

FOOD HABIT OF GREATER ONE-HORNED RHINO (*Rhinoceros unicornis*) IN BABAI VALLEY OF BARDIYA NATIONAL PARK, NEPAL



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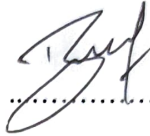
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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has been not submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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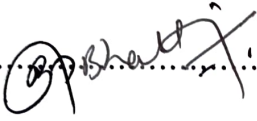
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This thesis work submitted by Bishnu Aryal entitled “**Food Habit of Greater One-Horned Rhino (*Rhinoceros unicornis*) in Babai Valley of Bardiya National Park, Nepal**” has been approved as a partial fulfillment for the requirements of Master’s Degree of Science in Zoology specializing in Ecology and Environment.

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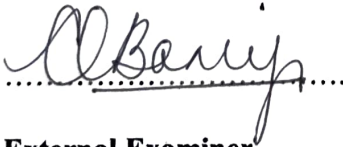
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LIST OF ABBREVIATIONS

BNP	: Bardiya National Park
B. T	: Broad Taxonomic Group
CNP	: Chitwan National Park
EN	: Endangered
F. G	: Functional Group
FO	: Frequency of Occurrence
GPS	: Global Positioning System
IUCN	: The International Union for Conservation of Nature
KNP	: Kaziranga National Park
RFO	: Relative Frequency of Occurrence

ABSTRACT

The Greater One-horned Rhino (*Rhinoceros unicornis*) is a globally vulnerable species threatened with habitat degradation and poaching. The species primarily resides in the lowlands of Nepal, in low-density but growing populations. Though being a globally threatened species, little is understood about its feeding ecology as understanding the foraging characteristics of this species is crucial for its management and conservation. The study investigated the dietary habits of the Great One-horned Rhino in Babai Valley of Bardiya National Park, Nepal, from 8-22 November 2022. The diet composition of this megaherbivore was estimated using a microhistological method based on the fecal samples. A total of 21 plant species from 15 different families were found consumed by the Rhino during the study period. The Rhino primarily feed on grasses rich in nutrients, with trees being their second preferred food followed by shrubs and forbs. The most frequently consumed plants by the Rhino included Kans (*Saccharum spontaneum*) (19.76%), Khar (*Eulaliopsis binata*) (12.76%), Siru (*Imperata cylindrica*) (9.51%), and Vellar (*Trewia nudiflora*) (7.07%). The study also found that the Graminae family accounted for the highest percentage contribution (47.24%) with a browse to grass ratio of 0.923. The standardized Levin's Measure of Niche Breadth (Bs) was calculated to be 0.528, indicating the Rhino in Bardiya National Park, Nepal, exhibit a generalized feeding strategy by consuming a diverse range of plant species from different categories, including grasses, trees, shrubs, forbs, and herbs, to meet their nutritional needs. This wider niche breadth of food items reflects their adaptable approach to acquiring necessary nutrients and to better understand the dietary habits of species like the Rhino, conducting comprehensive yearlong studies with sufficient sample sizes in Bardiya National Park and other landscapes is crucial. Additionally, evaluating the impact of ongoing grassland management on the feeding ecologies of large herbivores and implementing consistent management practices can help mitigate the negative effects of invasive species and enhance forage availability.

INTRODUCTION

1.1 Background

The Greater One-horned Rhino (*Rhinoceros unicornis*), also known as Indian Rhino is the second-largest of the five living species of Rhino around the world; Black rhinoceros (*Diceros bicornis*), White rhinoceros (*Ceratotherium simum*), Javan rhinoceros (*R. sondaicus*), Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and Greater one-horned Rhino (*R. unicornis*) (Tougard et al. 2001; Orlando et al. 2003). Rhino have a global distribution, with African rhinos (black and white) primarily found in sub-Saharan Africa, and Asian rhinos (Greater One-Horned, Sumatran, and Javan) limited to specific regions in Asia. African rhinos inhabit countries like Namibia, South Africa, Zimbabwe, and Kenya, while Asian rhinos are found in Nepal, India, Bhutan, Bangladesh, Sumatra (Indonesia), and Ujung Kulon National Park (Java, Indonesia) (IUCN 2022). At present, the *R. unicornis* is distributed in the Indian subcontinent (India and Nepal) with a population of only ~ 3600 individuals (Subedi et al. 2013; Das et al. 2015; Borthakur et al. 2016; Jhala et al. 2021). With a gradual decrease in population size throughout its present distribution range, Rhino has become extinct in Bhutan and Bangladesh (Jhala et al. 2021). The Rhino is listed as vulnerable species globally by International Union for Conservation of Nature's Red List of Threatened Species (Ellis and Talukdar 2019) and categorized as endangered by National Red List series of Nepal (Jnawali et al. 2011).

The main protected areas that currently shelter the majority of populations of Rhino are the Pobitora Wildlife Sanctuary and Kaziranga National Park in India, the Bardiya National Park and Chitwan National Park in Nepal (Jhala et al. 2021; Ellis and Talukdar 2019; Subedi et al. 2013). About 70% of the woods were destroyed during the 1960s in the Chitwan Valley alone (Dinerstein 2003). At the same time, Rhino populations in Nepal also noted a catastrophic collapse in the 1960s, when they were reduced to just 100 animals and restricted to the Chitwan Valley (Subedi et al. 2013). The Rhino population experienced a gradual increase to approximately 612 individuals by 2000 because of the implementation of strict laws and the establishment of Chitwan National Park in 1973 (Dinerstein 2003).

At present, the Rhino population in Nepal reached to 752 in numbers across different protected areas that includes Chitwan National Park and nearby forests (694), Bardiya

National Park and nearby forests (38), Shukla Phanta National Park and nearby forests (17), Parsa National Park and nearby forests (three) according to the results of the National Rhino Count 2021, (DNPWC, 2022). The count revealed an encouraging 16% increase in the population of Rhino in the Terai Arc Landscape of Nepal, showing growth from an estimated 645 individuals counted in 2015.

Feeding ecology

Mammal feeding behaviors are a key topic of study for population biology and ecology (Green 1987). One of the important steps in researching animals' ecology is to quantify their diets. Diet has an impact on species population dynamics, as it is required for species development, reproduction, and survival (Pekins et al. 1998). As the diets are crucial for animals to thrive and maintain their health, it is imperative for them to consume food that contains a sufficient amount of nutrients to fulfill their physiological needs (Hazarika and Saikia 2012). Thus, understanding herbivore diet composition is vital for regulating their effects on vegetation and ecosystems along with their feeding ecology (Barcia et al. 2007).

