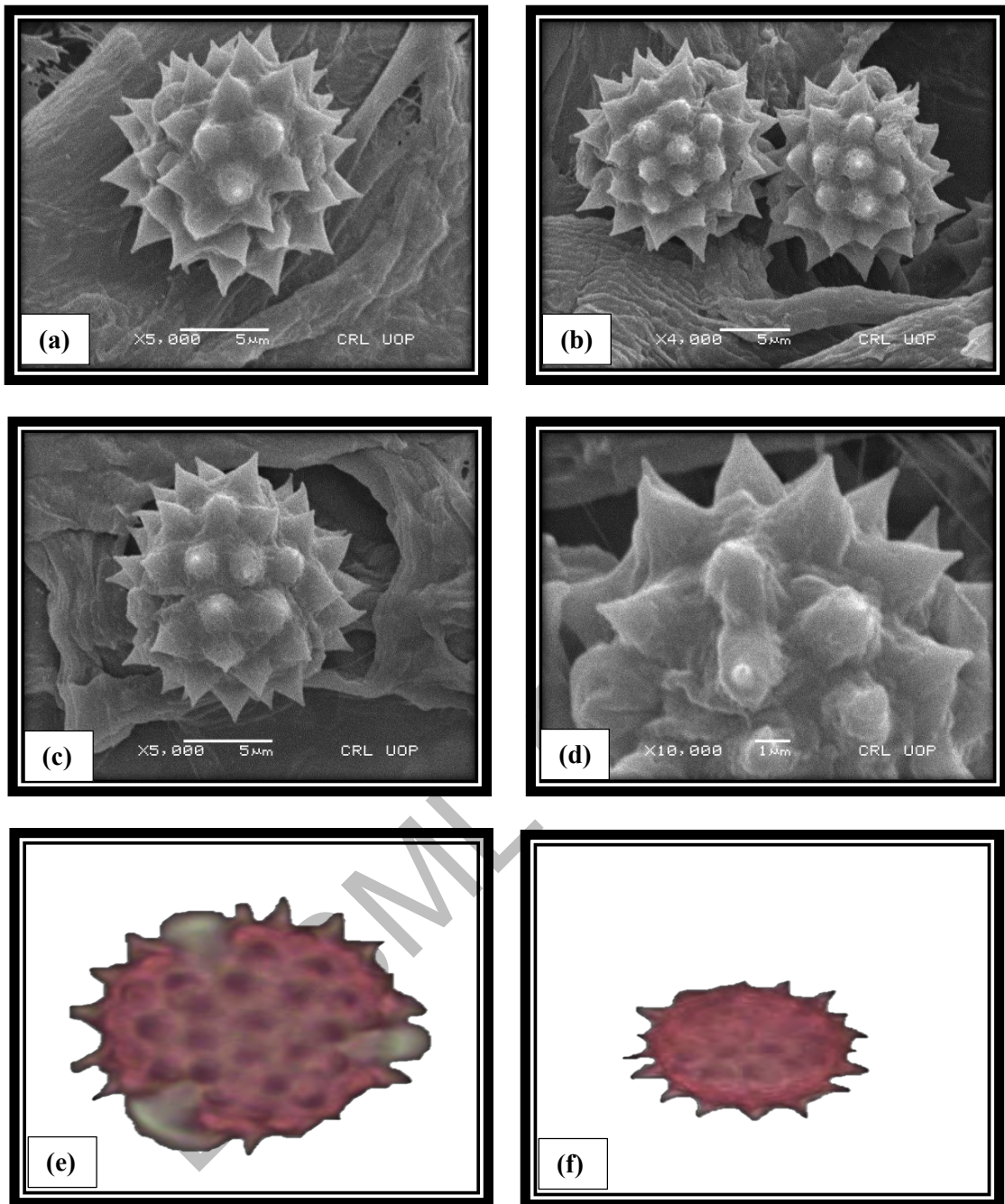


Plate 24. Pollen Microphotographs of *Parthenium hysterophorus* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

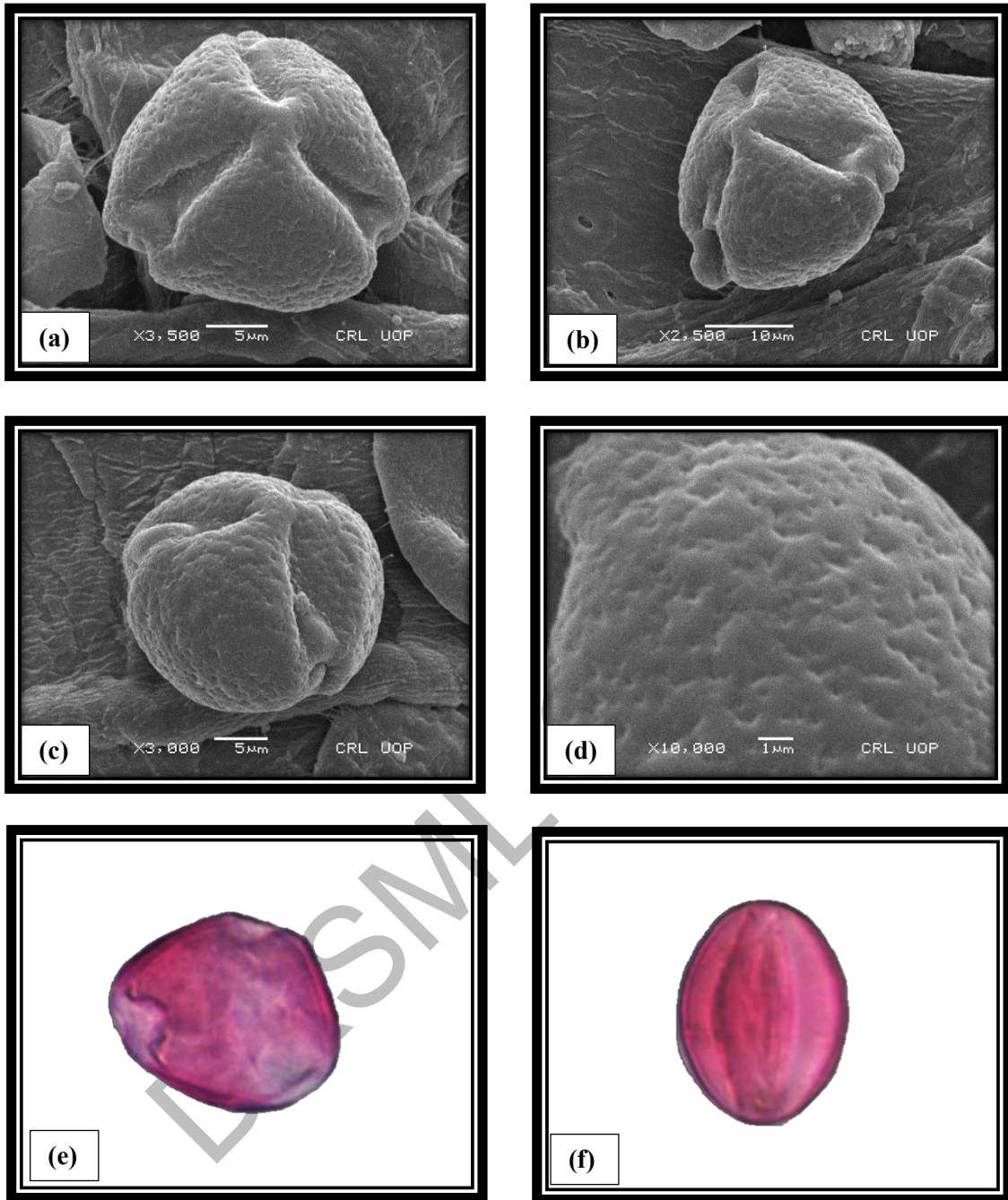


Plate 25. Pollen Microphotographs of *Prosopis juliflora* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

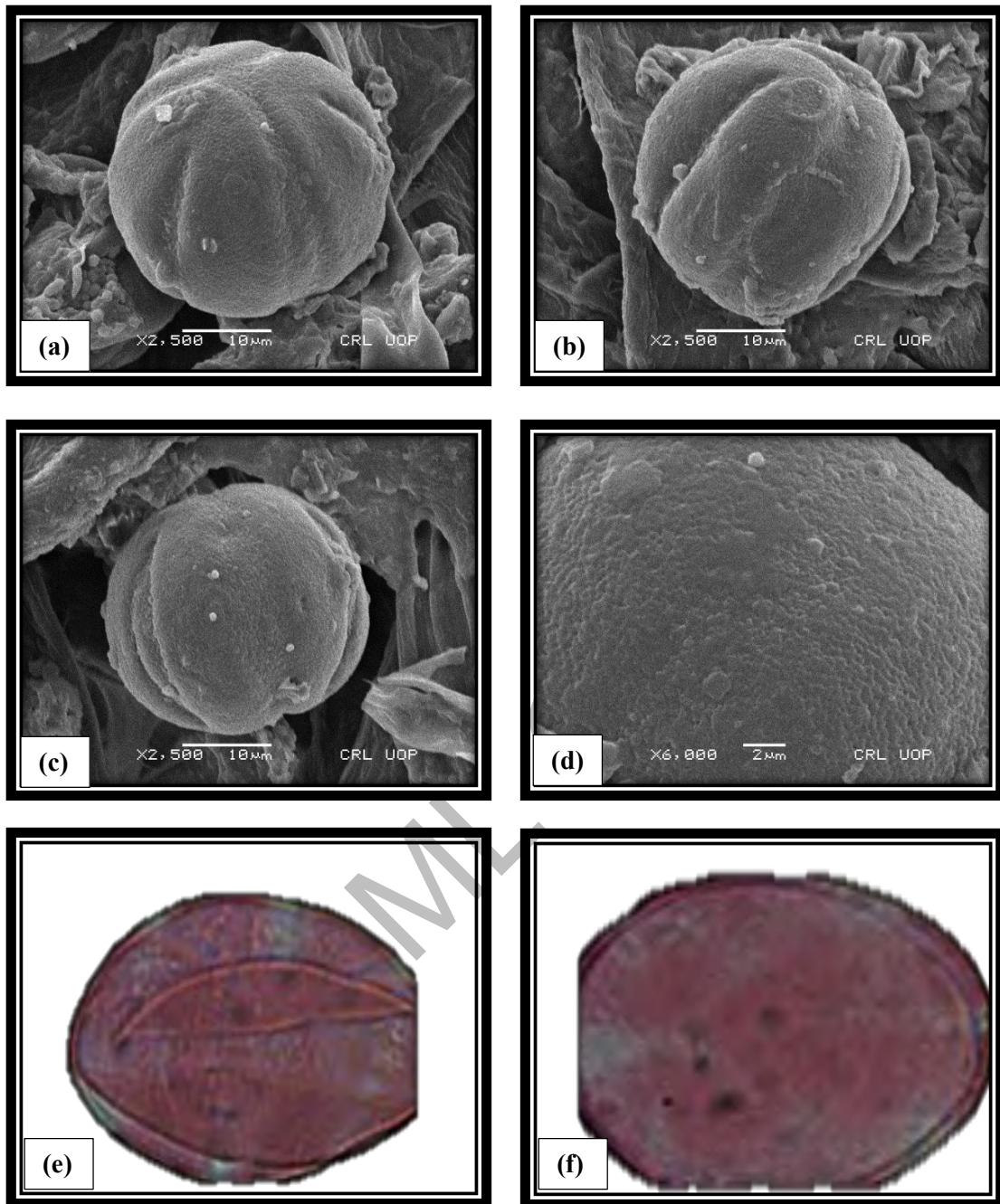


Plate 26. Pollen Microphotographs of *Quisqualis indica* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

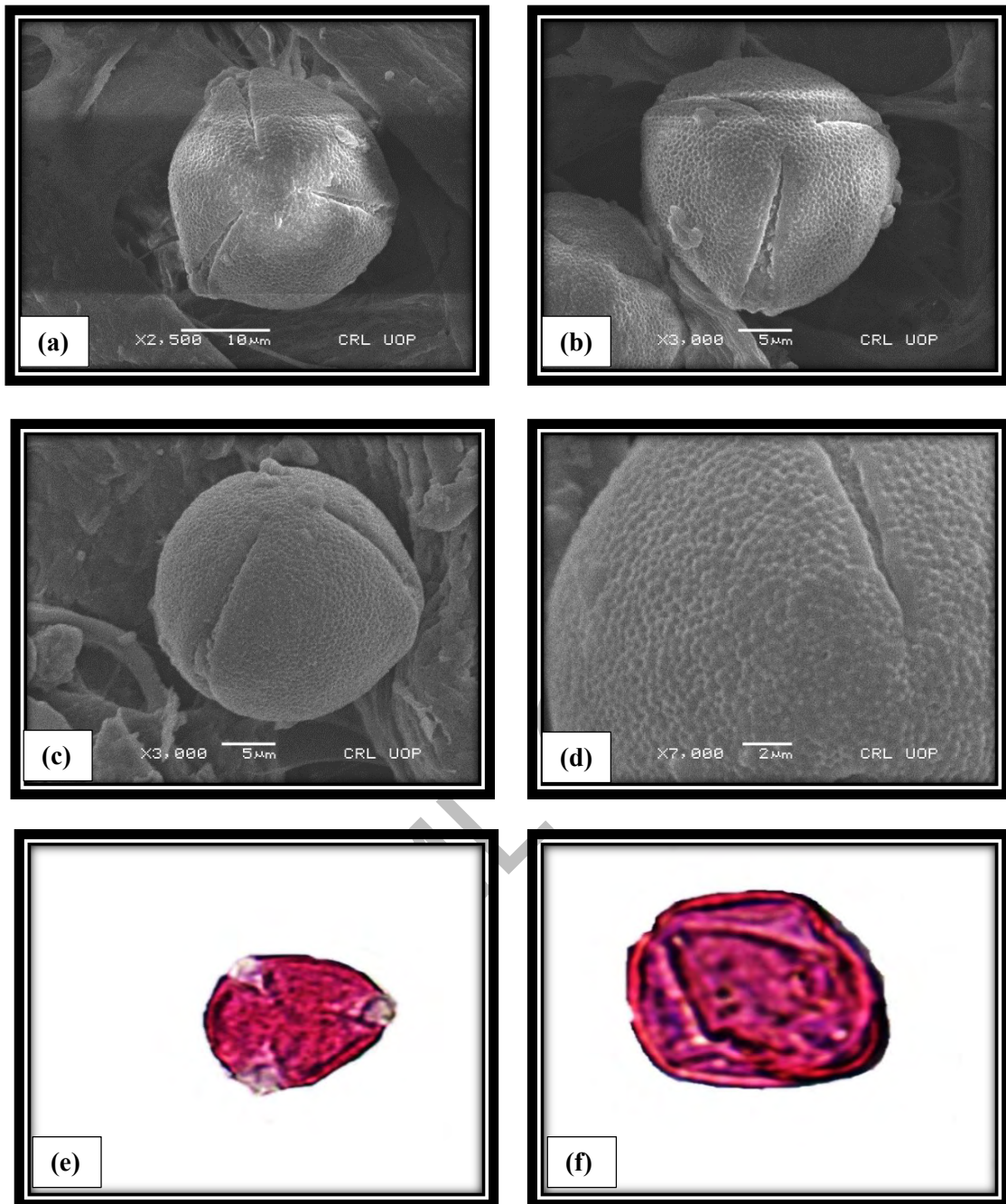


Plate 27. Pollen Microphotographs of *Ricinus communis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

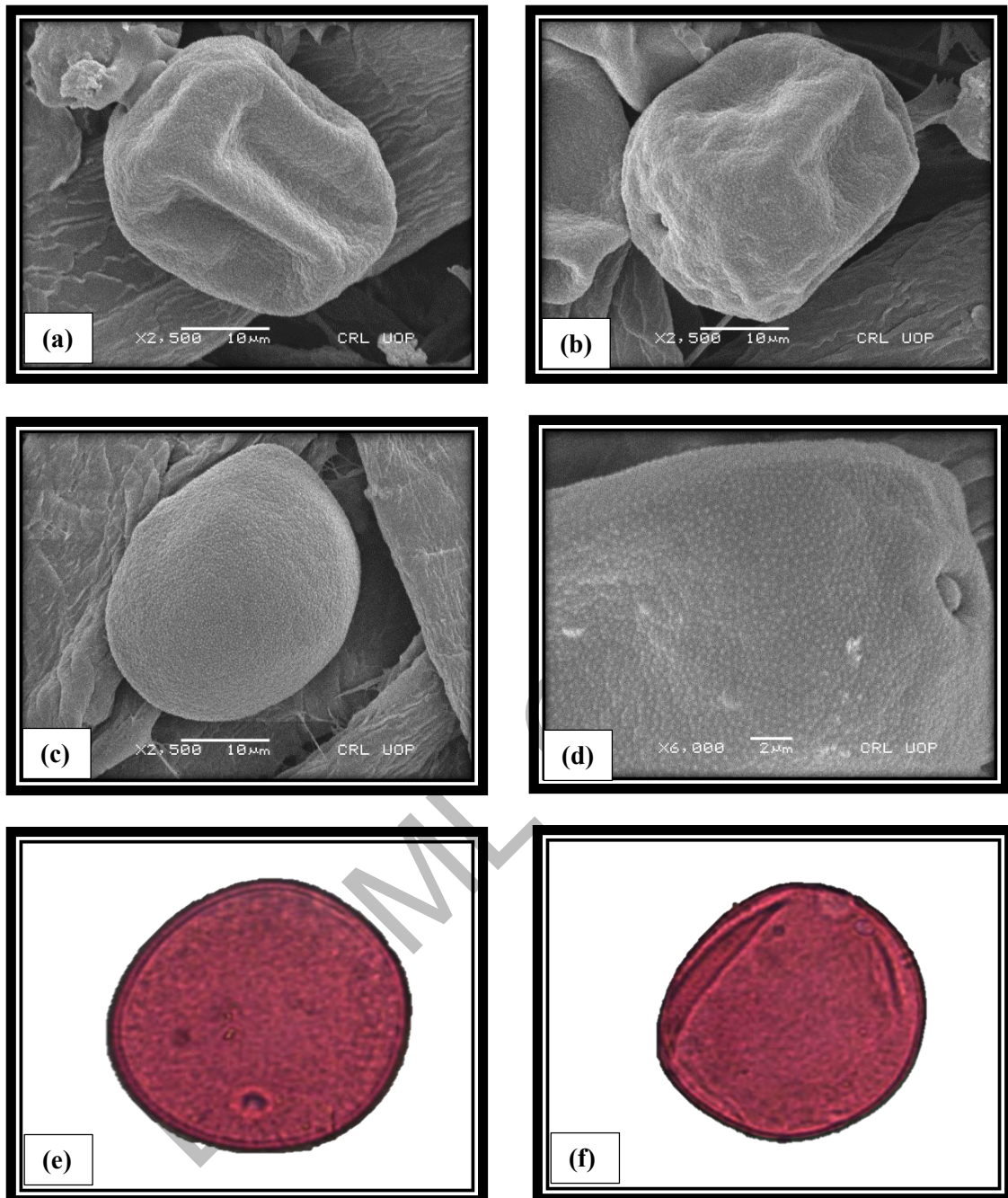


Plate 28. Pollen Microphotographs of *Sorghum halepense* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