Fecal analysis using microhistological identification of epidermal tissues of plants in the stomach content is the most popular indirect method for identifying the diet composition of herbivores (Dusi 1949; Prince 2013). It is frequently approach, especially for some rare, endangered, and elusive wild herbivores for which it is impossible to acquire ruminal samples (Hernandez and Dunate 2007). Additionally, it is non-invasive technique, as it does not require any direct contact between focal species and the researcher. In order to identify species by their distinctive cell and structural characteristics, the microhistological procedure requires mounting a sample of pellet contents on microscope slides (Sparks and Malechek 1968).

The Rhino is primarily a grazer than a browser (Dutta et al. 2016). The species mostly prefer tall grasses, primarily *Saccharum* species (Jnawali 1995) and sedges small grasses as other sources of nourishment (Deka et al. 2003). The variations in food availability in different seasons and geographical areas might cause differences in consumption rate and food ratio (Laurie 1982). During rainy seasons, the species is mostly observed to graze on grasses whereas during dry season majority of food consists of browse species as well as tree barks (Ghosh and Das 1970).

A number of articles and investigation related to Rhino was about their population, habitat selection, habitat destruction and threats to the Rhino (Laurie 1982; Subedi et al. 2013;

Kafley et al. 2015; Aryal et al. 2017; Bhandari et al. 2022). However, relatively low proportion of study have been found on feeding ecology and diet composition of Rhino (Hazarika & Saikia, 2012; Pradhan et al., 2008). Along with this reason other important facts to conduct the study was the globally vulnerable species, missing of frequent study and a large research gap accounted in the food habit and the isolated population of Rhino in Babai Valley. The current study's purpose was to give data evidence to design an effective conservation strategy by determining the feeding patterns and food preferences of Rhino in Babai Valley of Bardiya National Park, Nepal.

1.2 Research Questions

The main research questions of this study were:

1. What types of plants do Rhino consume?
2. What can be the diet selectivity (Grass/Browse) of Greater One-horned Rhino?

1.3 Objectives

1.3.1 General objective

The general objective of the study was to investigate the Food habit of Greater One-horned Rhino in Babai Valley of Bardiya National Park, Nepal.

1.3.2 Specific objectives

1. To examine the dietary composition of Greater One-horned Rhino in Babai Valley of Bardiya National Park.
2. To investigate the diet selectivity (Grass/Browse) of Greater One-horned Rhino in Babai Valley of Bardiya National Park.

1.4 Rationale of the Study

It is important to understand the ecology of animals and their feeding habits, which includes resource use, habitat use, and competitive interactions. Food plays a critical role in an animal's survival, growth, and reproduction, and therefore, understanding its composition is crucial for managing viable populations in the wild. Furthermore, Rhino is categorized as endangered by National Red List series of Nepal (Jnawali et al. 2011). So, the effort of our study will somehow convey the importance of Rhino and their conservation. As Rhino share their homes with other valuable plants and animals, conservation of Rhino will be the conservation of other species as well. On the other hand, a large study gap on food habits of mega herbivores and an isolated population of Rhino was one of the major attractions of

the study in the study area. Identification of the feeding patterns is one of the first tasks in the conservation of this species. Realizing these realities, the study's focus is on the Rhino feeding habits in order to advance biological understanding of the species and support management strategies.

1.5 Limitations

First of all, the actual sample size for the accurate estimation was unknown. The study was conducted only in a single season. Isolated population of Rhino in Babai Valley puts low sample numbers as they were frequently found around the two stations of Babai Valley. Furthermore, collected samples needs immediate dried up, but due to lack of drying time some samples were decayed. Lastly, all the reference plant samples could not be classified to the respective categories.

2. LITERATURE REVIEW

The Rhino once inhabited the whole northern part of the Indian subcontinent, stretching from Pakistan to the Indian-Myanmar border, including the Ganges, Indus, and Brahmaputra River basins (Gee 1952; Choudhury 1985; Poudyal et al. 2009; Li et al. 2015; Puri and Joshi 2018; Talukdar 2018). However, their population and range experienced a significant decline in the early 20th century due to habitat loss caused by human activities and climatic changes (Gee 1952; Choudhury 1985; Poudyal et al. 2009; Li et al. 2015; Puri and Joshi 2018; Talukdar 2018). Presently, the Rhino distribution is confined in only some protected areas of Nepal and India, encompassing an estimated total area of 20,000 square kilometers (Pant et al. 2020). During the early 20th century, the species was on the verge of extinction, with only around 200 individuals remaining in India (Rookmaaker et al. 2016). Kaziranga National Park (KNP), which houses a significant population, had less than twenty individuals of Rhino when hunting was prohibited in 1908 (Laurie 1982). The Rhino population in KNP had a significant growth, reaching around 1800 individuals by 2006 as a result of effective conservation measures. Moreover, this increase led to their expansion into adjacent regions like Orang and Pabitora Wildlife Sanctuaries (Talukdar 2018). Similarly, in Chitwan Valley of Nepal, a thriving population of more than 1000 rhinoceroses once existed until the year 1950 but drastically declined to 60-80 individuals by 1962 due to poaching and land clearing (Laurie 1982; Dinerstein and Mccracken 1990). However, effective anti-poaching measures led to a recovery, with the population reaching approximately 600 animals in 2000 (Thapa et al. 2013). The current global population of wild Rhino is estimated to be around 3550 individuals (Rookmaaker et al. 2016; Talukdar 2018).

2.1 Microhistological analysis

Giant free-ranging herbivores' dietary data has grown massive as a tool for resource management. The microhistological method developed by (Baumgartner and Martin 1939) has been widely used over the past seven decades to ascertain the botanical component of herbivore diets. The microhistological method given by Norbury (1988) is another highly used method now a days as this one seems to be less time-consuming, laborious, and the slides were clearer and easier to distinguish in comparison to the first method. The microhistological method involves collecting samples of the food, which can be from feces, the rumen or esophagus of fistulated animals, or the intestinal digesta of deceased animals.

While fossilized fecal samples dating back more than 10,000 years have been utilized to evaluate animal diets, fresh pellets provide the most dependable outcomes in determining dietary preferences (Hansen 1978). Using reference slides, microphotographs, or hand-drawn illustrations as a guide, it is possible to identify vegetative fragments in samples (Johnson et al. 1983).

Microhistological analysis has been employed to ascertain the botanical composition of the diets of various mammalian families, such as Cervids (e.g., deer, elk, and moose), Leproids (e.g., rabbits and hares), (O'Bryan 1983; Kirchhoff and Thomson 1998) and Equids (e.g., wild horses), (Hansen et al. 1977). This method has been used in Nepal to analyze the diets of various animals, including the rhinoceros (Sr 1995; Pradhan et al. 2008), Asian elephant (Steinheim et al. 2005; Pradhan et al. 2008), Gaur (Chetri 2006) , Barking deer (Nagarkoti and Thapa 2007), Wild sheep (Shrestha et al. 2005), Mountain domestic and Wild ungulates (Shrestha et al. 1997).

2.2 Food habit of Greater One-horned Rhino

Large herbivores, whose population is hardly regulated by natural predation, probably have limited food resources (Sinclair 1975; Owen-Smith 1988; Sukumar 1992). Megafauna like elephant and Rhino consume variety of foods, and their seasonal food consumption varies significantly depending on the species (Laurie, 1982; Sukumar 1992; Williams 2003). According to a study conducted by Pradhan et al. (2008), which focused on the feeding habits of two endangered mega herbivores, the Asian elephant (*Elephas maximus*) and Rhino in lowland Nepal, it was observed that *S. spontaneum*, a tall grass, constituted 18.5–31.5% of the Rhino diet across all three seasons. Both Elephant and Rhino frequently consumed grasses such as, *Desmostachya bipinnata*, *I. cylindrica*, *Arundo donax*, *Narenga porphyrocoma* and *S. bengalensis*. Additional grasses that served as significant food sources for rhinos included *Themeda arundinacea*, *Chrysopogon zizanioides*, *Cynodactylon*, and various species of *Cymbopogon*. Browsing species consumed by Rhino encompassed *Mallotus*, *Dalbergia sissoo*, *Calamus tenuis*, *Ehretia laevis*, *Bombax ceiba* and *Callicarpa macrophylla*. Similarly, in a study conducted by (Hazarika and Saikia 2012) in Rajiv Gandhi Orang National Park, it was found that the Indian Rhino's diet consisted of nine aquatic plant species, 75 grass species, 27 herb-shrub species, and 27 tree species. Among the top 10 food plants were *Hemarthria compressa* (11.63%), *Hymenachne pseudointerrupta* (10.64%), *Leersia hexandra* (8.00%), *Arundo donax* (6.38%),

Chrysopogon aciculatus (4.60%), *Phragmites karka* (4.42%), *Brachiaria ramosa* (3.83%), *Cynodactylon* (2.11%), and *S. spontaneum* (2.0%).

The diet composition of Rhino in the Babai Valley, as revealed by (Wegge et al. 2006) in their study on the dry season diets of sympatric ungulates in lowland Nepal, consisted mainly of graminoids (45.5%), followed by woody plants (33%) and other plants (3%). The study identified 11 species of woody plants, 10 species of graminoids, and 1 species of herb in the Rhino's diet. The primary source of fodder was *S. spontaneum*, accounting for 18.5% of the diet. The only herb species detected in the Rhino's diet was *Cirium wallichii*, comprising 3% of the overall diet.

According to studies, one of the main obstacles to maintaining Rhino conservation is the lack of enough habitat (Kafley et al. 2015; Ellis and Talukdar 2019). Due to the encroachment of woodland, the invasion of alien species into grasslands, and the silting up of wetlands, Chitwan National Park (CNP) in Nepal has seen a dramatic loss in both the quantity and quality of Rhino habitat (CNP 2013, 2016). The expansion of plant species such as Mikania (*Mikania micrantha*) has raised significant concerns due to its adverse impact on the local plant community and the subsequent reduction in the availability of food, particularly grasses, for Rhino populations. This issue has been addressed in recent studies conducted by (Lahkar et al. 2011; Murphy et al. 2013; Khadka 2017). The study in riverine forest of CNP revealed that with increase in *M. micrantha* cover percent species richness was decreased. It was also revealed that with increase in tree crown cover and distance from forest edge invasion of *M. micrantha* decreased (Shrestha and Dangol 2014; Baidar et al. 2017) found that in CNP the land cover of riverine forest was most affected with 85.98% of presence points and Sal Forest was least affected by invasion of *M. micrantha*. The authors also showed agricultural land as potential habitat for weed, although it was less affected due to human intervention and removal of weed. In Kumroj buffer zone, negative effect of *M. micrantha* on regeneration of major tree species was indicated such that in non-invaded areas substantial numbers of regeneration of tree species were found as opposed to the invaded area with few regenerations of tree species (Ulak et al. 2016). Similar study in sal forest of CNP revealed that germination and growth of saplings and seedlings were restricted by *M. micrantha* leading to less plant diversity in invaded area (Basnet et al. 2016). They found that density of seedlings in non-invaded area was almost six times more than in invaded area which made the conclusion that *M.*

micrantha coverage decreases the density of seedlings and saplings of tree species by restricting growth and germination of seedlings.

Growing invasion by invasive alien plant species in Nepal has seriously altered the feeding ecology and preference of wild herbivores. This is even more evident in case of large herbivores like Rhino and Elephant. Such scenario is a growing issue across the protected areas of Nepal like CNP (Lahkar et al. 2011; Murphy et al. 2013; Khadka 2017).

In conclusion, Rhino-related articles and investigations have predominantly focused on topics such as rhino population, habitat selection, habitat destruction, and threats to their survival (Laurie 1982; Subedi et al. 2013; Kafley et al. 2015; Aryal et al. 2017; Bhandari et al. 2022) . However, there has been a comparatively smaller amount of research dedicated to studying the feeding ecology and diet composition of rhinos (Pradhan et al. 2008; Hazarika & Saikia 2012). As Babai Valley has an isolated population of Rhino with only eight individuals in two groups (DNPWC 2022). Most of the dietary study of Rhino in BNP are concentrated in Karnali floodplain and very little study has been done in Rhino of Babai Valley. This study will help to fulfill this gap. Nevertheless, the proportion of research specifically addressing the feeding ecology and diet composition of rhinos remains limited.

3. MATERIALS AND METHODS

3.1 Materials used for sampling

Different equipment and chemicals were used for the completion of the proposed study.

- | | |
|---|-------------------------------|
| a. GPS (Garmin eTrex 7) | i. Compass (Silva) |
| b. Binoculars | j. Zip-lock bags |
| c. Silica gel | k. Herbarium sheets |
| d. Stationary materials (pen, pencil, papers) | l. Camera for photographs |
| e. Compound Microscope (Proway) | m. Sodium Hypochlorite (4%) |
| f. Distilled water | n. Petri discs |
| g. Slides and coverslips | o. Dropper, spatula and brush |
| h. Mesh sieve (1mm and 0.3mm) | p. Gentain violet |

3.2 Study area

3.2.1 Geographic location

Bardiya National Park (28.3649° N and 81.5596° E) is situated in the lowland region of Tarai. It spans a vast area of 968 km², making it largest national park in that area. The park situated at Western Tarai region of Nepal was created a reserve with the purpose to preserve the tiger and its prey species' habitat. The enchanting Babai Valley offers a captivating experience where visitors can witness rhinos, tigers, and elephants thriving in their native environment.