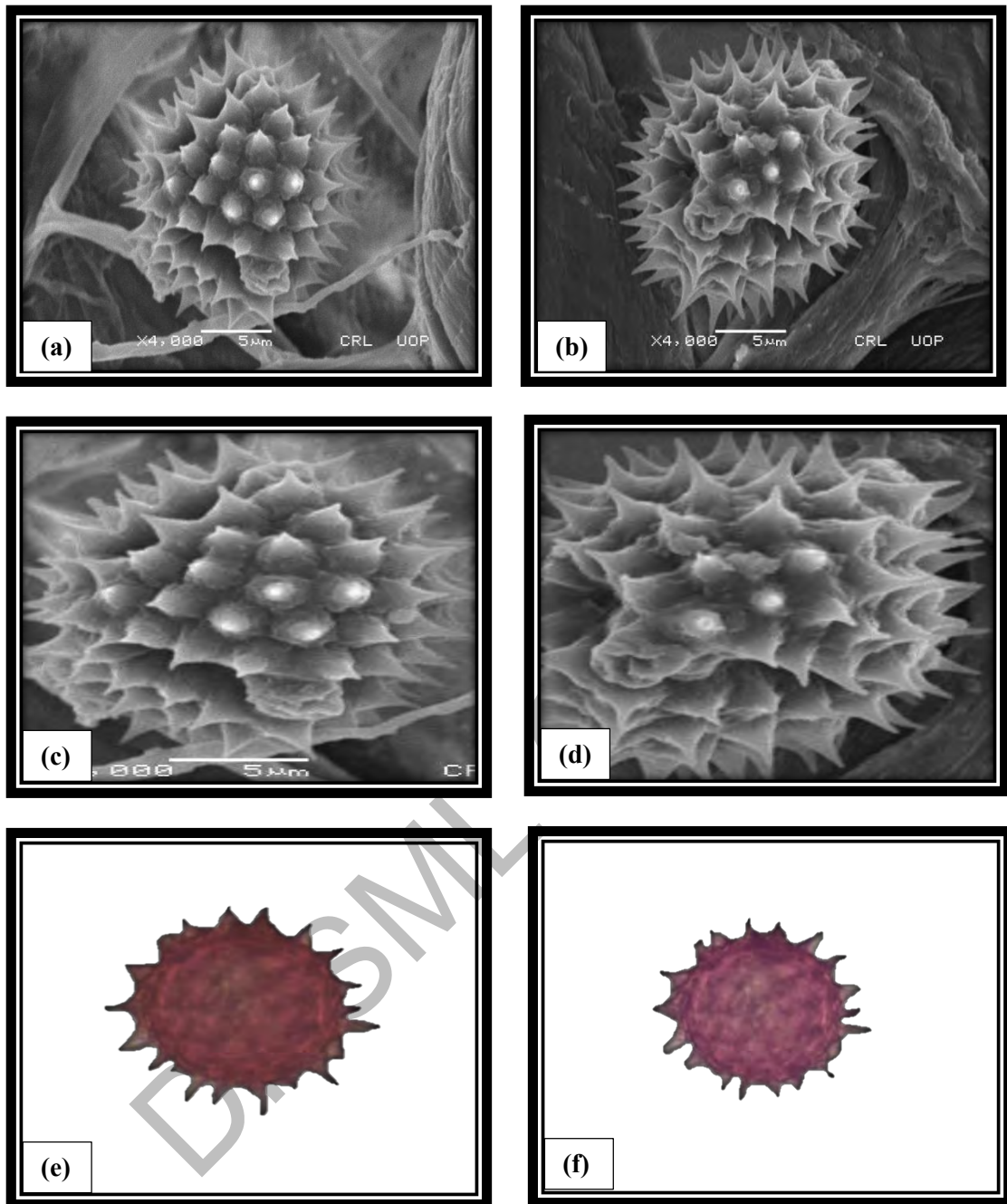


Plate 29. Pollen Microphotographs of *Taraxacum campylodes* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

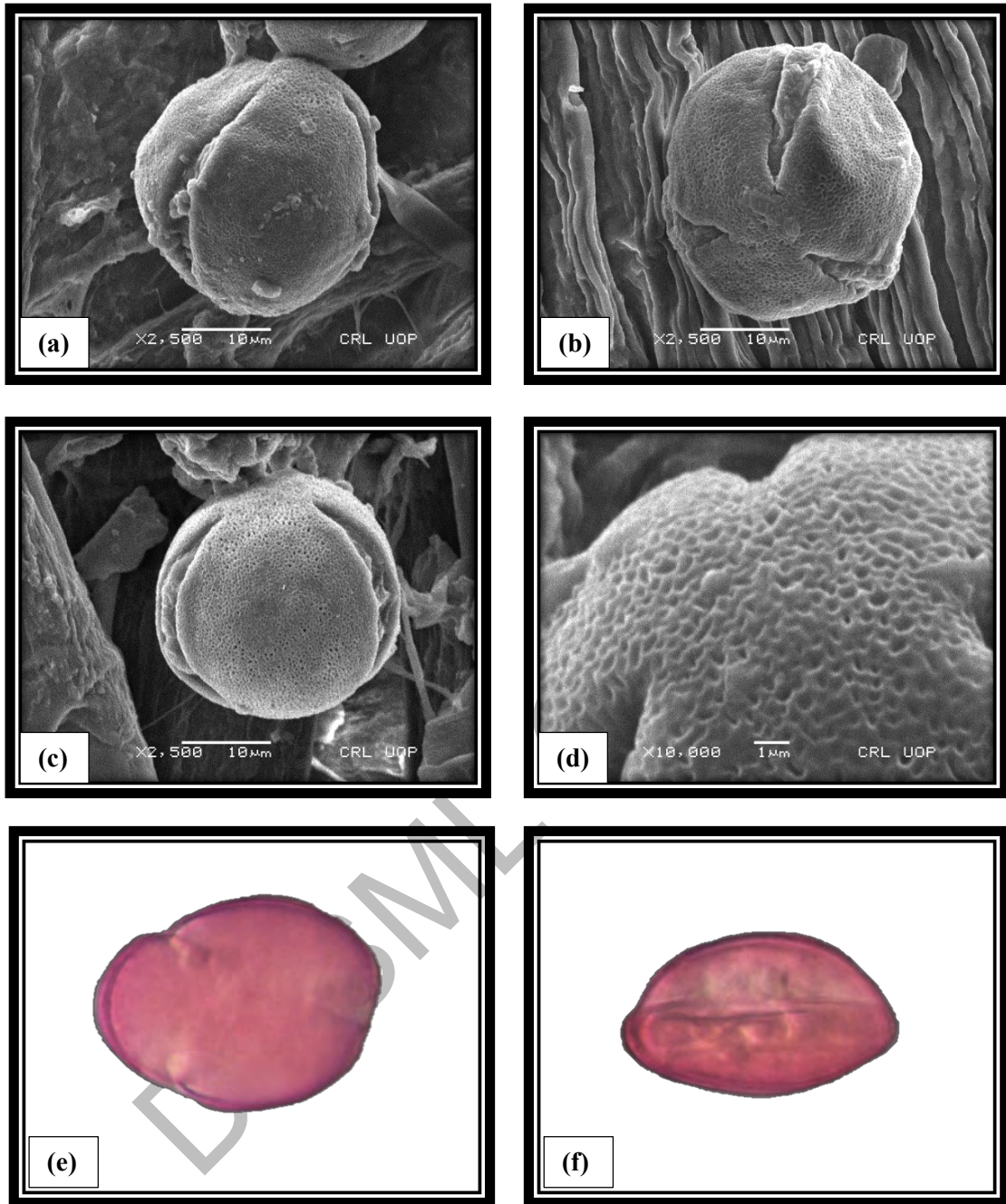


Plate 30. Pollen Microphotographs of *Tecoma stans* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view (40X)

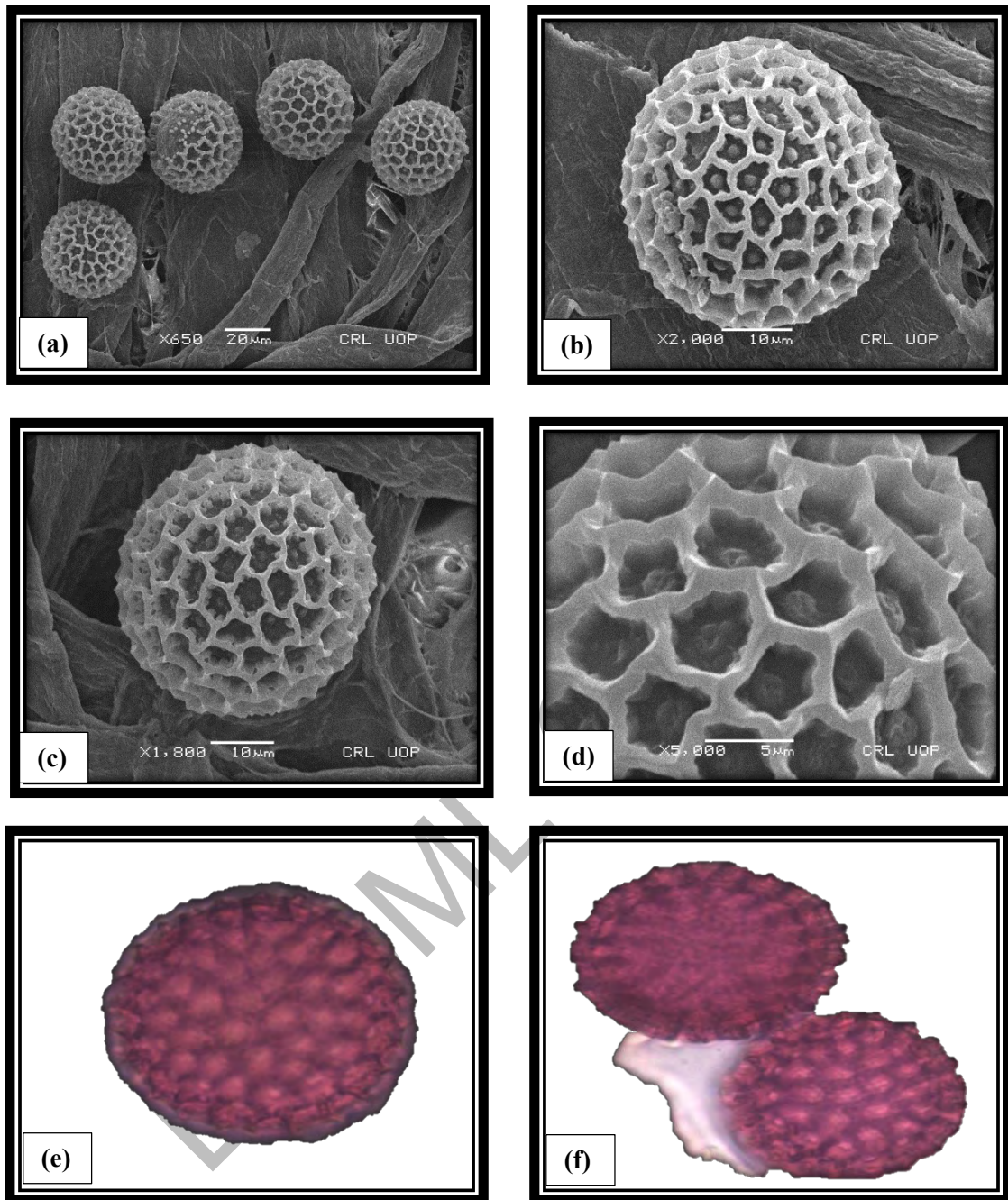


Plate 31. Pollen Microphotographs of *Tribulus terrestris* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view

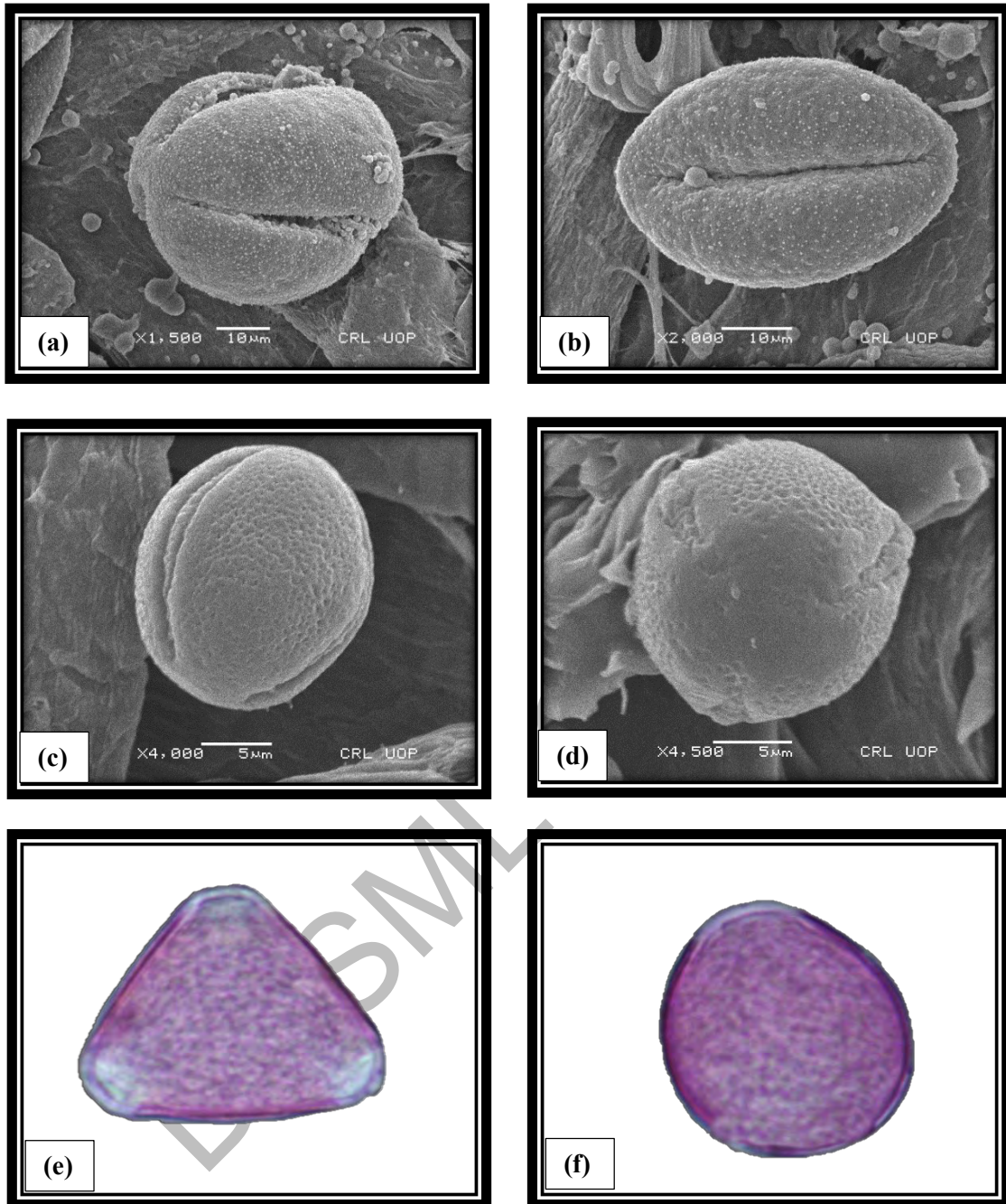


Plate 32. Pollen Microphotographs of *Tropaeolum majus* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view

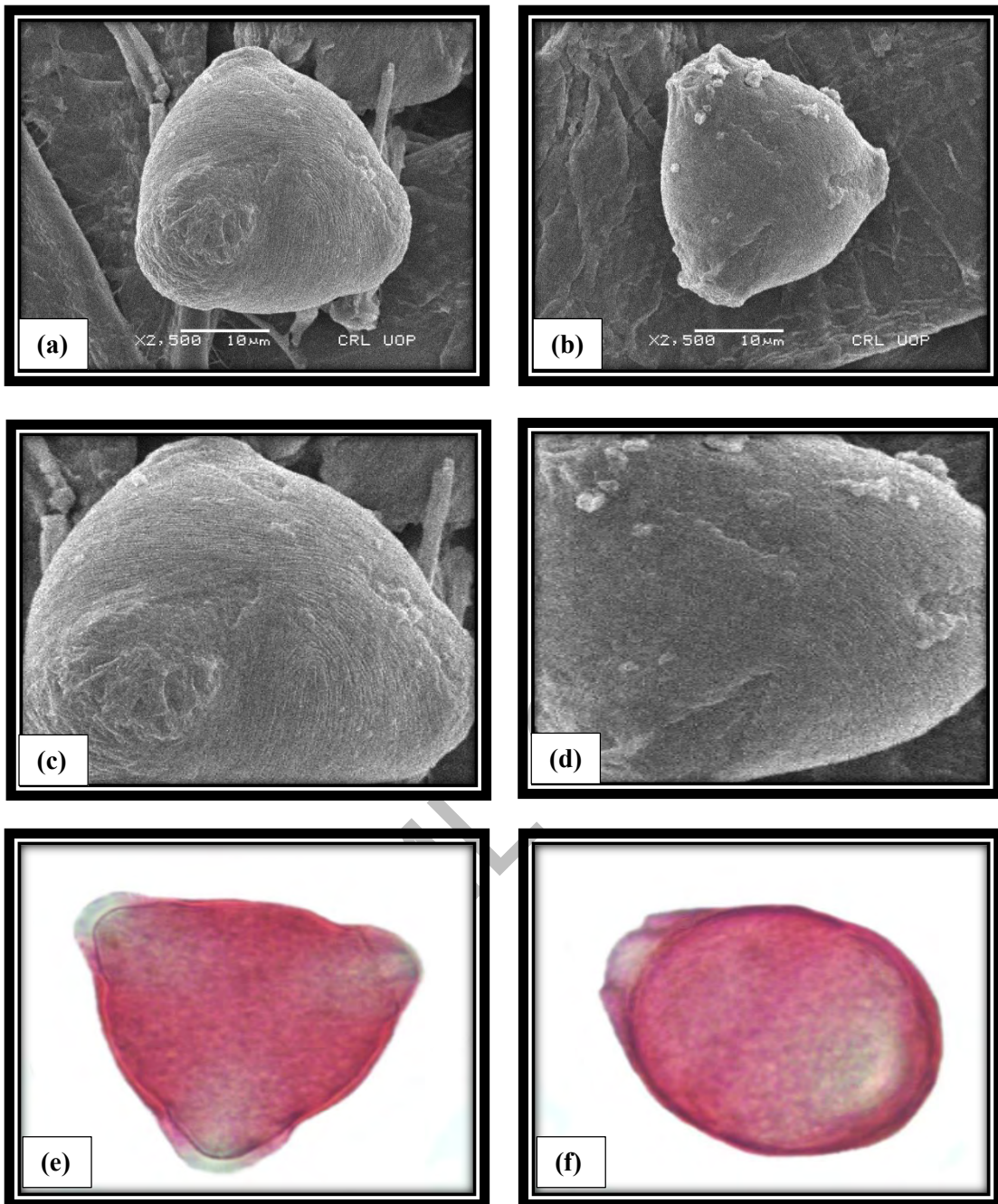


Plate 33. Pollen Microphotographs of *Verbina officinalis* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view