The study was conducted in the Babai Valley, located in the southeast of Bardiya National Park (28°23'0"N, 81°30'0"E). The enchanting Babai Valley offers a captivating experience where visitors can witness rhinos, tigers, and elephants thriving in their native environment. The Babai Valley is surrounded by the Churia range, also known as the Siwalik Hills. The park experiences three seasons; monsoon, winter and autumn, with a subtropical monsoonal climate. The temperatures in the region vary throughout the year, with an average monthly low of 10°C in January and a high of 45°C in June. The monsoon season, from June to September, brings the most rainfall, while the period from October to early

June remains dry with warm days and cool nights, (DNPWC, 2022).

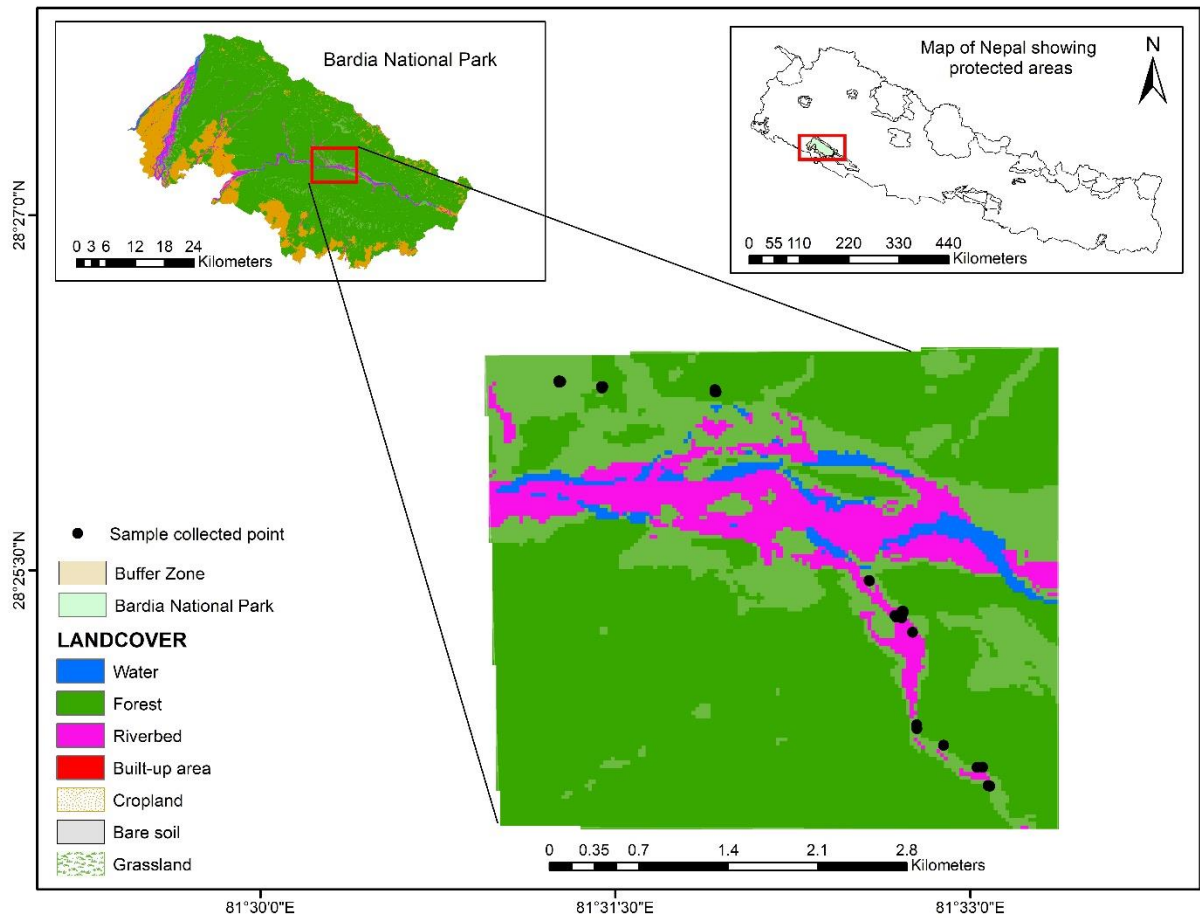


Figure 1. Study area map showing Babai Valley of Bardiya National Park, Nepal

3.2.2 Vegetation

The Babai River and its tributaries create a diverse floodplain landscape with different stages of succession, resulting in a mosaic-like pattern. The higher, drier areas are characterized by extensive forests dominated by Sal trees (*Shorea robusta*), representing mature and stable ecosystems. BNP is composed of two primary ecoregions: Tarai-duar Savannas and Grasslands, and Himalayan Subtropical Broad Leaved Forests, according to (Malla 2009). The park is predominantly covered by forests, accounting for 76% of the area. Trees make up 52%, shrubs 20%, and herbs 8% of the vegetation, as reported by Dinerstein (1979) and Bhujju et al. (2006). The park's vegetation was initially classified into six main types, later expanded to seven types by (Sr 1993), including Sal forest, mixed hardwood forest, forested grasslands, Riverine forest, tall alluvial Floodplain grassland, Phantas and other vegetation types. Notable

flora within the park includes *S. robusta*, *Dillenia pentagyna*, *Mallotus phillippensis*, *Terminalia tomentosa*, *Dalbergia sissoo*, *Bombax ceiba*, *Pinus roxburghii*, *Colebrookea oppositifolia*, *Pogostemon benghalensis*, *Imperata cylindrica*, *Buchanania latifolia*, *Acacia catechu*, *Murraya koenigii*, and various species of *Saccharum* (Shrestha et al. 1997).

3.2.3 Fauna

BNP hosts a diverse range of wildlife, with over 30 mammal species including the Royal Bengal Tiger (*Panthera tigris tigris*), Asian Elephant (*Elephas maximus*), Greater One-horned Rhino (*R. unicornis*), Swamp Deer (*Cervus duvauceli*), Blackbuck (*Antelope cervicapra*), Gharial Crocodile (*Gavialis gangeticus*) and Marsh Mugger Crocodile (*Crocodylus palustris*), and Gangetic dolphin (*Platanista gangetica gangetica*), more than 230 bird species including endangered bird species such as the Bengal Florican (*Houbaropsis bengalensis*), Lesser Florican (*Sypheotides indicus*), and Sarus Crane (*Grus Antigone*) and various snakes, lizards, and fish species. Furthermore, the park also attracts migratory birds in addition to its resident species (DNPWC2022).

3.3 Methods

3.3.1 Sampling design

As the Rhino population of Babai Valley is an isolated one, the species were frequently found only around two stations, the Thulo-shree station and the Guthi station. On the other hand, Rhino are latrine animals (Laurie 1982), means they used to defecate on same site for a longer time. So, due to their isolated population and frequently available on same area, the Rhino dung samples were collected as per the guidance of the game scout team and the army patrolling team of the respective stations. Along with this, 10m ×10m quadrat was drawn keeping the dung sample as a center point. All plants that present under the quadrat were taken as the reference sample for the later confirmation of diets. The GPS location of every sample point was also taken into consideration.

3.3.2 Field survey

Fecal samples were collected in the winter season from 8-22 November 2022. Field survey started from Chepang Valley and ends at Parewaodar camp. Four persons including a staff of the BNP were included for the sample collection. Nine stations

inside the Babai Valley were visited thoroughly but the samples were found on only two stations.

3.3.3 Microhistological analysis

The microhistological technique, first introduced by Baumgartner and Martin (1939), was used to analyze the plant composition of Rhino fecal matter. This method relies on microscopic identification of undigested plant fragments, particularly epidermal features that are indicative of specific plant groups. The process involves preparing reference and fecal slides and interpreting their findings, as described by Metcalf (1960). It is particularly valuable for quantifying diets, as it avoids the need to harm or kill animals (Sparks & Malechek, 1968).

3.3.4 Sample collection

Fresh dung samples, less than two days old, identified on the basis of texture and moisture content were collected in paper bags to avoid moisture and labeled with GPS location, date and status of the sample. The samples were air dried to remove moisture and to prevent fungal growth.

Parts of potential food plants that could be reached by the Rhino for feeding and encountered into the quadrat during survey were collected for the preparation of reference slides. The plant species were labeled with their local name. All the collected plant materials were identified as possible with the help of various literature (Dangol and Shivakoti 2001; NTNC-BCC and CNP 2020) and collected reference sample from the field were brought to National Herbarium and Plant Laboratories, Lalitpur, for further identification and confirmation.

3.3.5 Slide preparation

The method introduced by Norbury (1988) was adopted to prepare the microhistological slides. The plant samples were identified up to species level and then dried in the oven at 60 °C for 48- 72 hours in the laboratory of the Central Department of Zoology. The dried samples were powdered separately through pestle and mortar and the powder was sieved in mesh of size 1 mm and 0.3 mm. The powder remained on the 0.3 mm sieve was chosen as final reference sample for slide preparation. The same procedure was followed for fecal samples. Each 0.5 gm of powdered sample was taken in a petri dish and bleached with 50 ml of 4% Sodium hypochlorite for 6-24 hours at

room temperature to remove mesophyll tissues and to render the epidermis identifiable. The bleached contents were then rinsed with distilled water thoroughly in a sieve and then treated with few drops of staining substance-gentian violet solution for 10 seconds and again well rinsed. The stained fragments were mounted on standard microscope slides in a glycerin medium and covered with a cover slip (Figure 2). Both reference slides and fecal slides were observed immediately after preparation at different magnifications; 10X and 40X with a compound microscope and each fragment were photographed using phone camera.

3.3.6 Slide interpretation

First the key features of the reference plants such as structure, shape, size and arrangement of epidermal cells, stomata, vascular vessels, trichomes, etc. were photographed through 10X and 40X microscope. Then for each fecal sample, non-overlapping and distinguishable 30 fragments were observed moving the slide from right to left in the microscope. Each fragment of the fecal sample slide was identified by comparing it with the reference plants photographs. Plants that could not be categorized into specific species or genera were grouped as unidentified. For the reference library, 1230 microhistological images were selected, each showing characteristics of 61 plant species. The microhistological properties of the reference plants' key diagnostic traits were categorized (Annex-II) to make it simple to identify the plants consumed.

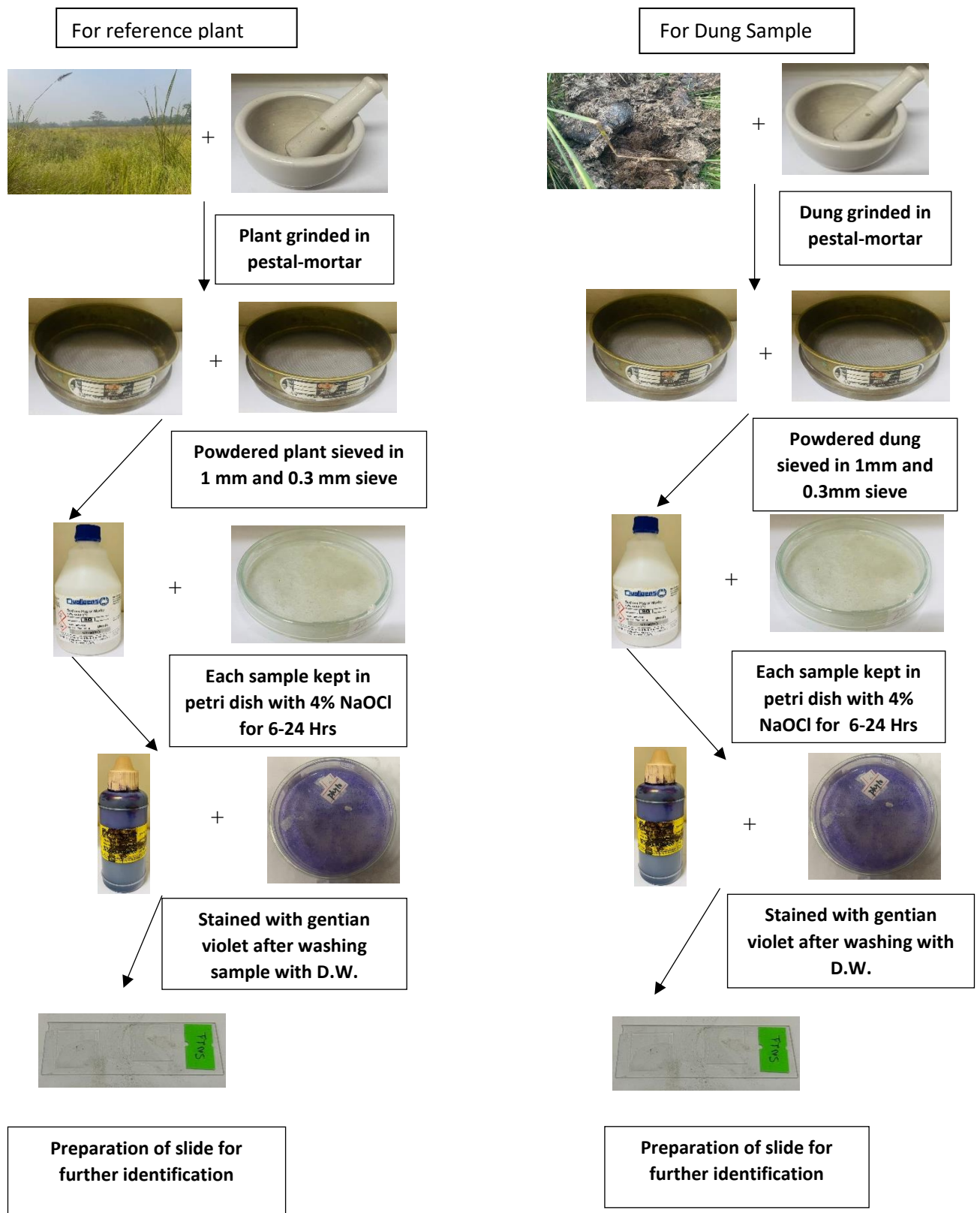


Figure 2. Flow chart of Slide preparation for microhistological analyses.