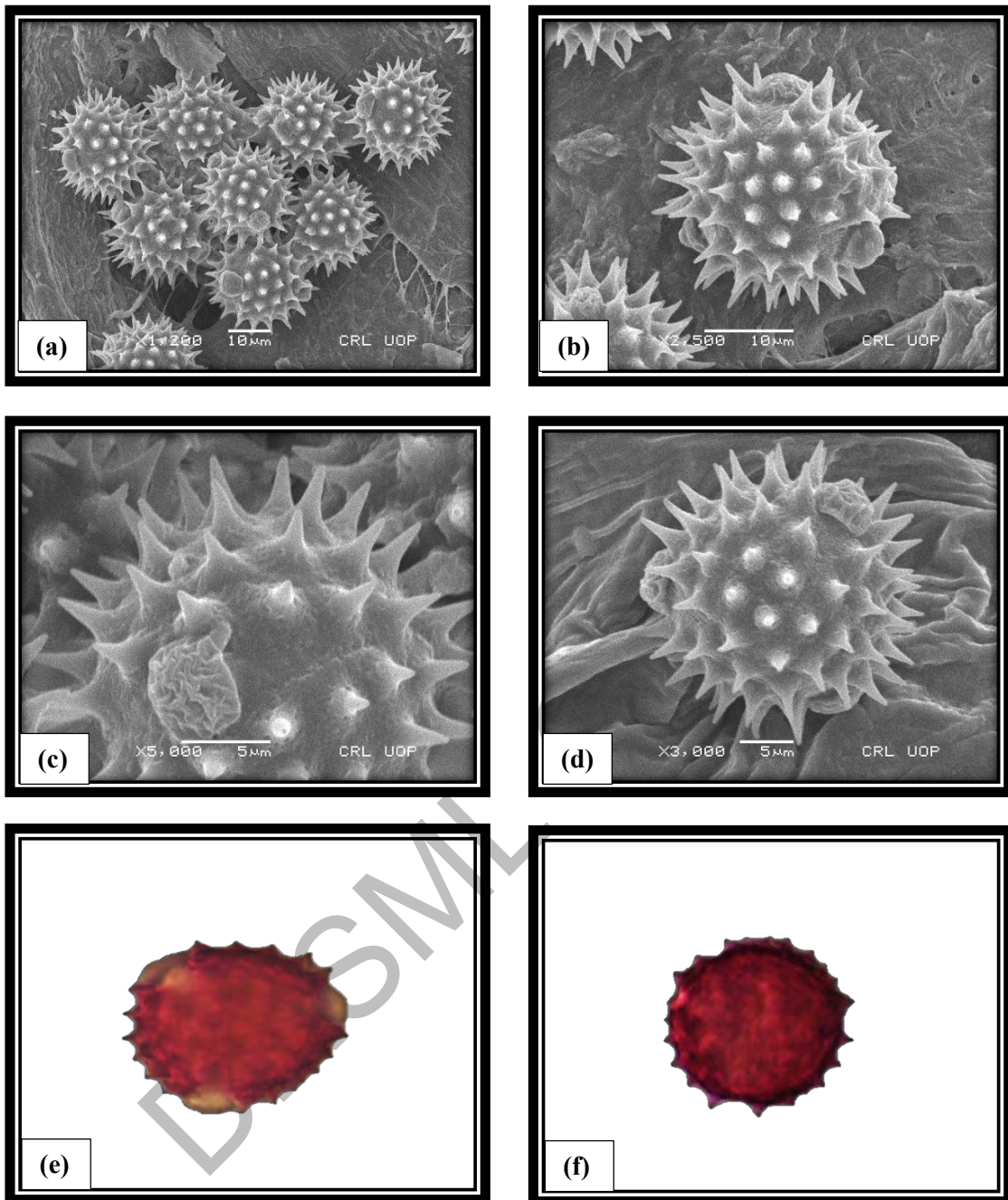


Plate 34. Pollen Microphotographs of *Verbisina eccelioides* showing (a, b, c & d) scanning microscopic imaging (e & f) light microscopic polar and equatorial view

**Section II: Seed Morphology of Invasive Plants of Lesser Himalayas-
Pakistan**

DRSML QAU

3.2 Seed Morphology of Invasive Plants of Lesser Himalayas-Pakistan

For plants seeds are means of survival of the species as they carry parent germplasm and protected against heat, cold, drought and water. In current study the macro and micro morphological characters of seeds of invasive plants of Lesser Himalayas, including seed shape, size, surface, seed inner color, position of hilum, compression, length, width and L/W ratio was observed using LM and SEM. Most taxonomists are in support of the idea that macro and micro structure of seeds are very useful for the classification of species and their correct identification. Dahlgren (1979-1980) stated the usefulness of micromorphology and ultrastructural data for evolution and classification of seed plants and play significant role in modern synthetic systems of angiosperms.

Morphological characters of seeds are used in for delimiting the taxa with quite conflicting morphological attributes. In present section morphology of twenty seeds of invasive plants were studied and data lies in Table 5. The Scanning Electron Microscopy (SEM) and Light Microscopy (LM) were applied. The current research study demonstrates the great variation in seed size, shape and ultra micromorphological features. The findings of research study showed that the seed morphological characteristics can be contribute as criteria to distinguish the taxa. Scanning electron microscopy is an innovative method used to study seed ornamentation, seed molding, and evaluation of micromorphological characters of seeds as compared to light microscopy findings (Môro et al., 2001). According to Luqman et al., 2018, seed size and shape along with micromorphological features can be used as diagnostic tools to identify the various plant species. Earlier literature has also proved that seed macro and micromorphological features can be used for correct identification purposes (Karakish et al., 2013) not only in native species but also in invasive plant species.

The collected seeds of invasive plants in present study were examined by using stereomicroscope. Kim et al. (2016) suggested that studies of seeds using stereomicroscope give an aid to facilitate morphological identification of these seeds. The shape of seeds among investigated taxa showed a great variation. In current study

the dominant seed was spheroidal followed by obovate and elliptical. Seeds of 12 invasive plants were found with smooth surface followed by 6 with rough surface and with ridges and furrows were followed by single invasive plant species. As far as position of hilum is concerned 15 invasive plant showed terminal while in 5 plants it was not clear. The seed of *Xanthium strumarium* L. was observed with maximum length of 13mm and with width of 8mm. Length by width ratio of seeds were also calculated it was found that maximum L/W ratio was observed in *Sonchus oleraceus* (L.) L i.e. 2.66 (Table 5). To understand the biological invasion process, the germination traits and seed properties for introduced and native populations of an invasive plant is essential.

3.2.1 *Ailanthus altissima* (Mill.) Swingle

In this study brownish color seeds with light brown internal color were observed. The seed shape was spherical-globose while seed texture was smooth. The compression was lateral while the hilum was not visible. The length of the seed was 8mm, whereas the width of the seeds was 6.5mm and length to width ratio was 1.23 was recorded. Plate 35 represents the SEM microphotographs of *Ailanthus altissima* that indicates smooth seed sculpturing with protruding periclinal walls. The morphological variation of the seeds represents the geographical distribution of taxa. *Ailanthus* is considered as highly invasive and there are number of characteristic which are associated with its invasiveness such as it produce up to 300,000 anemochorous seeds (Bory and Clair Maczulajtys, 1980). According to the investigation of Hu (1979) seeds of this plant have capability of germinating in a verity of habitats and in soil conditions as well. Kota et al. (2007) indicated that *Ailanthus* seeds have high germination rates in disturbed environments.

3.2.2 *Argemone ochroleuca* Sweet, Brit. Fl. Gard.

The *A. ochroleuca* belongs to family Papaveraceae is native to Mexico, West India, Bulgaria, Spain, Pakistan, African countries, and Europe. The seeds were golden yellow in color and the internal color was creamy white. The seed was obovate with smooth texture. The compression was absent while the hilum was not visible. The length of the seed was 4.5mm, whereas the width of the seeds is 2.5mm and

length to width ratio was 1.8 was recorded. Seed sculpturing was rugose without any projections with flat to concave or slightly convex periclinal wall was observed in *A. ochroleuca* (Plate 36). These findings match with the results of Munir et al. (2019) but they described seed sculpturing in this invasive plant as smooth which differs from present study. The findings of research study by Abid et al. (2016) revealed that in *Argemone* species seeds were non compressed, dark brown in color with reticulate surface and basal hilum, which is slightly different from present study. Variations in seed morphology not only helpful in correct identification but these characters also contributes in expansion and establishment of invasive species.

3.2.3 *Azadirachta indica* A.Juss.

In *Azadirachta indica* seed coat was grey in color while internal color was copper brown. The shape of seeds were oblong and seed texture had ridges. The compression was lateral while the hilum was terminal. The length of the seed was 9mm, whereas the width of the seeds was 3.5mm and length to width ratio was 2.57 was recorded. The seeds of *A. indica* were observed using SEM and surface with irregular reticulate ridges were noted (Plate 37). Arshad et al. (2019) also described seed surface as irregular reticulate ridges in this invasive plant species.

3.2.4 *Calotropis procera* (Aiton). Dryand

The brown seeds in *Calotropis procera* were observed while internal color of seeds was light brown. The seed shaped was flat, broadly ovate with smooth texture. The compression was dorsoventral while the hilum was terminal. The length of the seed is 5.5 mm, whereas the width of the seeds were 4.5mm. Therefore length to width ratio of 1.2 was recorded. Plate 38 represents the SEM microphotographs results of *Calotropis procera* that indicates that surface of seed was striate ornamentation with irregular arranged granules like structures and tiny apertures. Anticlinal walls were smooth and thin while periclinal walls were raised with thick irregular cell arrangement. Aziz et al. (2022) found similar seed sculpturing in species of *Calotropis*. Seed morphology and seed coat anatomy were studied by Dalia Gabr (2014) in some species of Apocynaceae and Asclepiadeceae using LM and SEM and found that potential taxonomic value of the recorded characters is indicated by the

richness of variation recorded in the sample of genera and species. He also investigated seeds of *Calotropis procera* with hairy texture and papillate coat that is contrary to present results. The microstructure of seeds provides data that is useful for determination of various environmental factors on the phenotypic variability of invasive species.

3.2.5 *Cannabis sativa* L.

Cannabis sativa of cannabaceae is considered as highly noxious weed. In present study the greyish-brown seeds with creamy white internal color was observed. The seed shape was oblong while seed texture was smooth. The compression was lateral while the hilum was terminal. The length of the seed was 5.5mm, whereas the width of the seeds was 3-4(3.5mm) and length to width ratio of 1.57 was recorded. Seeds were analyzed using SEM and findings showed that seed surface pattern was smooth but more or less papillate (Plate 39). An apical and almost circular hilum in *Cannabis sativa* was studied by Piluzza et al. (2013).

3.2.6 *Cassia occidentalis* L.

This plant is a part of family Fabaceae and this genus is mostly distributed in South America, Brazil and in Pakistan. The color of seed coat was grey and the internal color was brown. The seed was ovate. The seed texture was smooth. The lateral compression with terminal hilum was observed in the seeds. The length of the seed was 4.5mm, whereas the width of the seeds was 3.5mm. Therefore length to width ratio of 1.28 was recorded. Plate 40 represents the SEM microphotographs of *Cassia occidentalis* that indicates seed surface pattern was reticulate with granular projections. Cell shaped was crooked angular while anticlinal walls with protrusions and periclinal walls with depressed elevations was observed. Aziz et al. (2022) also described seeds of *Cassia occidentalis* with reticulate with granular projection crooked shaped cells.

3.2.7 *Chenopodium ambrosioides* L.

In present study the seeds of *Chenopodium ambrosioides* were green in color and the internal color was blackish brown. The seed shape was spherical. The seed

texture was rough. The compression was absent while hilum was not visible. The length of the seed was 2mm, whereas the width of the seeds was 1.5mm while length to width ratio of 1.33 was recorded. The scanning electron microscopic analysis revealed that seeds surface punctuate and sculptured with ruminant lines (Plate 41).

3.2.8 *Datura innoxia* Mill

D. innoxia is placed in family Solanaceae, the plants of this family is widely distributed in South Western USA, South and Central America, Western India, Afghanistan, Pakistan, India, and Malaysia. The seeds were brown in color and the internal color was creamy white. The seed shaped was broad kidney while rough seed texture was noted. The compression was dorsoventral while the hilum was not visible. The length of the seed was 4.5mm, whereas the width of the seeds was 3mm. Therefore length to width ratio of 1.5 was recorded. Seeds of *D. innoxia* were visualized using SEM and results revealed that sculpturing was smooth-rough with elongated to polygonal cells (Plate 42). Wrinkled or ridges with small projections and dentate or slightly straight margins were observed. Deep anticlinal walls while flat to concave periclinal wall was noted in seeds of this species. In *D. innoxia* similar patterns were observed by Munir et al. (2019).

3.2.9 *Dodonaea viscosa* (Linn.) Jacq.

Dodonaea viscosa belongs to family Brassicaceae. The seeds were black in color and the internal color was yellow. The seed was spherical. The seed texture was smooth. The compression was lateral while the hilum was terminal in the seed. The length of the seed was 3.5mm, whereas the width of the seeds was 3-4(3.5mm). On the basis of measurements of length and width of seed, the length to width ratio of 1 was recorded. Plate 43 represents the SEM microphotographs results of *Dodonaea viscosa* which showed that surface was reticulate with variously shaped depression in succession while anticlinal walls were elevated. Seed coat of *Dodonaea viscosa* showed concave periclinal walls. The concave periclinal surface walls of seed coat have also been shown in Brassicaceae (Kasem et al., 2011).

3.2.10 *Lantana camara* L.

Lantana camara is evergreen shrub commonly known as tick berry. This plant species was introduced as ornamental plant but now due to its vigorous growth it become invasive. It is native to Southeast Asia, India and Australia. The seeds were brown in color and the internal color was light yellow. The seed shape was spheroid-ovate. The seed texture was wrinkled. The compression was absent while the hilum was terminal. The length of the seed was 5mm, whereas the width of the seeds was 4.5mm. Therefore length to width ratio of 1.11 was recorded. The seeds showed striate surface pattern with rectangular cell shape. Smooth, depressed, thick anticlinal walls while deeply groove, rough periclinal walls were noted in *L. camara* (Plate 44). Kanwal et al. (2022) investigated the seed morphology of Verbenaceae and found similar results as in present research study.

3.2.11 *Lantana urticoides* Hayek

Lantana urticoides is commonly known as Texas lantana or, widely distributed in Central America Pakistan and Mexico. The seeds were brown in color and the internal color was light yellow. The seed shape was spheroid-ovate. The seed texture was wrinkled. The compression was absent while the hilum was terminal. The length of the seed was 5mm, whereas the width of the seeds was 4.5mm and length to width ratio of 1.11 was recorded. SEM results of showed that surface sculpturing was reticulate with granular projections (Plate 45). The cell arrangement was irregular and anticlinal walls were smooth and elevated while periclinal walls were thin, faintly raised and flat Kanwal et al. (2022).

3.2.12 *Nasturtium officinale* R.Br.

Nasturtium officinale is placed in family Brassicaceae which is one of the largest family in the angiospermae and it can be easily distinguished by its flower and fruits characteristics. According to Warwick et al. (2006) it is a family of cosmopolitan plants mainly distributed in temperate zones and Mediterranean region; this family is represented by 338 genera and 3709 species. The seeds were brown in color and the internal color was light yellow. The seed shape was elliptical. The seed

texture was wrinkled. The compression was dorsoventral while hilum was terminal. The length of the seed was 9mm, whereas the width of the seeds is 7mm. The length to width ratio of 1.28 was recorded. Micro morphological analysis revealed that cell arrangement was reticulate with well-defined outer boundaries and anticlinal walls were raised and straight (Plate 46). These findings are correlates with findings of Morozowska (2015). Sirin (2020) also observed reticulate sculpturing in some *Barbarea* taxa of family Brassicaceae.

3.2.13 *Ricinus communis* L.

Ricinus communis belongs to family Euphorbiaceae, a diverse family of flowering plants which are economically very important. The seeds were brown in color and the internal color was white. The seed shape was oblong. The seed texture was smooth. The compression was dorsoventral while the terminal hilum was observed in seeds. The length of the seed was 14mm, whereas the width of the seeds were 6mm. Therefore length to width ratio of 2.33 was recorded. SEM results indicates that seeds of *R. communis* (Plate 47) were spherical, rough sculpturing, asymmetrical and with reticulate cell arrangement with minute apertures, thick anticlinal walls and raised smooth periclinal walls. Similar findings were noted in seeds of *Ricinus communis* by Aziz et al. (2022).

3.2.14 *Robinia pseudoacacia* L.

In *Robinia pseudoacacia* black-brown seeds with light brown internal color was observed. The seed was kidney-shaped. The seed texture has furrows. The compression was lateral while the hilum was not visible in seeds. The length of the seed was 5.5mm, whereas the width of the seeds was 4mm while length to width ratio of 1.37 was recorded. The seed surface pattern was regulate (Plate 48), Lersten (1981) also observed regulate seed coat surface in this invasive plant species.

3.2.15 *Sapium sebiferum* (L.) Roxb.

The seeds were white in color and the internal color was brown. The seed shape was globular-spheroid. The seed texture was smooth (shiny). The compression was ventral while the hilum was terminal. The length of the seed was 9.5mm, whereas

the width of the seeds was 6mm and L/W ratio of 1.58 was recorded. Smooth seed surface with reticulate sculpturing with protruding periclinal walls were studied via SEM (Plate 49). Can and Küçüker (2015) studied the reticulate seed surface pattern in some Euphorbia taxa distributed in Turkey.

3.2.16 *Sesamum indicum* L.

The seeds were grey in color and the internal color was white. The seed shaped was obovate. The seed texture was smooth (shiny). The compression was lateral while the hilum was terminal. The length of the seed was 4.5mm, whereas the width of the seeds was 3mm and length to width ratio of 1.5mm was recorded. Seed coat surface in *S. indicum* varied from reticulately rough to smooth at two extremes (Plate 50). Reticulate seed surface in *S. indicum* was also observed by Prasad et al. (2013).

3.2.17 *Silybum marianum* (L.) Gaertn.

The seeds was grey in color and the internal color was white. The seed shaped was oblong and seed texture was smooth (shiny). The compression was dorsoventral while the hilum was terminal. The length of the seed was 6mm, whereas the width of the seeds were 2.5mm and L/W ratio of 2.4mm was recorded. Plate 51 represents the SEM microphotographs of *Silybum marianum* which showed that seed surface was reticulate-rugose, thin raised anticlinal cells. Smooth and convex periclinal walls were noted by Dalia Gabr (2015) in *S. marianum* these findings differs from our results.

3.2.18 *Solanum surattense* Burm. f.

The Solanaceae is one of the most important and large family of flowering plants. The family is widely distributed in a diversity range of ecological habitats in both tropical and temperate regions. It consists of about 98 genera and 2700 species (Olmstead and Bohs, 2007). The seeds were brown-orange in color and the internal color was yellow. The seed shape was discoid-obovate. The seed texture was smooth (shiny). The compression was lateral while terminal hilum was observed in seeds. The length of the seed was 3.5mm, whereas the width of the seeds was 2.25mm. So on the basis of length and width, L/W ratio was calculated i.e.1.55. Ridged with irregular and

reticulate surface was noted in seed surface of *Solanum surattense* (Plate 52). Samai and Shaheen (2002) and Khafagi et al. (2018) also noted reticulate irregular surface in seed coat of native and naturalized species of *Solanum* species.

3.2.19 *Sonchus oleraceus* L.

The seed was dark brown in color and the internal color was creamy white. The seed shape was ovate. The seed texture was smooth. The compression was dorsoventral while the hilum was terminal. The length of the seed was 4mm, whereas the width of the seeds was 1.5mm and length to width ratio of 2.66 was recorded. The SEM results (Plate 53) showed that seed surface was reticulate and anticlinal walls were thin, wavy and grooved while periclinal walls were striated and grooved. These results matches with findings of Dalia Gabr (2015).

3.2.20 *Xanthium strumarium* L.

The seed was brownish in color and the internal color was grey. The seed shape was oblong-ellipsoid. Seed texture was rough (spiny). The compression was dorsoventral while hilum was terminal in seeds. The length of the seed was 13mm, whereas the width of the seeds was 8mm while L/W ratio of 1.625 was recorded. Scanning electron microscopy (Plate 54) results indicates that seed surface was spiny and rough with tetra to polygonal anticlinal cell walls while shallow periclinal walls were observed.

Form present findings it can be observed that distinction can be made between taxa based on external seed morphology. Manos et al., (2001), suggested that seed morphological characters provide an aid in distinguishing the various species and also support their placement in different clades. The study indicates that use of seed morphological characters is a useful parameter for species identification of invasive plants. Seeds texture and sculpturing features proved very important systematically (Al-Gohary & Mohamed, 2007). According to Song et al. (2015) the investigation of the micro-morphological characteristics of seeds using SEM has been used to identify and distinguish species. It can be concluded from the current study that macro and micro morphological characteristics of seeds are considered diagnostic at both generic and specific level of invasive plant species amongst the wide range of taxa.

Table 5: Qualitative and Quantitative Characters of Seeds of Invasive Plant Species

Sr. No.	Plant Name	Seed color	Inner color	Seed shape	Texture/surface	Hilum	Compression	Length (mm)	Width (mm)	L/W ratio
1.	<i>Ailanthus altissima</i> (Mill.) Swingle	Brown	Light brown	Spherical-Globose	Smooth	Not visible	Lateral	7-9 (8)	5-8 (6.5)	1.23
2.	<i>Argemone ochroleuca</i> Sweet, Brit. Fl. Gard.	Golden yellow	Off-white	Obovate	Smooth	Not visible	Absent	4-5(4.5)	2-3(2.5)	1.8
3.	<i>Azadirachta indica</i> A.Juss.	Grey	Copper brown	Oblong	Ridges	Terminal	Lateral	8-10(9)	3-4(3.5)	2.57
4.	<i>Calotropis procera</i> (Aiton) Dryand.	Brown	Light brown	Flat, Broadly Ovate	Smooth	Terminal	Dorsoventral	5-6(5.5)	4-5(4.5)	1.2
5.	<i>Cannabis sativa</i> L.	Greyish brown	Off-white	Oblong	Smooth	Terminal	Lateral	5-6(5.5)	3-4(3.5)	1.57
6.	<i>Cassia occidentalis</i> L.	Grey	Brown	Ovate	Smooth	Terminal	Lateral	4-5(4.5)	3-4(3.5)	1.28
7.	<i>Chenopodium ambrosioides</i> L.	Green	Blackish brown	Spherical	Rough	Not Visible	Absent	1-3 (2)	0.5-2.5 (1.5)	1.33
8.	<i>Datura innoxia</i> Mill	Brown	Off-white, creamy	Broad kidney	Rough	Not visible	Dorsoventral	4-5(4.5)	2-4(3)	1.5
9.	<i>Dodonaea viscosa</i> (Linn.) Jacq.	Black	yellow	Spherical	Smooth	Terminal	Lateral	3-4(3.5)	3-4(3.5)	1
10.	<i>Lantana camara</i> L.	brown	Light yellow	Spheroid-ovate	Wrinkled	Terminal	Absent	4-6(5)	4-5(4.5)	1.11
11.	<i>Lantana urticoides</i> Hayek	brown	Light yellow	Spheroid-ovate	Wrinkled	Terminal	Absent	4-6(5)	4-5(4.5)	1.11

12.	<i>Nasturtium officinale</i> R.Br.	Brown	Light yellow	elliptical	Wrinkled	Terminal	Dorsoventral	8-10(9)	6-8(7)	1.28
13.	<i>Ricinus communis</i> L.	Brown	White	Oblong	Smooth	Terminal	Dorsoventral	13-15(14)	5-7(6)	2.33
14.	<i>Robinia pseudoacacia</i> L.	Brown to black	Light brown	Kidney shape	Furrows	Not visible	Lateral	5-6(5.5)	3-4(3.5)	1.37
15.	<i>Sapium sebiferum</i> (L.) Roxb.	White	Brown	Globular-Spheroid	Smooth (shiny)	Terminal	Ventral	8-1.1 (9.5)	5-7 (6)	1.58
16.	<i>Sesamum indicum</i> L.	Grey	White	Obovate	Smooth (shiny)	Terminal	Lateral	4-5(4.5)	2-4(3)	1.5
17.	<i>Silybum marianum</i> (L.) Gaertn.	Grey	White	Oblong	smooth(shiny)	Terminal	Dorsoventral	5-7(6)	2-3(2.5)	2.4
18.	<i>Solanum surattense</i> Burm. f.	Brown-orange	yellow	Discoid-Obovate	Smooth (shiny)	Terminal	Lateral	3-4 (3.5)	2-2.5 (2.25)	1.55
19.	<i>Sonchus oleraceus</i> (L.) L.	Dark brown	Off - white	Ovate	Smooth	Terminal	Dorsoventral	3-5(4)	1-2(1.5)	2.66
20.	<i>Xanthium strumarium</i> L.	Brown	Gay	Oblong-ellipsoid	Rough (spiny)	Terminal	Dorsoventral	12-14 (13)	7-9 (8)	1.625

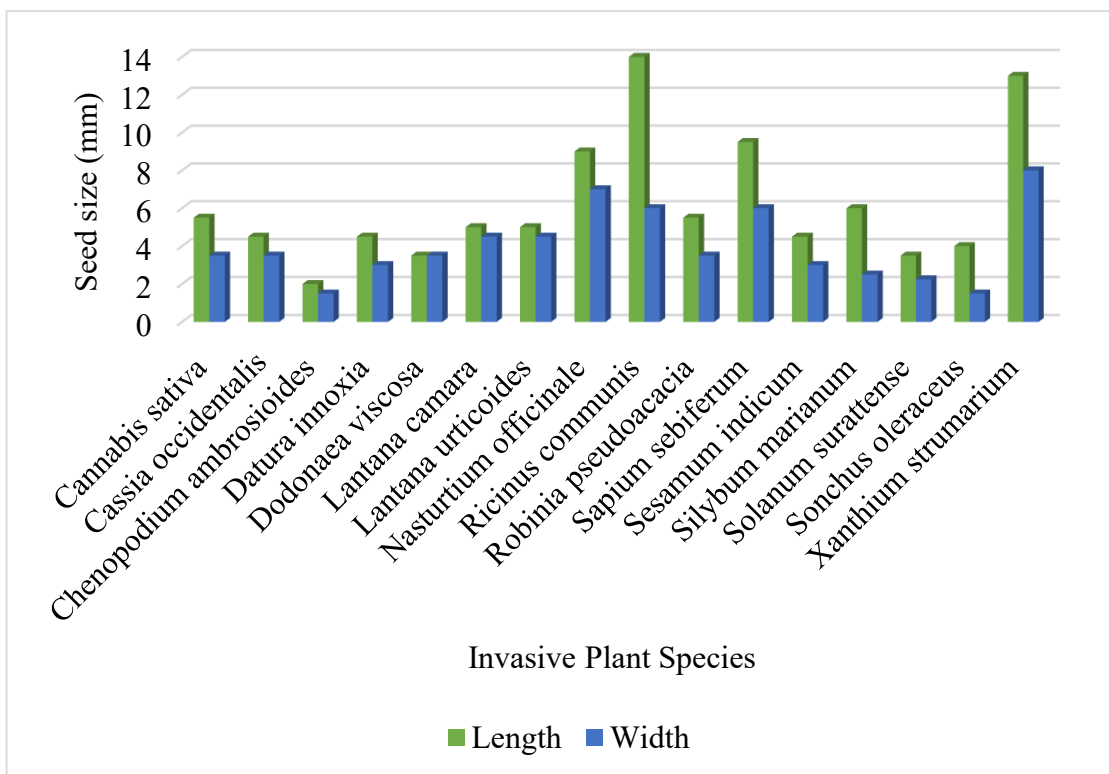


Figure 55. Comparison of seed length and width among Invasive species

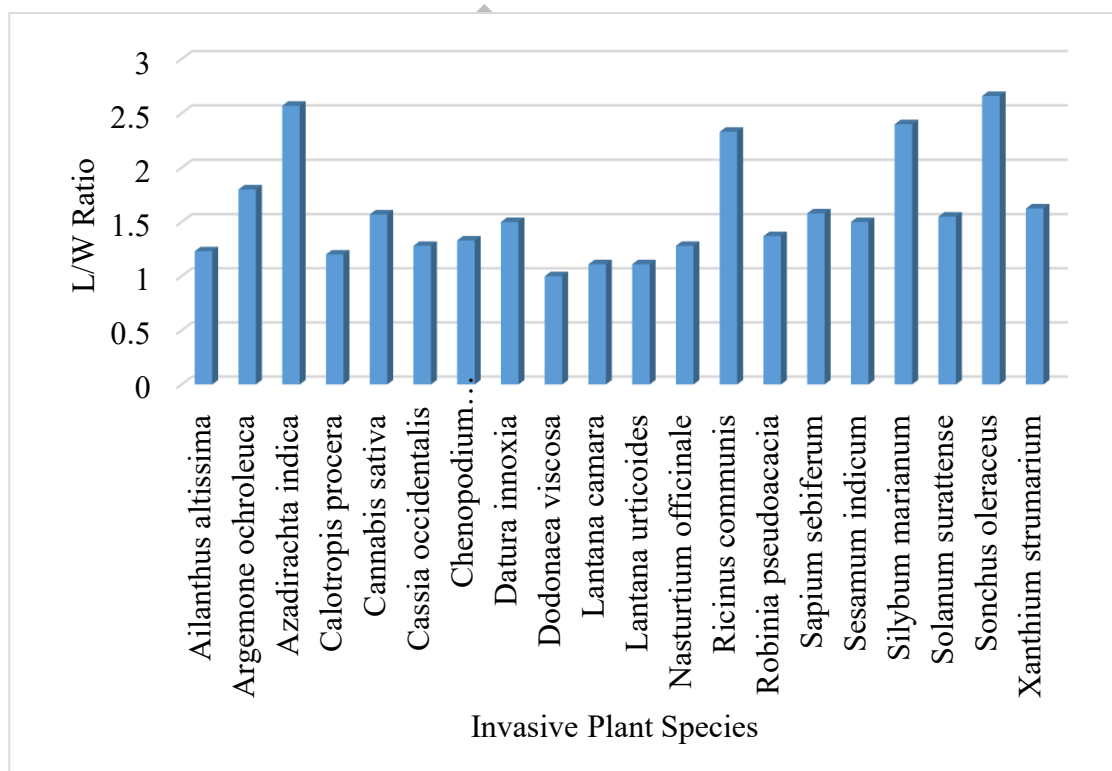


Figure 56. Length to width ratio among Invasive species

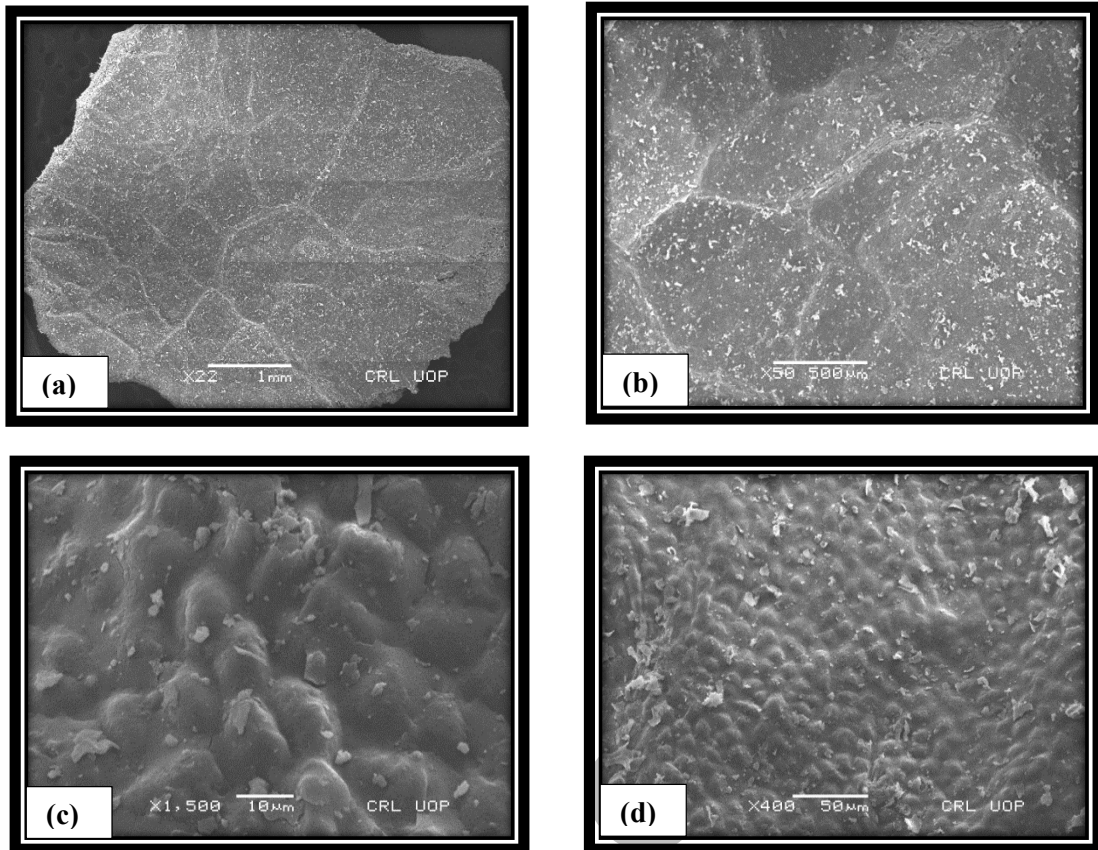


Plate 35: Scanning Electron Microscopy (SEM) micrographs of seed of *Ailanthus altissima* (a) General view (b, c & d) Surface sculpturing



Plate 36: Scanning Electron Microscopy (SEM) micrographs of seed of *Argemone ochroleuca* (a) General view (b, c, d & e) Surface sculpturing

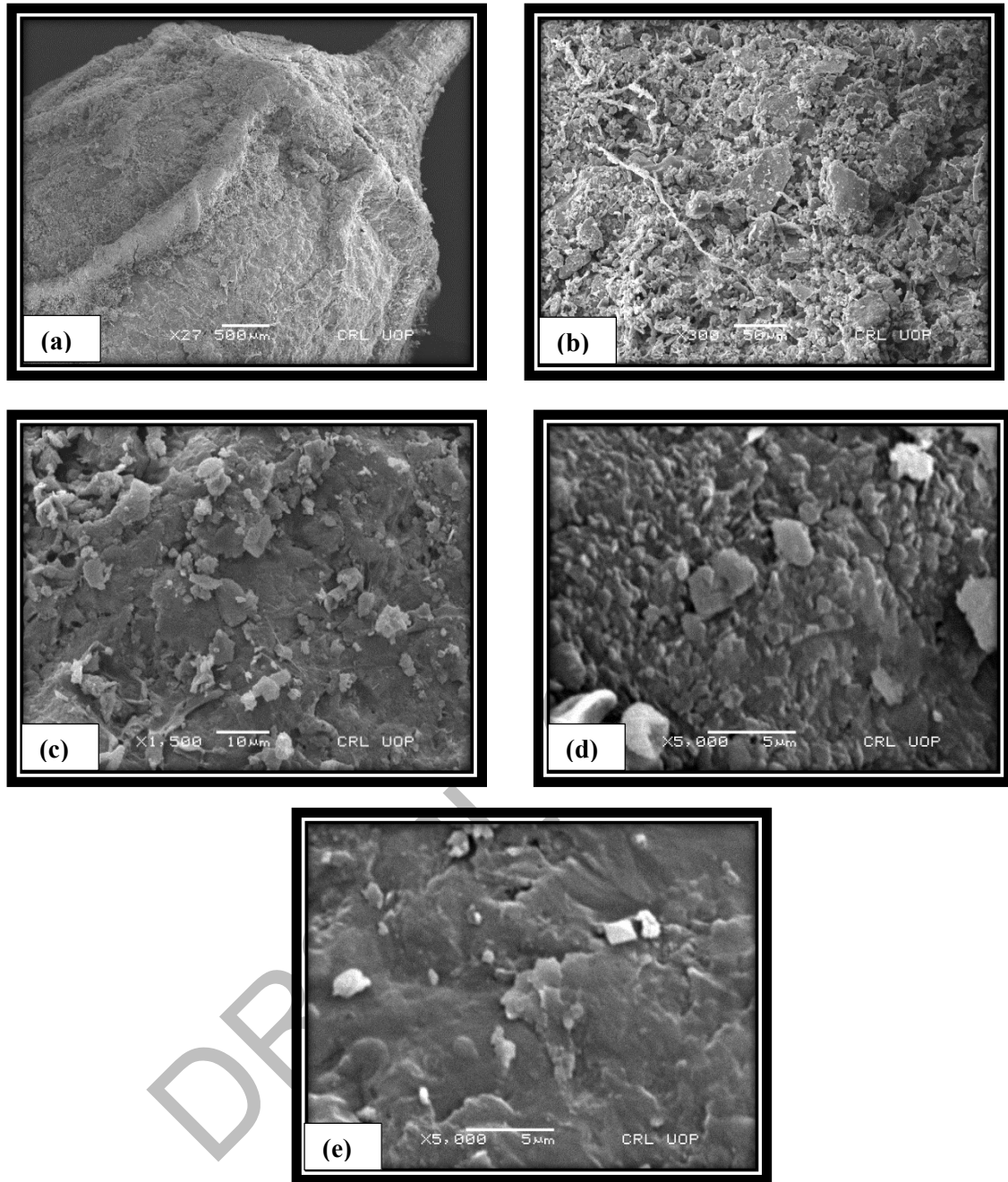


Plate 37: Scanning Electron Microscopy (SEM) micrographs of seed of *Azadirachta indica* (a) General outline (b, c, d & e) Surface sculpturing

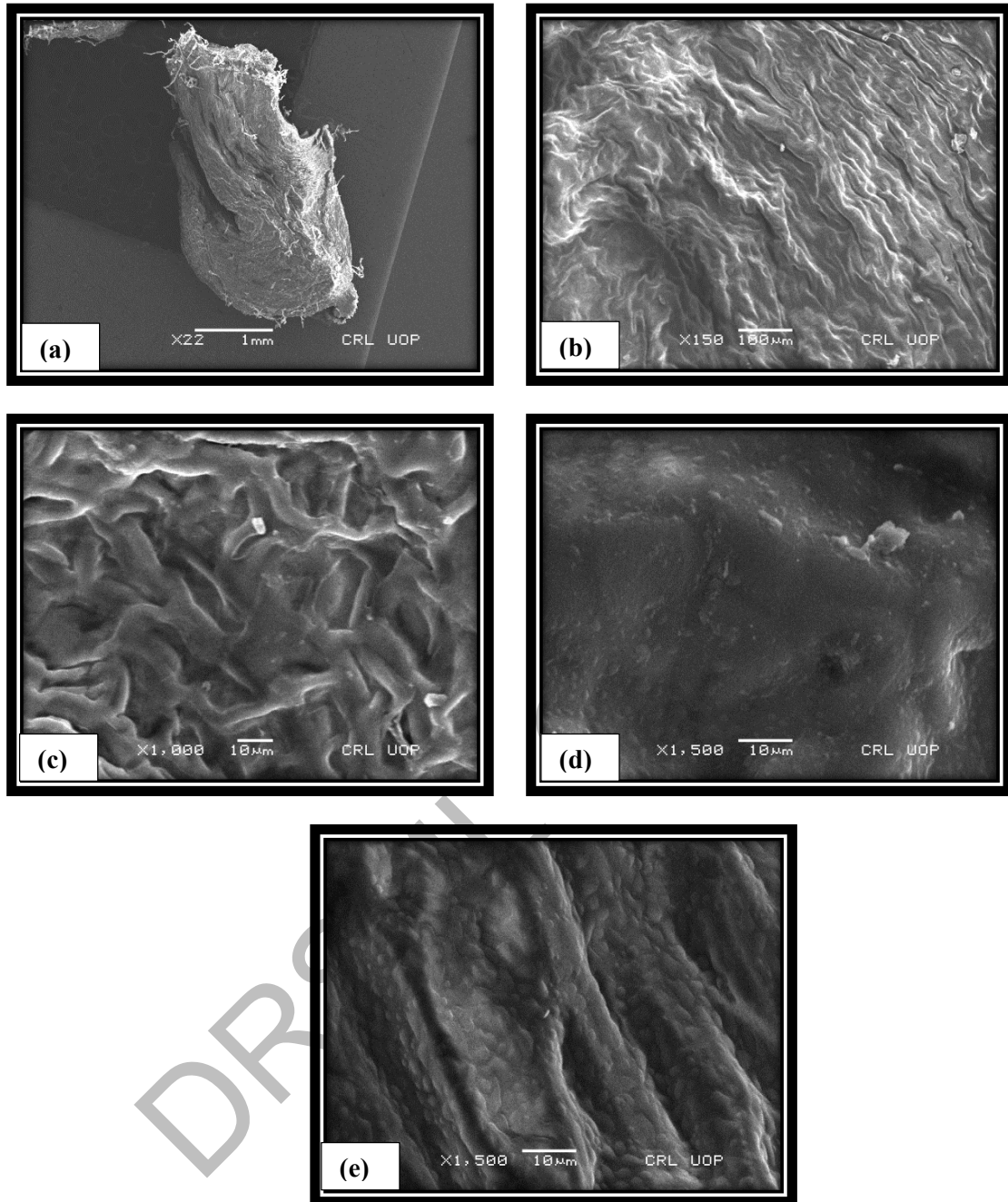


Plate 38: Scanning Electron Microscopy (SEM) micrographs of seed of *Calotropis procera* (a) General outline (b, c, d & e) Surface sculpturing