3.4 Data analysis

Plant fragments were categorized into different (1) Functional groups (grasses, shrubs, trees, forbs, and climbers) (2) Broad taxonomic group (monocots and dicots) (3) family and (4) species. MS Excel was used for data analysis.

3.4.1 Frequency of occurrence

Frequency of occurrence provides information about how many times the same events occur into the total sample unit. Frequency of occurrence was calculated as,

$$\text{Frequency of occurrence} = \frac{\text{number of times the same fragments occur}}{\text{total number of sample unit}}$$

3.4.2 Relative frequency of occurrence

Relative frequency of occurrence was expressed into percentage of occurrence (O %) Cavallini and Lovari (1991).

$$\text{Percentage of Occurrence (O \%)} = \frac{\text{number of fragments of each food}}{\text{Total number of plant fragments read}} \times 100\%$$

3.4.3 Niche Breadth

Levin's measure of Niche Breadth (Levins 1968), as described by Krebs (1999), which assesses how consistently resources are being utilized, was used to assess the degree of selectivity of plant species included in the diet.

The equation is.

$$B = \frac{1}{\sum_{i=1}^n p_i^2}$$

Where, B= c

p_i = Percentage of total samples belonging to species i ($i= 1, 2, \dots, n$)

n = total number of plant species in all samples.

Diversity was standardized to a scale of 0.0 to 1.0 by using Hurlbert's method (Krebs 1999).

$$B_s = \frac{B-1}{n-1}$$

Where, B_s = Levins's standardized niche breadth, and n is the number of possible resource states.

A value of 1 indicates generalist taxa that are similarly preferred by the rhino, whereas a value of 0 suggests specialist taxa that prefer specific fodder (Levins 1968).

3.4.4 Browse to grass ratio

Plants were further grouped into grass and browse to determine whether the rhino predominantly eats grass or browse, following the methods of Jarman (1974) and Shipley (1999). The ratio of these two types of plants in the rhino's diet was then calculated.

$$\text{Browse to grass ratio (B/G)} = \frac{\sum(Bp)}{\sum(Gp)}$$

Where; B_p = Percent occurrence of all browse plant species in the diet

G_p = Percent occurrence of all grass plant species in the diet

4. RESULTS

4.1 Diet composition

The diet of Rhino comprised of a wide variety of food plants. Twenty-seven plant species (six grasses, six trees, four shrubs, four forbs, one fern and seven unknown species) were recorded (Annex-I). However, the majority (more than 60%) of the diet's volume came from just seven species, (three grass species; *S. spontaneum*, *I. cylindrica*, *Eulaliopsis binata* and four browse species; *Ageratum conyzoides*, *S. robusta*, *Clerodendrum viscosum* and *Trewia nudiflora*. On an average grass species was found to be dominant over browse species. But the diverse composition of the food demonstrates that the Rhino are both grazers and browsers.

4.2 Relative frequency of occurrence

Microhistological analysis of 1230 plant fragments from 41 dung samples collected throughout the field survey (n=41) led to the identification of 21 plants from 15 distinct family groups in the dung samples of Rhino (Table 1).

Among the identified food plants Kans (*S. spontaneum*) accounted the highest percentage of relative frequency (19.76%) followed by Khar (*Eulaliopsis binata*) (12.76%), Siru (*I. cylindrica*) (9.51%), Vellar (*Trewia nudiflora*) (7.07%) which were the dominant food plants found whereas Kadam (*Adina cordifolia*) (0.89%), Rudilo (*Pogostemon benghalensis*) (0.81%), Gandhe jhar (*A. conyzoides*) (0.41%) contributed the least RFO value. Likewise, in case of frequency of occurrence as well; Kans (*S. spontaneum*) accounts the highest frequency of occurrence (5.93) while Gandhe jhar (*A. conyzoides*) (0.12) shows the least (Table 1).

Table 1. Categorization of the plant fragments into their 1; local name, 2; Scientific name, 3; Family, 4; Functional Category, 5; Broad Category, 6; FO value and 7; RFO% found in the dung samples of Rhino.

SN	Local name	Scientific name	Family	Functional category	Broad category	FO	RFO (%)
1	Kans	<i>Saccharum spontaneum</i>	Gramineae	Grass	Monocots	5.93	19.76
2	Siru	<i>Imperata cylindrica</i>	Gramineae	Grass	Monocots	2.85	9.51
3	Khar	<i>Eulaliopsis binata</i>	Gramineae	Grass	Monocots	3.83	12.76
4	Dhaddi	<i>Themeda triandra</i>	Gramineae	Grass	Monocots	1.00	3.33
5	-	<i>Cyperus</i> sp.	Cyperaceae	Grass	Monocots	0.93	3.09
6	-	<i>Themeda</i> sp.	Poaceae	Grass	Monocots	1.10	3.66
7	Vellar	<i>Trewia nudiflora</i>	Euphorbiaceae	Trees	Dicots	2.12	7.07
8	Sadan	<i>Eugenia</i> sp.	Myrtaceae	Trees	Dicots	1.37	4.55
9	Khanyeu	<i>Ficus semicordata</i>	Moraceae	Trees	Dicots	1.46	4.88
10	Kadam	<i>Adina cordifolia</i>	Rubiaceae	Trees	Dicots	0.27	0.89
11	Sal	<i>Shorea robusta</i>	Dipterocarpaceae	Trees	Dicots	0.61	2.03
12	Jamun	<i>Syzygium cumini</i>	Myrtaceae	Trees	Dicots	0.41	1.38
13	Dhairo	<i>Woodfordia fruticosa</i>	Lythraceae	Shrubs	Dicots	0.56	1.87
14	Rudilo	<i>Pogostemon benghalensis</i>	Labiatae	Shrubs	Dicots	0.24	0.81
15	Banmara	<i>Eupatorium adenophorum</i>	Oleaceae	Shrubs	Dicots	1.05	3.50
16	Bhati	<i>Clerodendrum viscosum</i>	Verbenaceae	Shrubs	Dicots	0.78	2.60