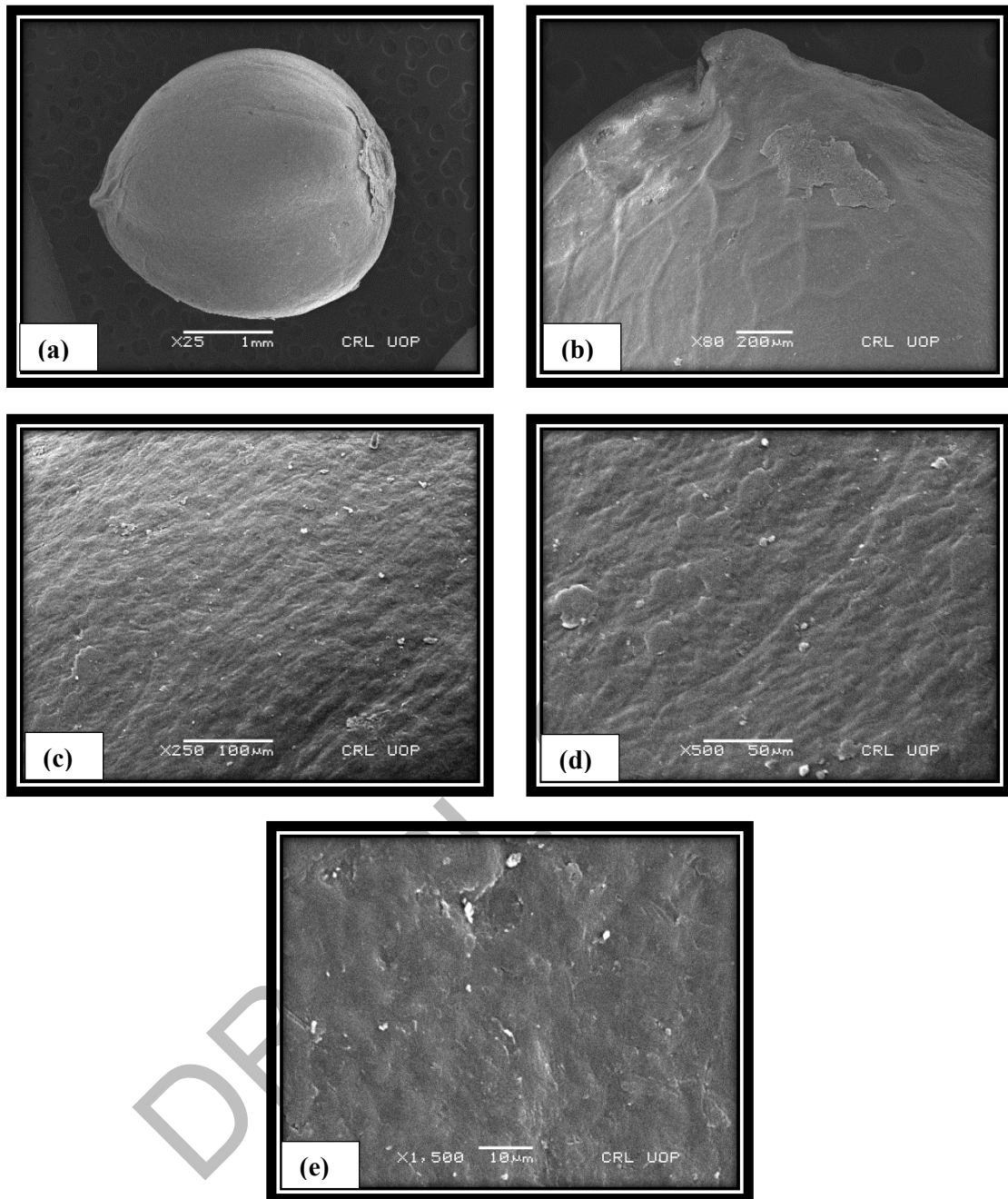


Plate 39: Scanning Electron Microscopy (SEM) micrographs of seed of *Cannabis sativa* (a) General outline (b, c, d & e) Surface sculpturing

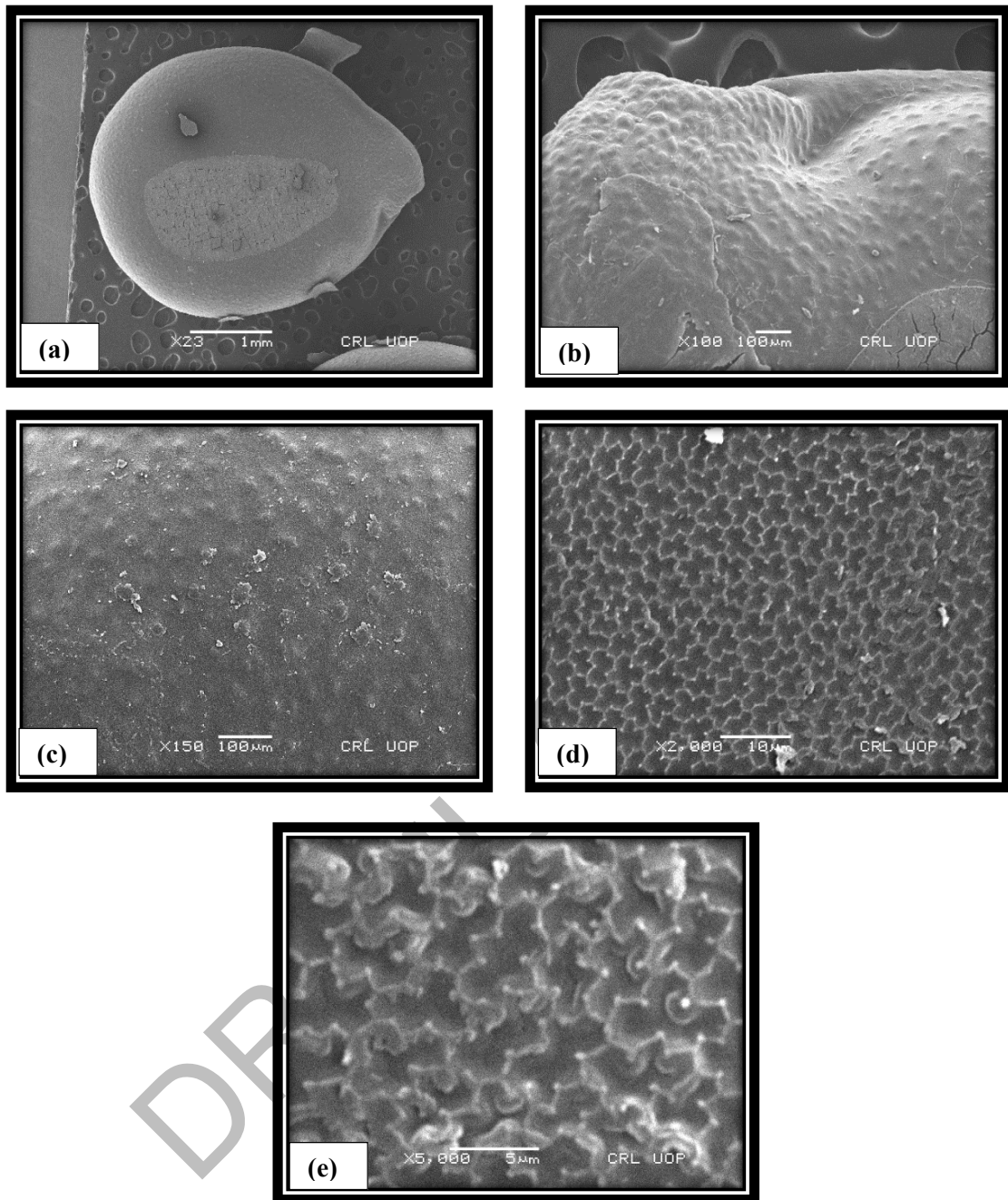


Plate 40: Scanning Electron Microscopy (SEM) micrographs of seed of *Cassia occidentalis* (a) General outline (b, c, d & e) Surface sculpturing

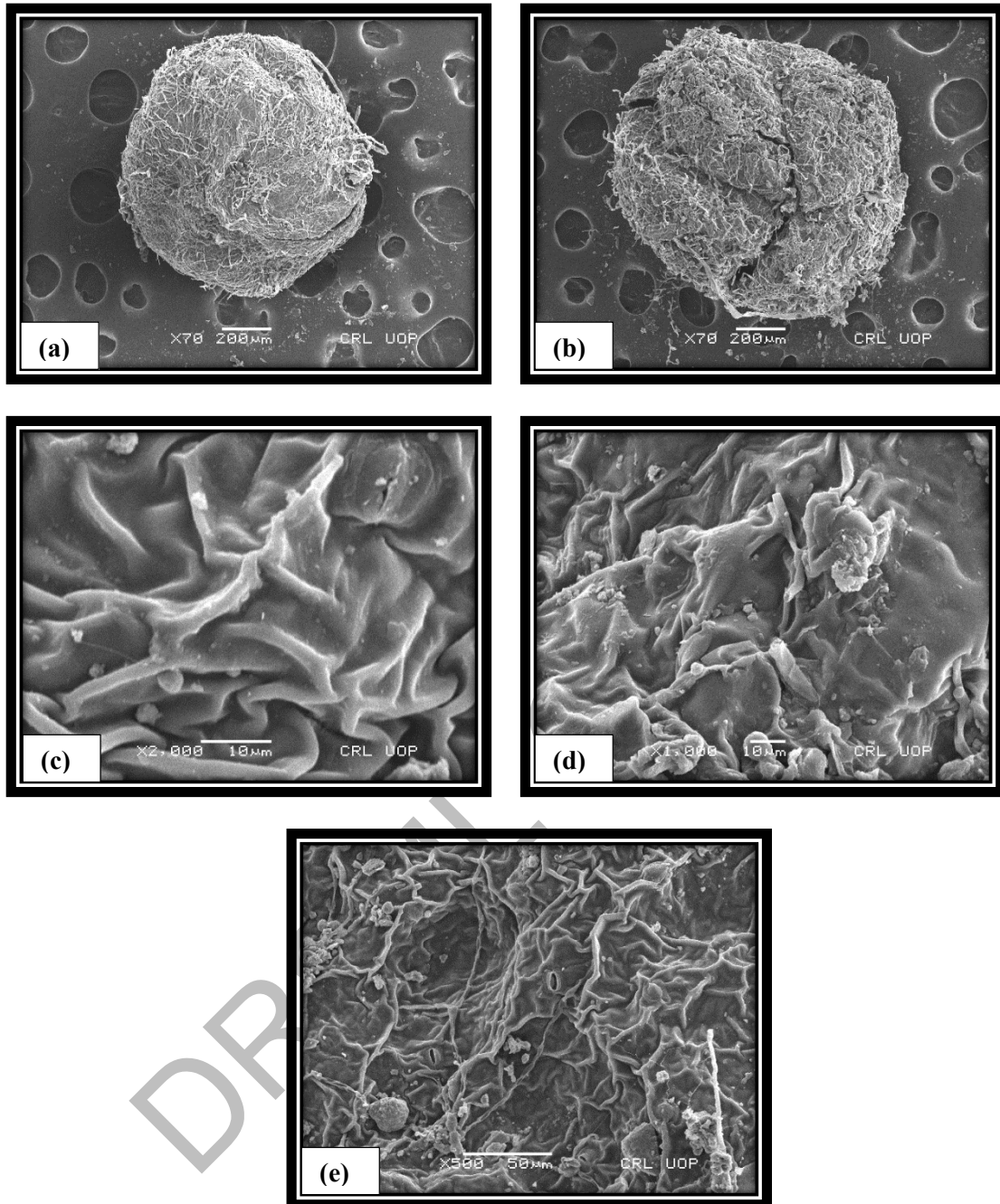


Plate 41: Scanning Electron Microscopy (SEM) micrographs of seed of *Chenopodium ambrosioides* (a) General outline (b, c, d & e) Surface sculpturing

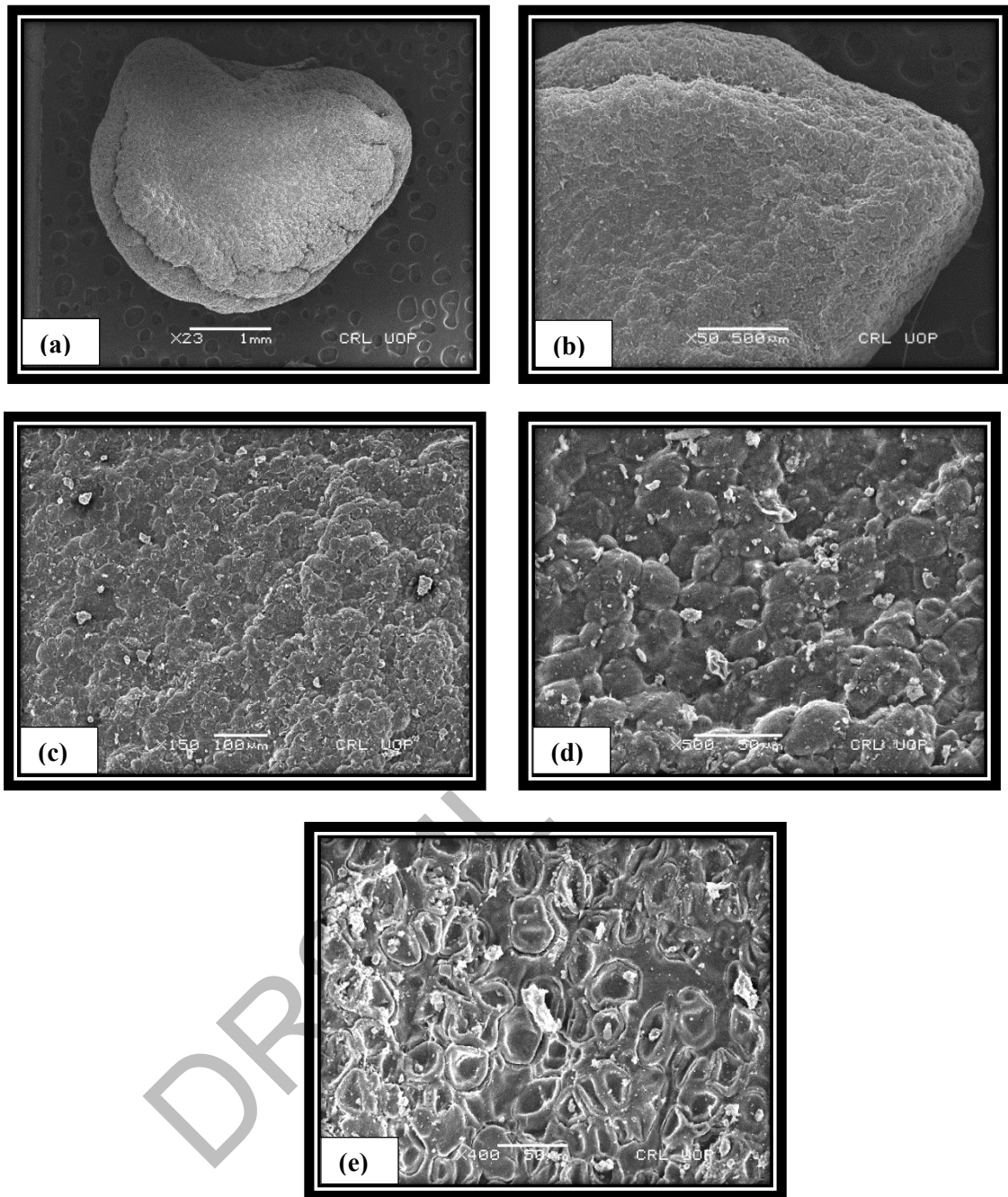


Plate 42: Scanning Electron Microscopy (SEM) micrographs of seed of *Datura innoxia* (a) General view (b, c, d & e) Surface sculpturing

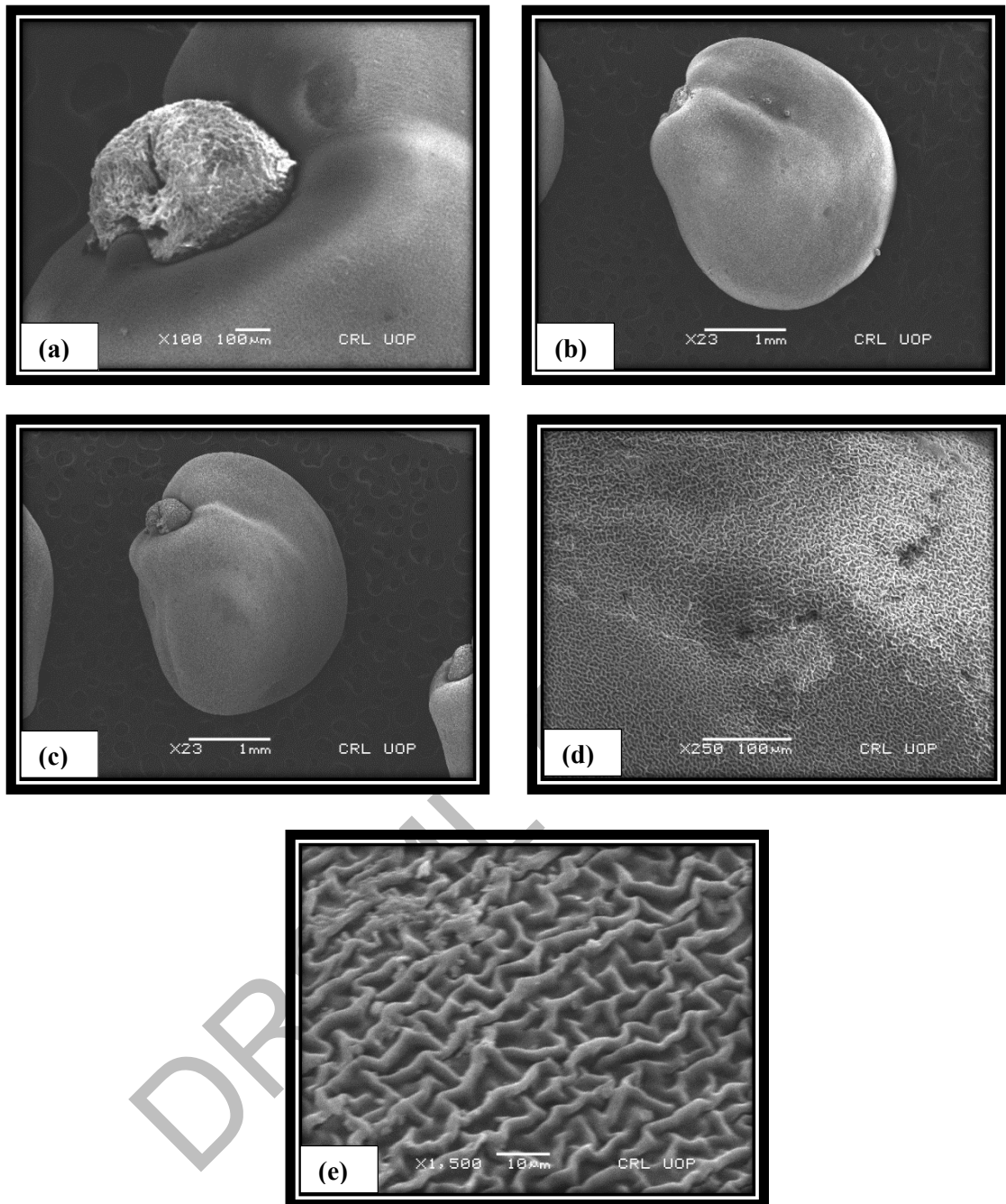


Plate 43: Scanning Electron Microscopy (SEM) micrographs of seed of *Dodonaea viscosa* (a, b & c) General view (d & e) Surface sculpturing

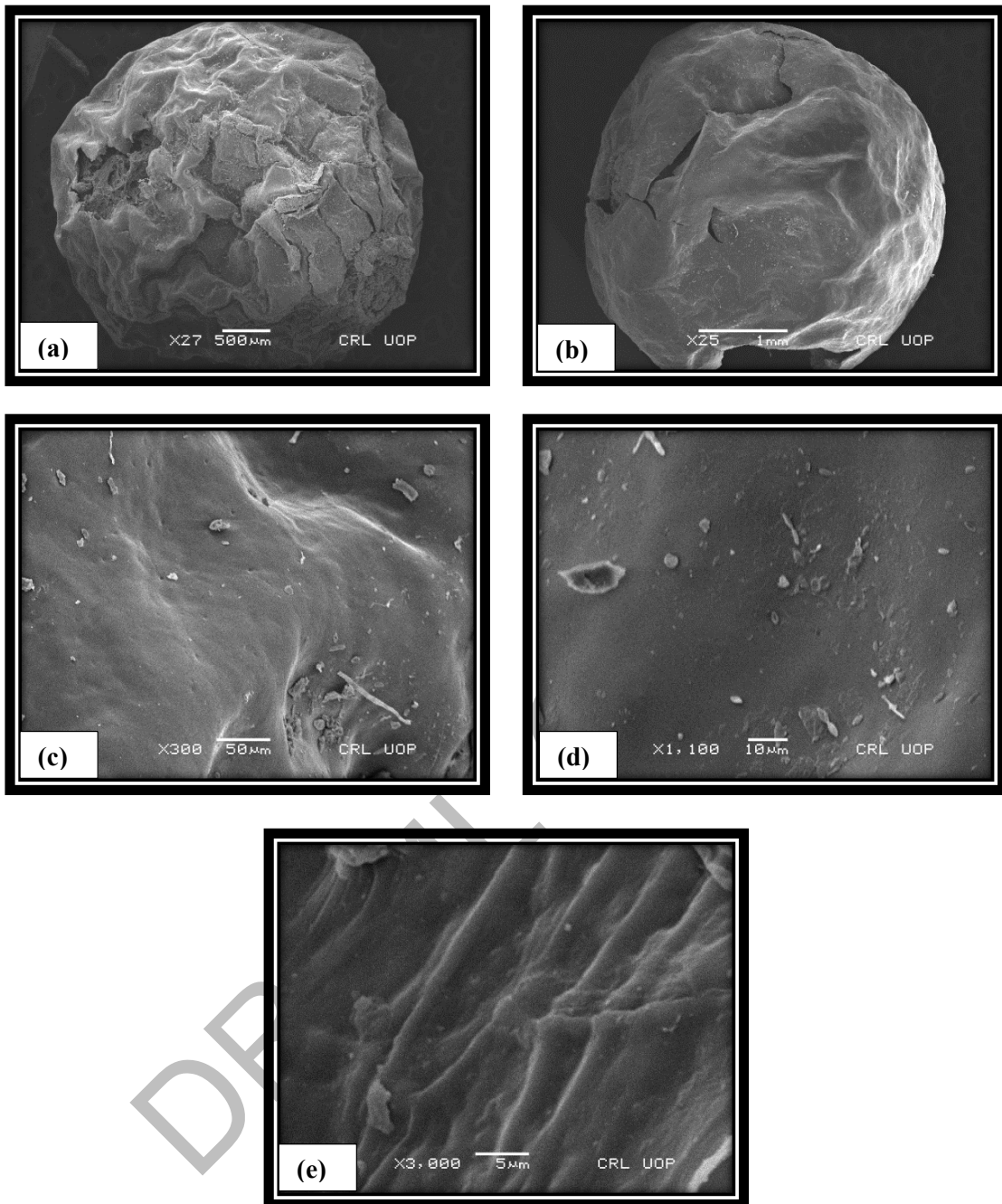


Plate 44: Scanning Electron Microscopy (SEM) micrographs of seed of *Lantana camara* (a & b) General view (c, d & e) Surface sculpturing

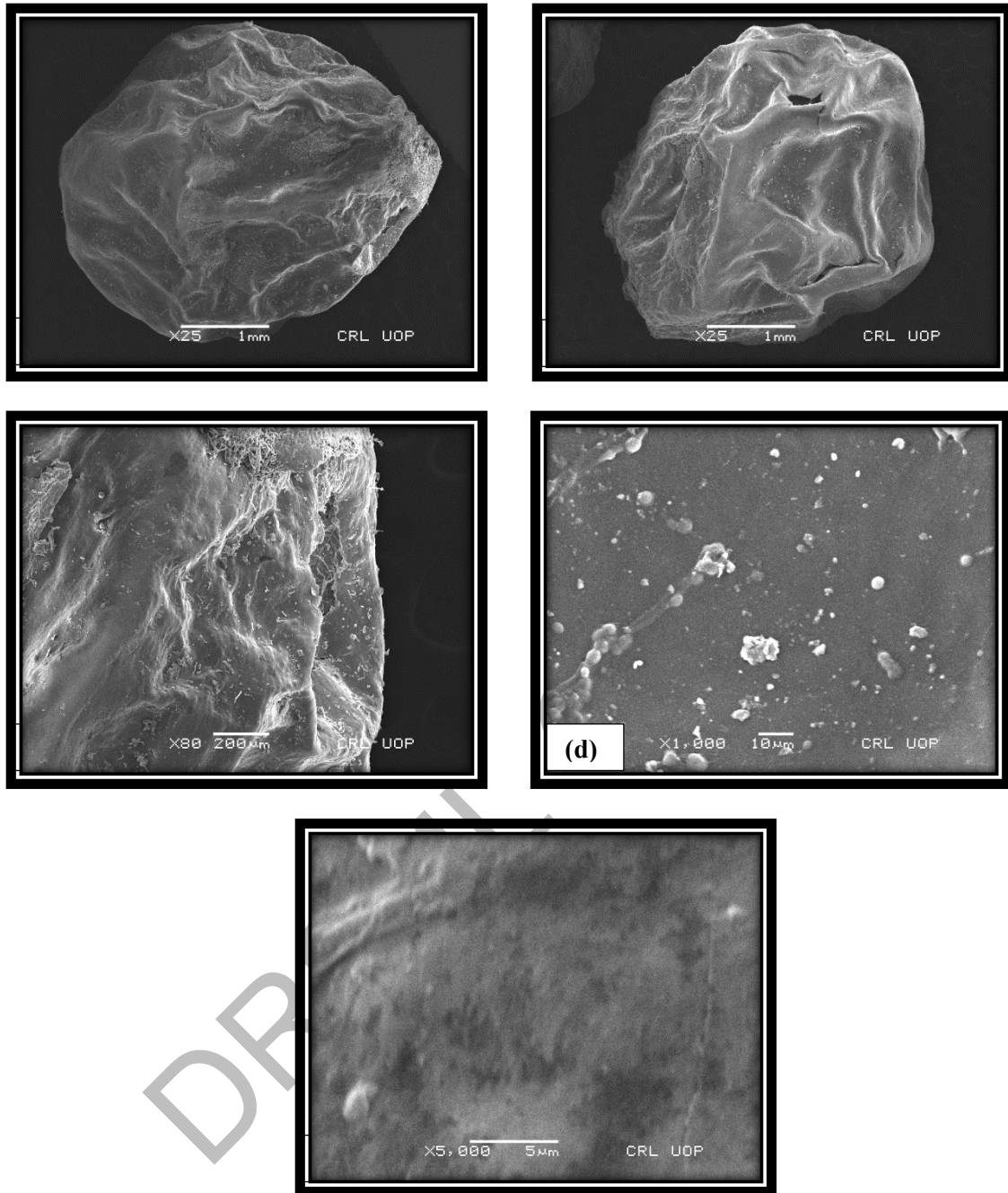


Plate 45: Scanning Electron Microscopy (SEM) micrographs of seed of *Lantana urticoides* (a & b) General view (c, d & e) Surface sculpturing

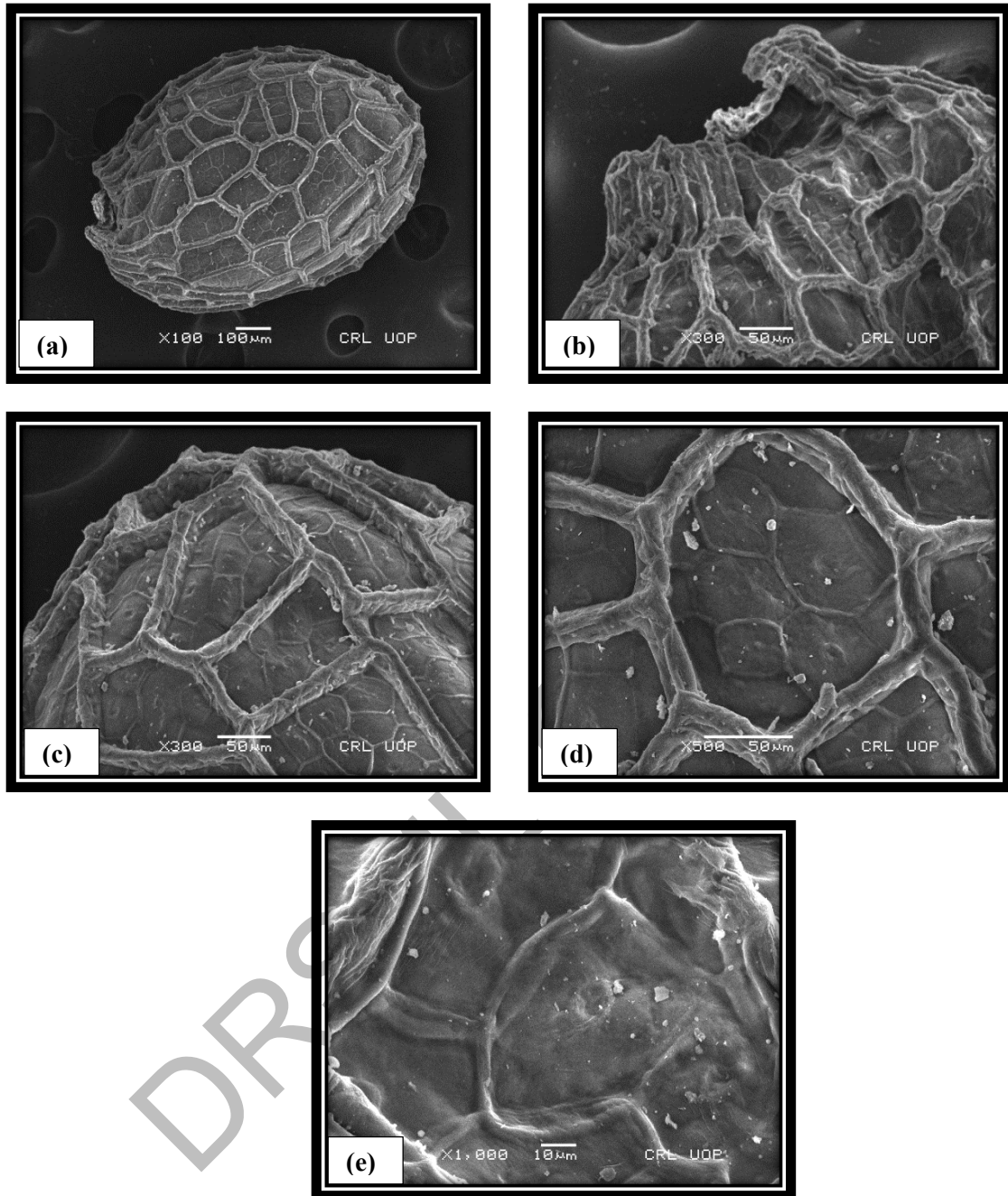


Plate 46: Scanning Electron Microscopy (SEM) micrographs of seed of *Nasturtium officinale* (a & b) General view (c, d & e) Surface sculpturing

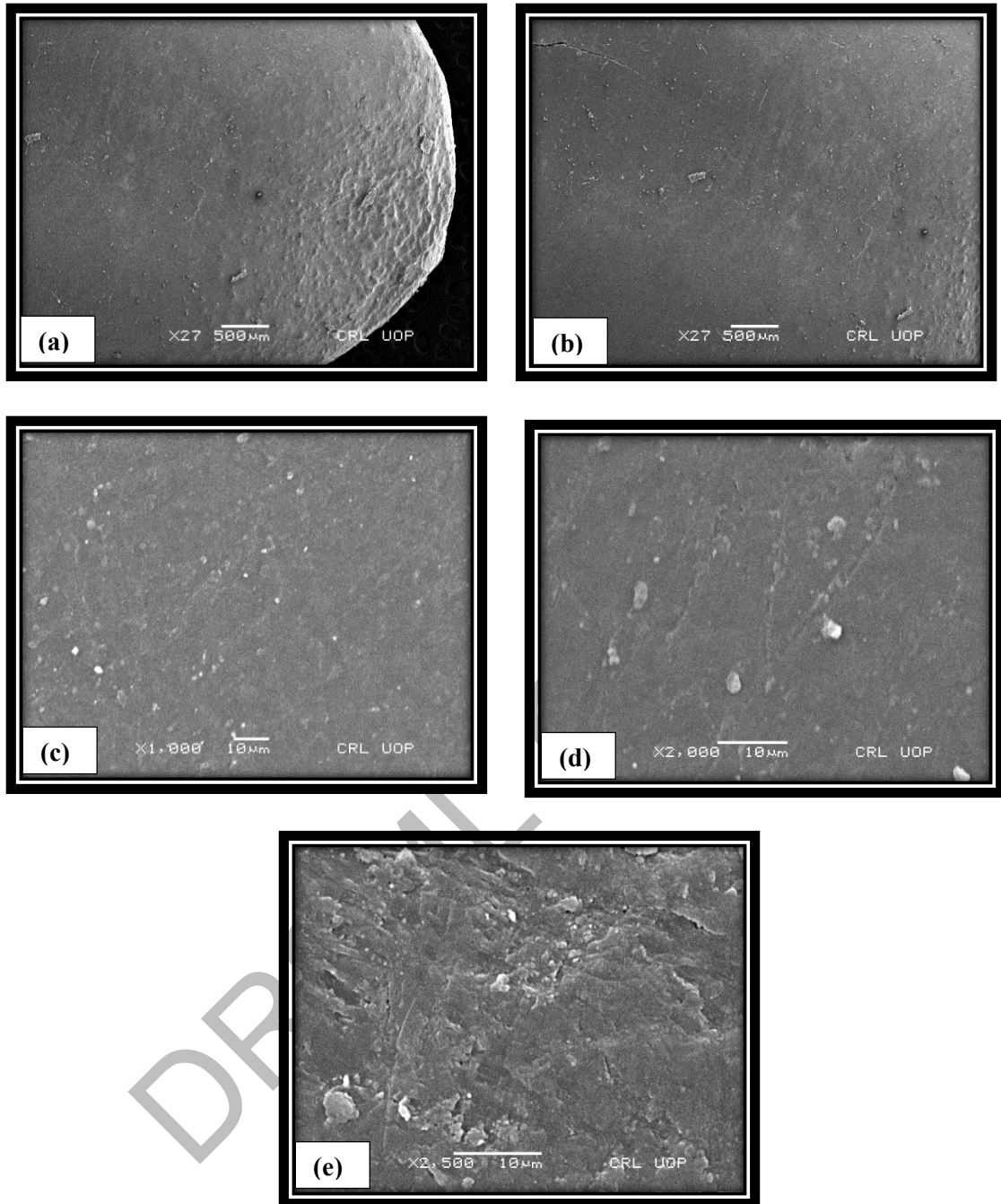


Plate 47: Scanning Electron Microscopy (SEM) micrographs of seed of *Ricinus communis* (a & b) General view (c, d & e) Surface sculpturing

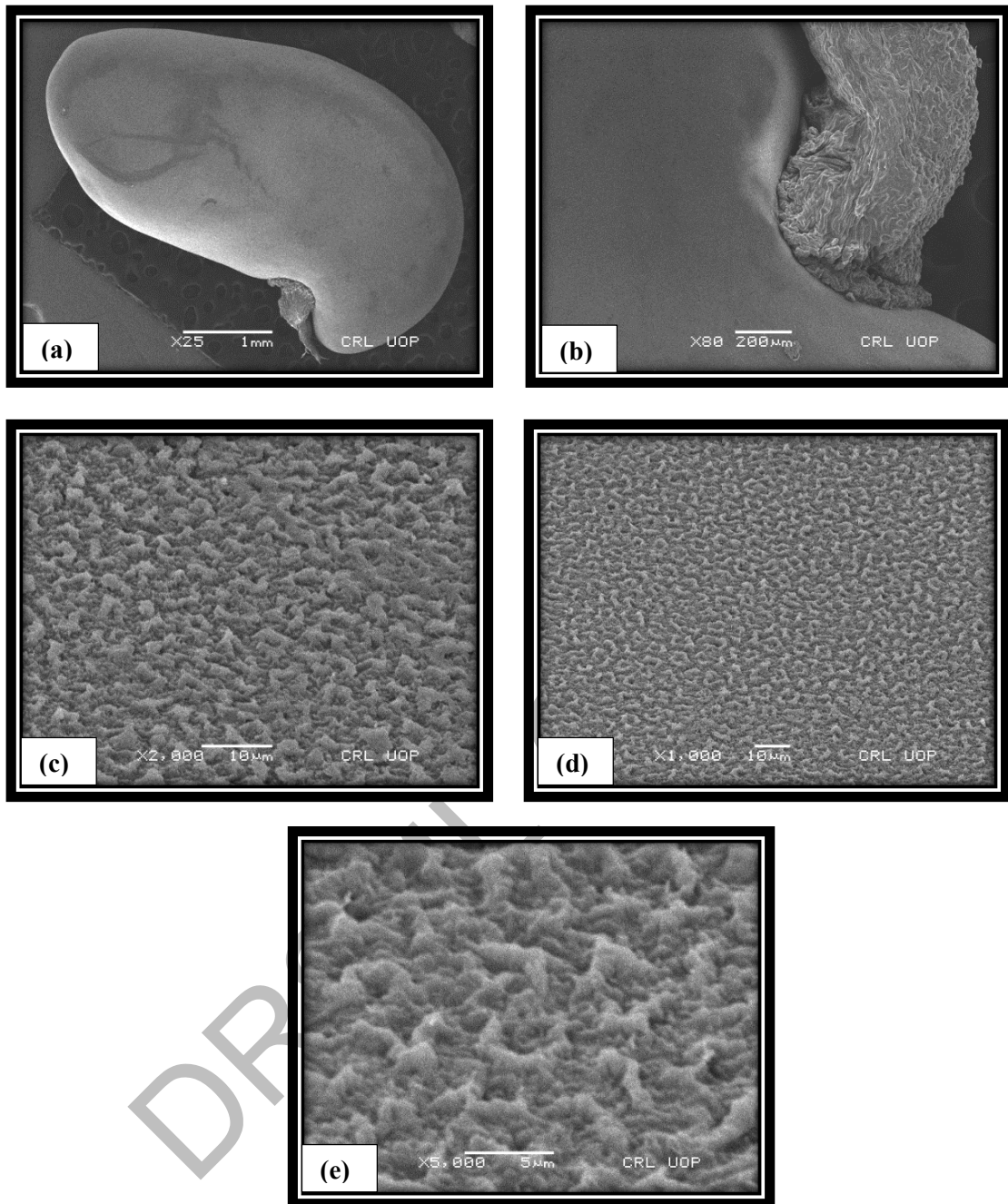


Plate 48: Scanning Electron Microscopy (SEM) micrographs of seed of *Robinia pseudoacacia* (a & b) General view (c, d & e) Surface sculpturing