17	Gandhe jhar	<i>Ageratum conyzoides</i>	Asteraceae	Forbs	Dicots	0.12	0.41
18	Ilame jhar	<i>Ageratum sp.</i>	Asteraceae	Forbs	Dicots	1.00	3.33
19	-	<i>Justicia sp.</i>	Acanthaceae	Forbs	Dicots	1.76	5.85
20	Dubo	<i>Cynodondactylon</i>	Gramineae	Forbs	Monocots	0.56	1.87
21	Unyo	<i>Drynaria mollis</i>	Polypodiaceae	Herbs	-	0.56	1.87

In terms of plant types, grass represented the largest share (52%) in the diets of Rhino, followed by trees (18%), shrubs (12%), and forbs (10%). The diets' lowest proportion of occurrence for herbs was (3%). Here, those plants which were unidentified listed under unknown groups which contributed (5%) (Figure 3).

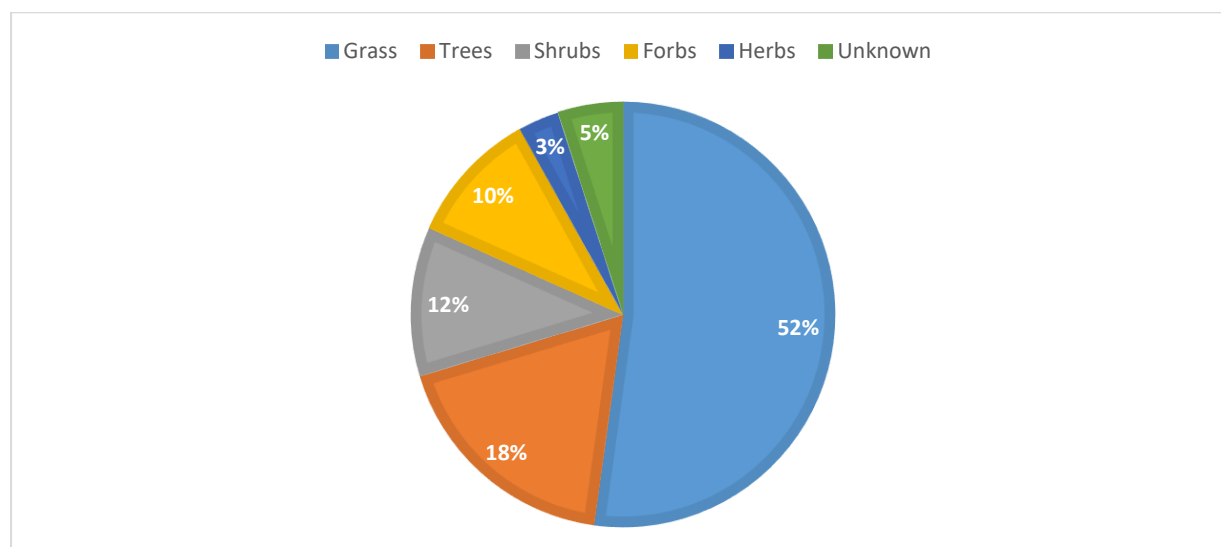


Figure 3. Number of species in different functional categories consumed by Rhino in BNP, Nepal. In Babai Valley, Rhino was observed to consume 15 distinct plant families (Table 1). Gramineae family had highest contribution and Acanthaceae had lower contribution on Rhino's diet. (Figure: 4).

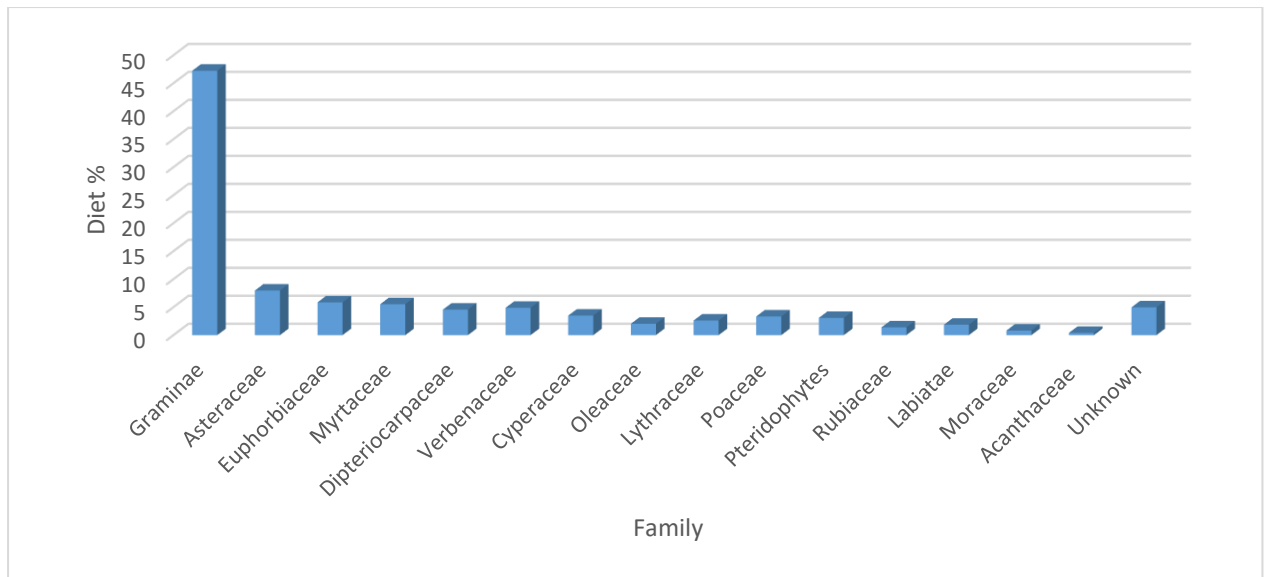


Figure 4. Relative frequency of occurrence of different plant families in the diet of Rhino in BNP, Nepal.

Native plant species had a higher contribution to the Rhino's diet compared to invasive species. Species that originated and developed in its surrounding habitat and had adapted to living in that particular environment were generally native species whereas species of plant that outcompetes other species, causing damage to an ecosystem were invasive species (Phinney 2021). Mean occurrence of invasive species was (2.952) whereas (27.1) for native species. The maximum and minimum number of observations in a sample for invasive species ranged from 0 to 8 whereas it was 22 to 30 for native species. This suggests that invasive species are not as significant in the diet of Rhino as compared to native species, which are more commonly consumed by Rhino. These findings highlight the importance of protecting and conserving native species that are essential to the Rhino's diet (Figure 5).