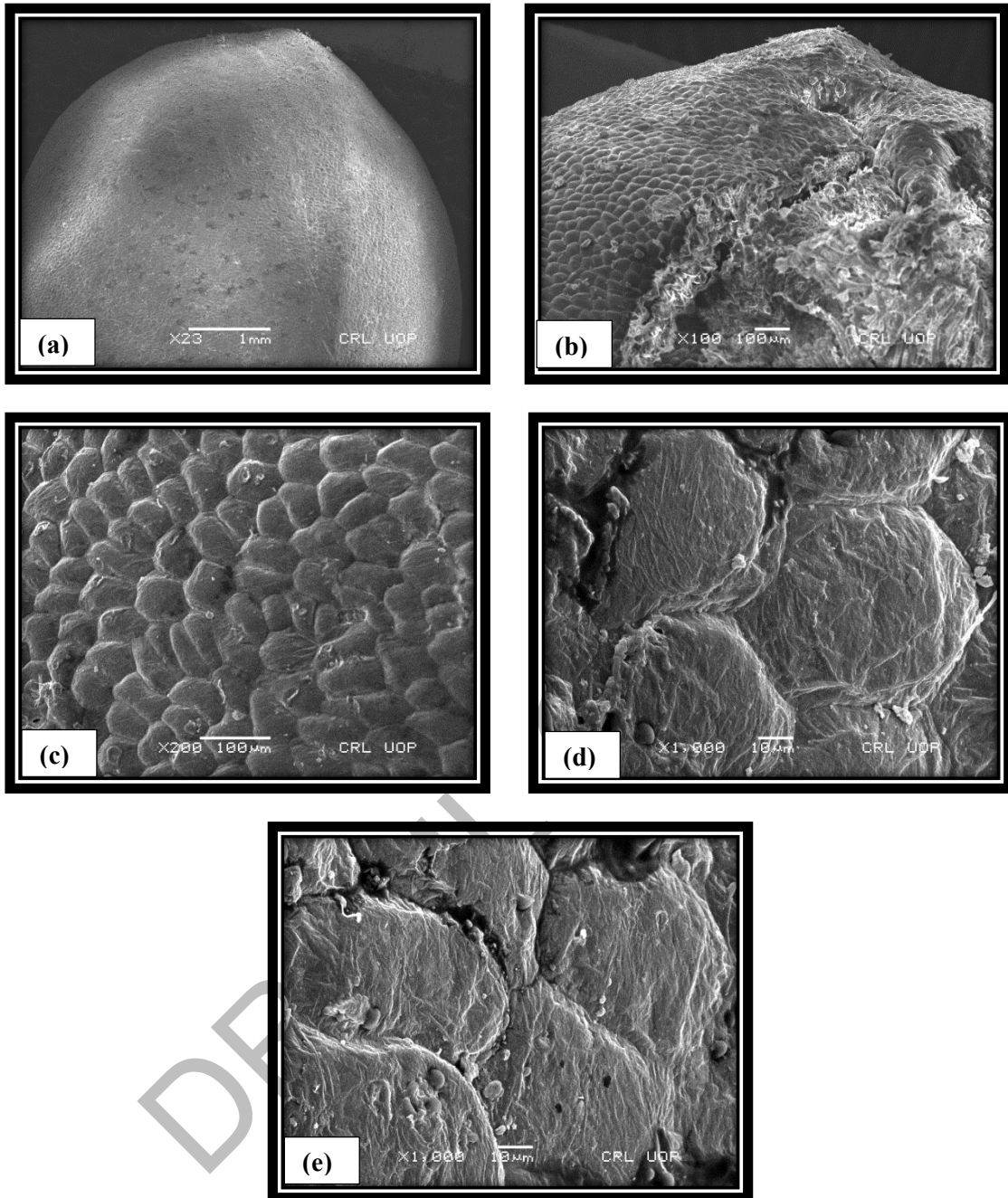


Plate 49: Scanning Electron Microscopy (SEM) micrographs of seed of *Sapium sebiferum* (a & b) General view (c, d & e) Surface sculpturing

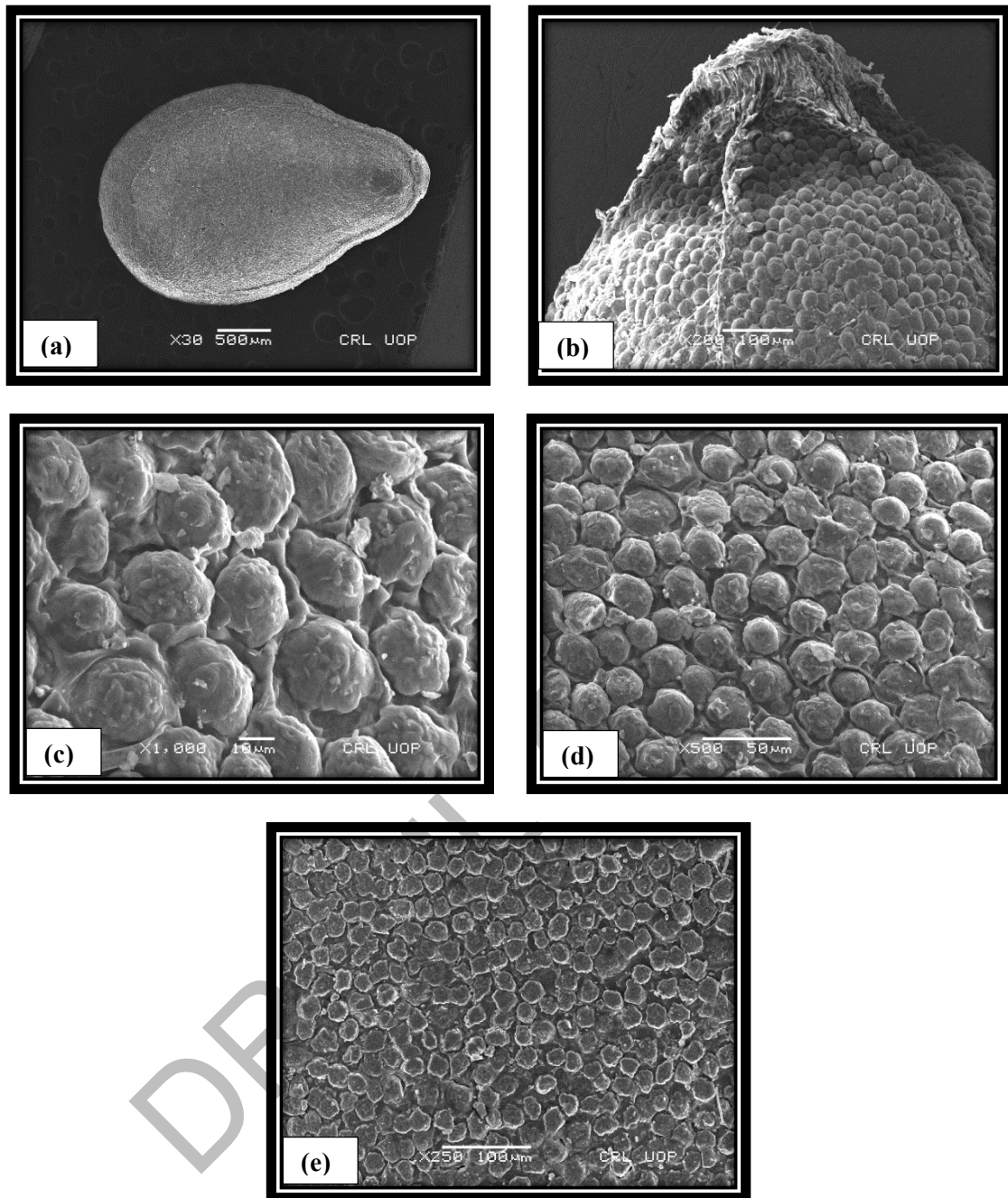


Plate 50: Scanning Electron Microscopy (SEM) micrographs of seed of *Sesamum indicum* (a & b) General view (c, d & e) Surface sculpturing

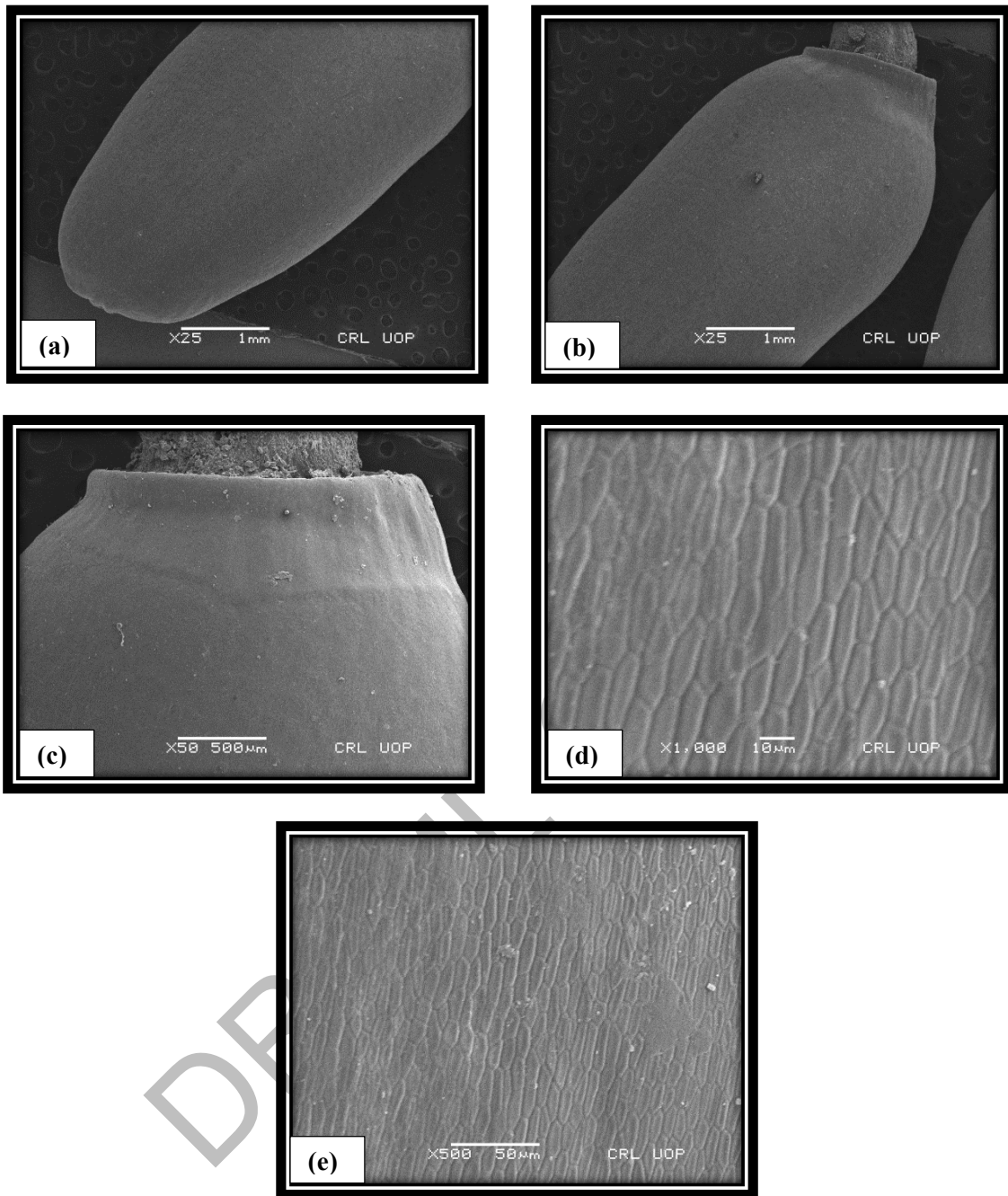


Plate 51: Scanning Electron Microscopy (SEM) micrographs of seed of *Silybum marianum* (a & b) General view (c, d & e) Surface sculpturing

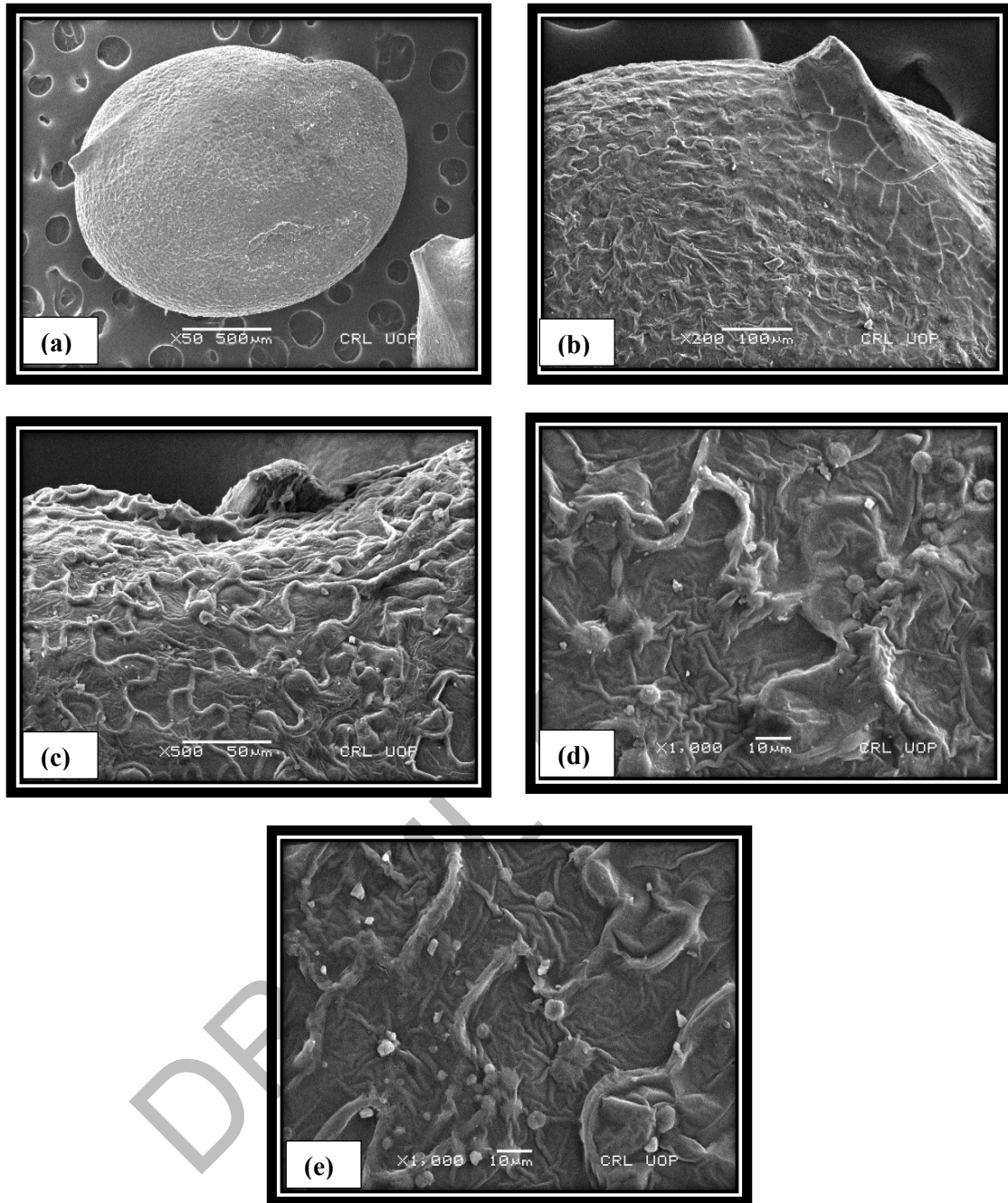


Plate 52: Scanning Electron Microscopy (SEM) micrographs of seed of *Solanum surattense* (a & b) General view (c, d & e) Surface sculpturing

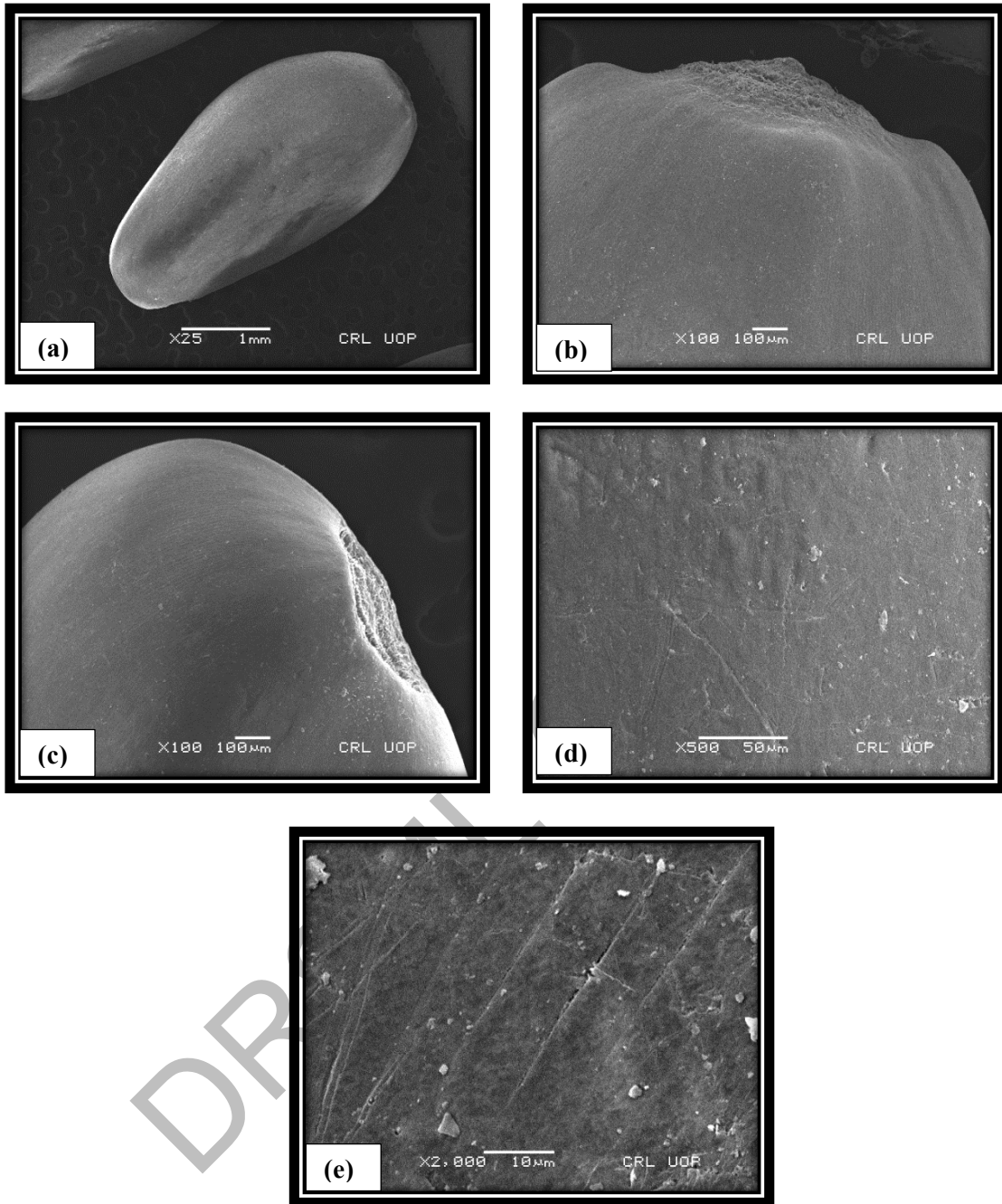


Plate 53: Scanning Electron Microscopy (SEM) micrographs of seed of *Sonchus oleraceus* (a & b) General view (c, d & e) Surface sculpturing

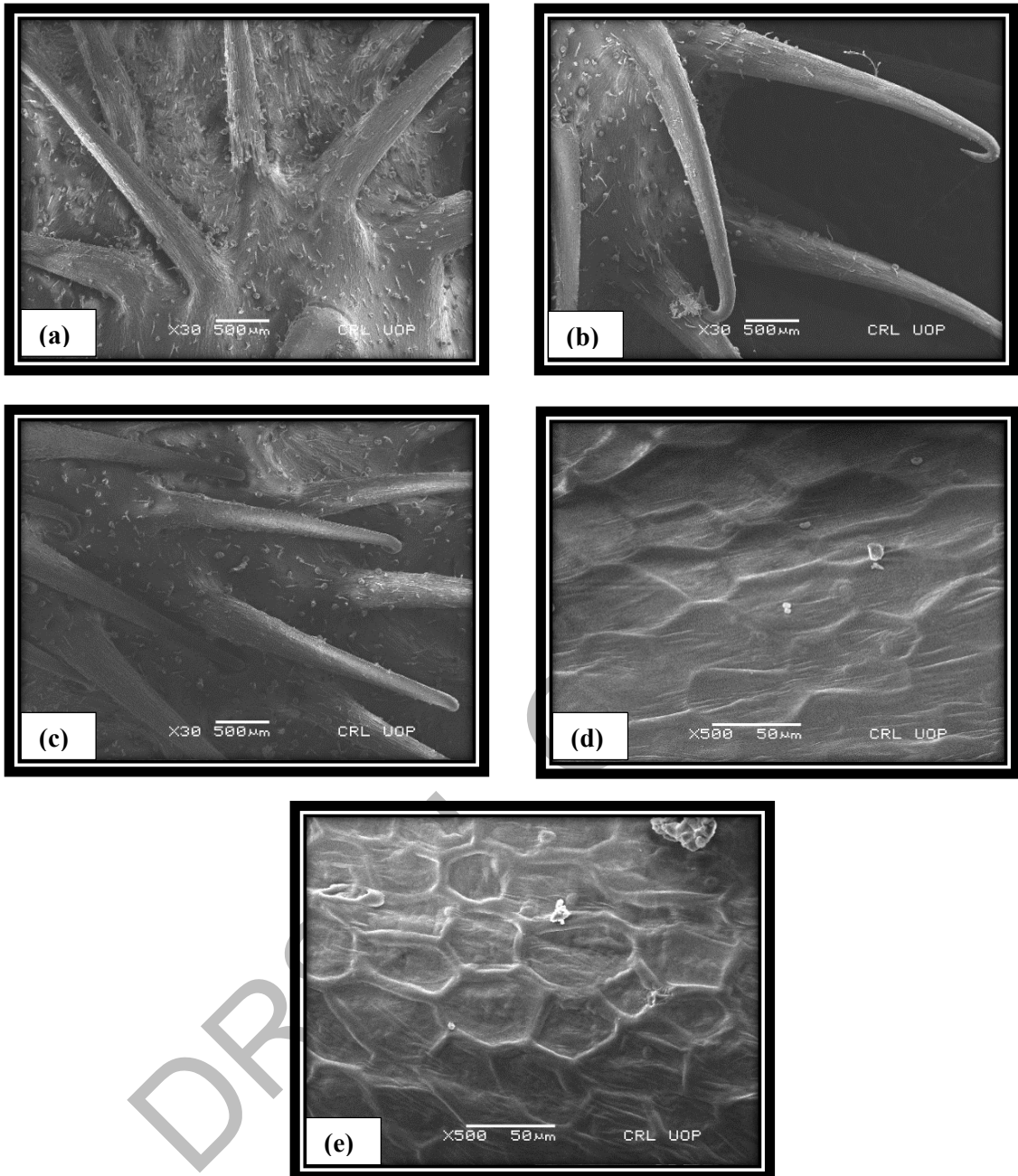


Plate 54: Scanning Electron Microscopy (SEM) micrographs of seed of *Xanthium strumarium* (a & b) General view (c, d & e) Surface sculpturing



**Conclusion & Future
Recommendations**

3.3 Control Mechanisms for Invasive Plant Species

Mechanical, chemical, biological, and ecological control methods are four broad categories to control invasive plants. Invasive plants can be pulled up or dug up by hands physically but it involves high labor inputs. They can be removed mechanically by bulldozing or ploughing but this control mechanism results in non-target damage and have higher capital costs. To kill the established herbs mowing is effective as it prevents flowering and also results in reduction of underground storage reserves (Auld et al., 2014)

Invasive plants can be controlled by use of herbicides which is less labor intensive than physical control. Decision to use herbicide to control invasive plants is carefully considered one. Major difficulties by the use of chemicals include non-target species damage, potential health risks to workers and the environment and herbicide resistance in target weeds by the continued use of one herbicide. As chemical control is costly and it may lead to environmental pollutions while mechanical control needs massive manpower, hence, biological and ecological control of invasive species have gained much more attention (Chen et al., 2013; Prider et al., 2008; Yu et al., 2011). Manipulation of environmental conditions to reduce the allelopathic potential of alien species and construction of resistance indigenous plants species are helpful in control of invasive species. Native plant species also have ability to produce allelochemicals which may act as novel weapon against the exotic species thus results in control of invasive plants. So native plants in this way strengthen the resistance of indigenous plant flora. Findings of research study by Zan and Li in 2010, indicates that some species of *Heteropanax fragrans* and *Hernandia Sonora*, native to southern China have allelopathic ability and have been used in controlling of *M. micrantha* i.e. invasive species.

Elimination of allelopathic effects of invasive plant species is helpful in the control of exotic plant invasion, this can be also achieved by enhancing the allelopathic effects of indigenous plant species. According to Kumari and Kohli (1987) autoallelopathy results in reduction of invasive species as these plants can also be affected by their own allelochemicals.

Exotic alien plants can also be control by use of microorganisms or insects i.e. biological control. But one limitation involves to use microorganisms is that it requires long-term investment in research before an agent is proven safe and potentially effective (Auld et al., 2014). Biological control is considered as one of the most significant approach to manage or control the invasive plants especially when this invasion spreaded on large scale. But for the management of invasive species the factors like funding availability, public support and strong institutional commitment are vital (Larson et al., 2011). Other factors which plays important role in successful implementation of any program may include Stakeholder involvement, effective administration, coordination and leadership (Downey 2010; Howell 2012).

3.4 Conclusion

The current study provides a detailed account of the systematics of invasive plants of Lesser Himalaya Pakistan. Systematics studies include the morpho-palynology and seed morphology (LM and SEM) of invasive plants that plays an important role in species identification at species, genera or family level. It is the first ever documented systematic exploration of invasive plants in the study area with special reference to pollen and seed morphology.

In total of 52 species belongs to 31 families of invasive plants are investigated using LM & SEM to characterize pollen and seed diversity. Palyno-morphological investigation revealed that tricolporate pollen is most dominant of the studied taxa while tricolpate pollen were noted in *Bryophyllum pinnatum* (Lam.) Oken, *Cannabis sativa* L., *Convolvulus arvensis* L., *Datura innoxia* Mill., *Digera muricata* (L.) Mart., and *Jasminum humile* Linn. The pantoporate pollen were shown by 13 % species. Trizonocolporate, Monoporate and Tetracolpate, Tricolpate were reported only 3%. The dominant four exine sculpturing pattern i.e. echinate, psilate, reticulate and psilate perforate respectively has been observed. Dominant pollen shapes were Prolate-spheroidal, sub oblate, spheroidal and prolate. Medium size pollen were investigated in 45% speices while 34 % were small size. The maximum polar to equatorial (P/E) ratio was calculated *Lanta camara* i.e. 1.42 while highest pollen fertility (97.7%) was documented in *Quisqualis indica* L. The findings of present study showed that pollen

morphological characters provides useful data about inter and intrageneric variations and geographical relationships of invasive taxa.

Seed germination process is rapid in invasive plants than of native species and invasion process is facilitated through this rapid process. Plant invasion success is linked with seed germination as reported in various studies so far. The seed morphology of invasive plants of study region showed a great variations. The seed morphological characters provide an aid in distinguishing the various species and also support their placement in different clades. In present study morphology of twenty seeds of invasive plants were studied. The spheroidal shape were observed as dominant while color of seeds showed great variation. Maximum length of 14mm was calculated in *Ricinus communis* L. while maximum width of 8mm was observed in *Xanthium strumarium* L. The maximum L/W ratio was recorded in *Sonchus oleraceus* i.e. 2.66. The SEM results showed significant taxonomic scorable diversity among various invasive species and give useful data on seed surface and shape in the context of phylogeny. The majority of seeds of invasive plants showed the reticulate seed surface. Invasive plants have higher growth rates, lower resistance and higher tolerance to herbivores and they also possess greater seed productivity, however there is little research have been conducted with reference to differences in seed morphology of native and introduced populations.

The analysis of present research study showed differences in size, shape and ornamentation of pollen grains and seeds between nonnative species. Thus it can be concluded from present study that pollen grains of invasive plants with smaller size and spherical shape could have better advantages than those of native plants species. Introduced plants also exhibit the variations in exine ornamentation, these differences in ornamentation can be related to their adaptation to specific pollinators in new ecosystems which is positively related with successful establishment and spread of introduced plants. Studied invasive plants also showed high percentage of fertile pollen which can be referred to their pollination and distribution success. Furthermore the seed physical properties like seed size, shape, coat thickness, hardness and seed cost surface have found to be influence on rate and speed of seed germination in introduced plants and may lead to successful invasion. In future further studied related to other aspects of

pollen and seed morphology are needed to evaluate the invasive success of the alien plants.

3.5 Future Recommendations:

- This systematics approach is valuable for the preparation of Atlas of invasive flora of this region for ecologist, botanist and taxonomist.
- The compilation of regional based flora is required immediately to enhance the research in biodiversity of invasive plants in Lesser Himalayas.
- This systematic study will be helpful for molecular biologists for the better and correct identification of invasive plants
- Conservation projects should be started with an aim to conserve and restore native biodiversity that are threatened by invasive alien plants.
- Long term control action programs should be developed.
- It is recommended that community awareness programs should be started through print and social media to discuss this hot issue.
- It is suggested that for identification of invasive alien flora there should be capacity building programs for the quarantine department in the country.
- It is also suggested that government should take measures to reestablishment of highly degraded and threatened native ecosystems.
- For the identification of invaded organisms, international cooperation and interdisciplinary coordinated research is recommended.

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DRSML QAU

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**Project
Outcomes**

List of Publications:

S. No.	Paper title	Year	Impact Factor
1.	Anjum, F, Ahmad, M., Shinwari, Z.K., Zafar, M., Sultan, S., Majeed, S., Usma, A., Mir, A. (2022). Diversity of Invasive Alien Plants Species of Lesser Himalayas-Pakistan. Pakistan Journal of Botany (Accepted).	2022	1.00
2.	Anjum, F, Ahmad, M., Zafar, M., Sultan, S., Mir, A., Ameen, M. (2022). Seed Coat Morphology and Sculpturing of Selected Invasive Plants from Lesser Himalaya Pakistan and their Systematic Implications. BioMed Research International (Accepted)	2022	3.41

DRSML QAU

Research Article

Seed Coat Morphology and Sculpturing of Selected Invasive Alien Plants from Lesser Himalaya Pakistan and Their Systematic Implications

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Invasive alien species (IAS) are considered as the second major threat to biodiversity after habitat destruction worldwide. They are aggressive competitors and dominate an ecosystem where they introduce and cause reduction in indigenous diversity. Invasive plants alter the evolutionary pathways of native species by competition, niche displacement, hybridization, introgression, predation, and ultimately extinction of native species. Biological invasion also results in economic and environmental damage and harm to human health. Invasive plants have an effective reproductive as well as dispersal mechanisms. Most invasive plants produce abundant fruits and seeds that are widely disseminated and remain viable in the soil for several years. Invasive plants may change their seed character in order to adapt themselves to the new environment and facilitate their performance. A study on seed coat sculpturing in invasive alien plants collected from Lesser Himalaya region, Pakistan, was conducted using scanning electron microscope to determine the importance of seed morphological characters as an additional tool for identification. Quantitative characters such as seed length and width, macromorphological characters including color, hilum position, and seed shape, and micromorphological characters of seed including surface patterns and periclinal and anticlinal wall of seeds were studied. Findings at the present indicate that most of the seeds were found spherical followed by ovate and elliptical in shape with smooth surface and showed terminal hilum. Almost reticulate seed patterns were observed in seeds. Majority of seeds showed raised anticlinal walls with protuberance periclinal walls. The seeds of *Xanthium strumarium* were observed with maximum length of 13 mm and with width of 8 mm. Length by width ratio of seeds was also calculated; it was found that maximum L/W ratio was observed in *Sonchus oleraceus* L., i.e., 2.66. Seed characters, both macro- and micromorphological, furnish useful data for classification and delimitation of invasive taxa. This study will help to understand the invasion mechanism in plants due to variations in seed surface, shape, and other characters. Adaptive behavior of the seed during the invasion process of the new ecosystem is also elaborated.

1. Introduction

Invasive plant species are species that are exotic to an ecosystem. They are aggressive in nature and adversely affect ecosystems and biodiversity. They also have negative effects on the environment and cause harm to the economy and human health as well [1]. Biological invasion can lead to alterations in species composition and changes

in nutrient cyclic and result in forest fire [2]. In Pakistan, alien invasive plants are considered as one of the biggest and neglected hazards to the native biodiversity [3]. The climatic characteristics like temperature, day length, and relative humidity act as an abiotic filter for newly introduced plants [4]. A study by [5] has also shown that there is no significant relationship between heat waves and alien plant species.

DIVERSITY OF INVASIVE ALIEN PLANTS SPECIES OF LESSER HIMALAYAS-PAKISTAN

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Abstract

The objective of current study is the comprehensive account regarding the diversity of invasive alien plants of Lesser Himalayas-Pakistan with special emphasis on family, habit, nativity, mode of introduction, and global distribution. A total of 73 invasive alien species under 65 genera and 30 families have been documented in present research study which is based upon field observations, herbarium, literature consultations and review. As far as mode of introduction of species is concerned 37% species, have been introduced intentionally while rest of species were unintentionally established via different means, the most common is trade. Analysis of status or life form of these plant species indicates that herbs are dominant (44), while shrubs (10 species), Grass (6 species), Trees (10 species), undershrub, climber, and Fern (1 species each). Perennial habit is represented by 43 invasive plant species followed by 25 annual and rest of species shows both annual and perennial habit. Nativity analysis revealed that majority of invasive alien plant species are reported from American subcontinent (51%). Plant invasions adversely affect indigenous flora, ecosystem functions which results in ecological and economic imbalance of a particular region. Impacts of biological invasions are complex as it alters the species composition and its dynamics. Due to biological invasions plant communities in an area become less diverse that is proved as a threat to native diversity of invaded region. A better planning strategies and appropriate control measures (indigenous biological and cultural weed control measures) is the urgent need of time. This will give a clear strategy to mitigate the existing and future adverse effects of alien plant invasions on ecological processes that are vulnerable.

Key words: Diversity, Invasive species, Biological invasions, Lesser Himalaya, Floristic inventory.

Introduction

Invasive alien species (IAPS) of plants are exotic species, established themselves outside their natural range that negatively affect native flora, environment, ecosystem processes, and habitat in invaded regions (Naz *et al.*, 2021). These species can be introduced either accidentally or intentionally (Masters & Norgrove, 2010). According to International Union for Conservation of Nature (IUCN) and Natural Resources, alien invasive species are foreign species that establishes itself in a semi natural ecosystem or environment and threatens native biological diversity.

Richardson & Rejmanek (2011) indicate that 0.5–0.7% of worldwide species tree and shrubs are invasive outside from their normal range. IAPS are very aggressive in nature and suppress the indigenous vegetation thus they are now serious threat causing to vegetation biodiversity. According to Pant & Sharma (2010), invasive species reduce biodiversity and change hydrology and ecosystem dynamics. Rate of invasive alien plant species have been rapidly increased all over the globe posing a great threat to indigenous biodiversity, native habitats, and ecological functions (Booth *et al.*, 2003; Hulme, 2003; Shinwari & Qaisar 2011). Biological invasions cause natural groups to change and ecological traits to change at an exceptional rate. Deleterious effects of IAPS ecosystem processes are linked with human well beings as they alter natural environment, ecosystem structure as well as economic cost (Crowl *et al.*, 2008; Mazza *et al.*, 2014). Grice in 2006 reported that IAPS are responsible in degradation of native plant species in ecosystems by outcompeting the available resources e.g., nutrients, space and as well as modified

ecological systems (Hameed *et al.*, 2020; Hussain *et al.*, 2020; Yaseen *et al.*, 2015; Yaseen *et al.*, 2019).

Rapid increase in biotic invasions have been observed over a due course of time, the main reason for this rapid increase is growing market globalization, increase in worldwide trade, travel and tourism (Abbasi *et al.*, 2012; Eiswerth *et al.*, 2005; Majeed *et al.*, 2021). Murphy *et al.*, (2013) and invasive plants have been expanding throughout protected areas in the Himalaya region, according to Aryal *et al.*, (2017), posing a threat to wildlife habit and nutrition availability. According to Alexander *et al.*, (2016), in mountain ecosystems IAPS are found in plentiful at lesser and mid elevation and this establishment of IAPS is due to ongoing climate drift (increased warming) and anthropogenic changes.

The Lesser Himalayan regions have always been encroached by invasive alien plants. As this region shows great variation in altitude and wide range of climatic zones which supports growth of diversified and rich vegetation in this region of world (Shaheen *et al.*, 2011; Khan *et al.*, 2020; Malik *et al.*, 2019; Sultana *et al.*, 2011). A very few studies have been conducted on the diversity and distribution of IAPS in Lesser Himalayas. However, a comprehensive study focusing on this vast territory is unavailable. It is necessary to implement effective IAPS management, and understanding on the diversity, habitat and origin of invasive species is required. Therefore, present study is conducted to enumerate the invasive alien plant species of Lesser Himalayan Region of Pakistan and its diversity with future implication of this region. This study will also be helpful for policy makers in developing the management approaches to control the aggression of alien invasive species.