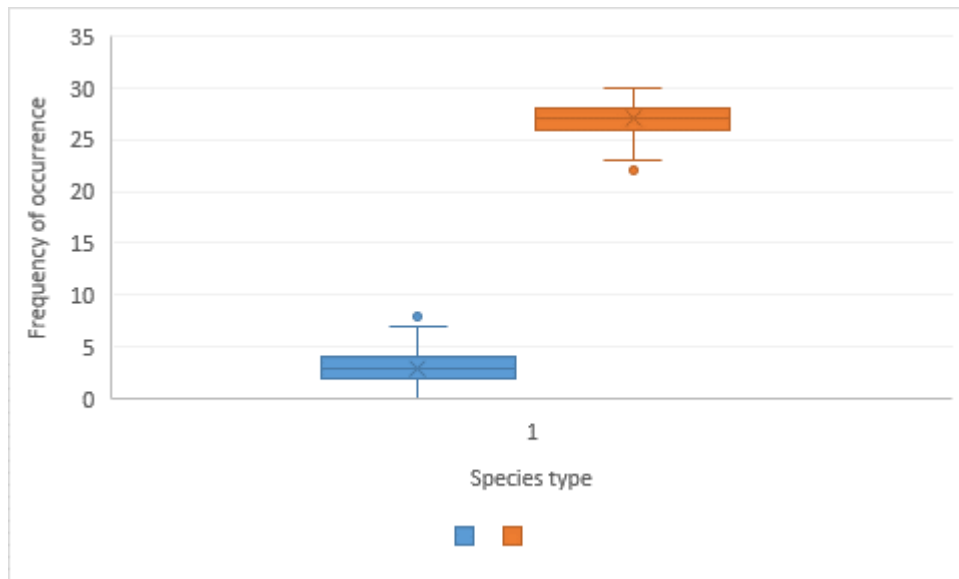


Figure 5. Boxplot demonstrating number of plant species occurrence per sample

In terms of diet contribution in percentage, the invasive species shares 7.24% whereas the native species represents the largest share of 92.76%. This result conveys that the invasive species has not overtook the Rhino's diet but has started to show up (Figure 6).

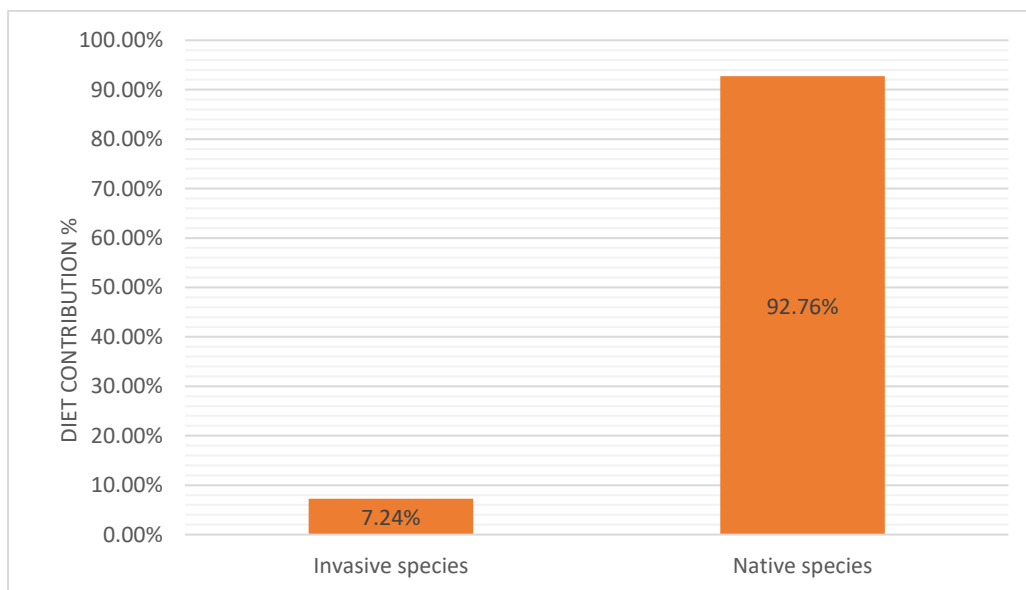


Figure 6. Diet contribution of two different species in terms of percentage

4.3 Niche Breadth

Levin's Measure of Niche Breadth (B_s) for the food plants in the Rhino's diet, was found to be 0.528. This value suggests that the Rhino demonstrates a diverse feeding behavior by consuming a broad variety of forage plants, indicating a generalized feeding pattern (Table 2).

Table 2. Niche Breadth (Bs) of plant species identified in dung of Rhino in BNP, Nepal.

S.N	Scientific name	Total count	Pi	Pi ²
1	<i>Saccharum spontaneum</i>	243	0.20787	0.04321
2	<i>Imperata cylindrica</i>	157	0.134303	0.018037
3	<i>Eulaliopsis binata</i>	117	0.100086	0.010017
4	<i>Themeda triandra</i>	41	0.035073	0.00123
5	<i>Cyperus</i> sp.	43	0.036784	0.001353
6	<i>Themeda</i> sp.	41	0.035073	0.00123
7	<i>Trewia nudiflora</i>	72	0.061591	0.003793
8	<i>Eugenia</i> sp.	23	0.019675	0.000387
9	<i>Ficus semicordata</i>	10	0.008554	7.32E-05
10	<i>Adina cordifolia</i>	17	0.014542	0.000211
11	<i>Shorea robusta</i>	56	0.047904	0.002295
12	<i>Syzygium cumini</i>	45	0.038494	0.001482
13	<i>Woodfordia fruticosa</i>	32	0.027374	0.000749
14	<i>Pogostemon benghalensis</i>	23	0.019675	0.000387
15	<i>Eupatorium adenophorum</i>	25	0.021386	0.000457
16	<i>Clerodendrum viscosum</i>	60	0.051326	0.002634
17	<i>Ageratum conyzoides</i>	87	0.074423	0.005539
18	<i>Ageratum</i> sp.	11	0.00941	8.85E-05
19	<i>Justicia</i> sp.	5	0.004277	1.83E-05
20	<i>Cynodondactylon</i>	23	0.019675	0.000387
21	<i>Drynaria mollis</i>	38	0.032506	0.001057
22	Total	1169	1	0.094637
	Levin's Breadth measure (B)			10.566
	Niche Breadth (1%) (Bs)			0.528

4.3 Browse to grass ratio

The Rhino consumed seven species of grass and 14 species of browse. Despite of 14 browse species, the browse to grass ratio was only 0.923, indicating higher preference towards grass species. Furthermore, grass species contributed 52% of the diet, while browse species reports 48% relatively (Figure 6).

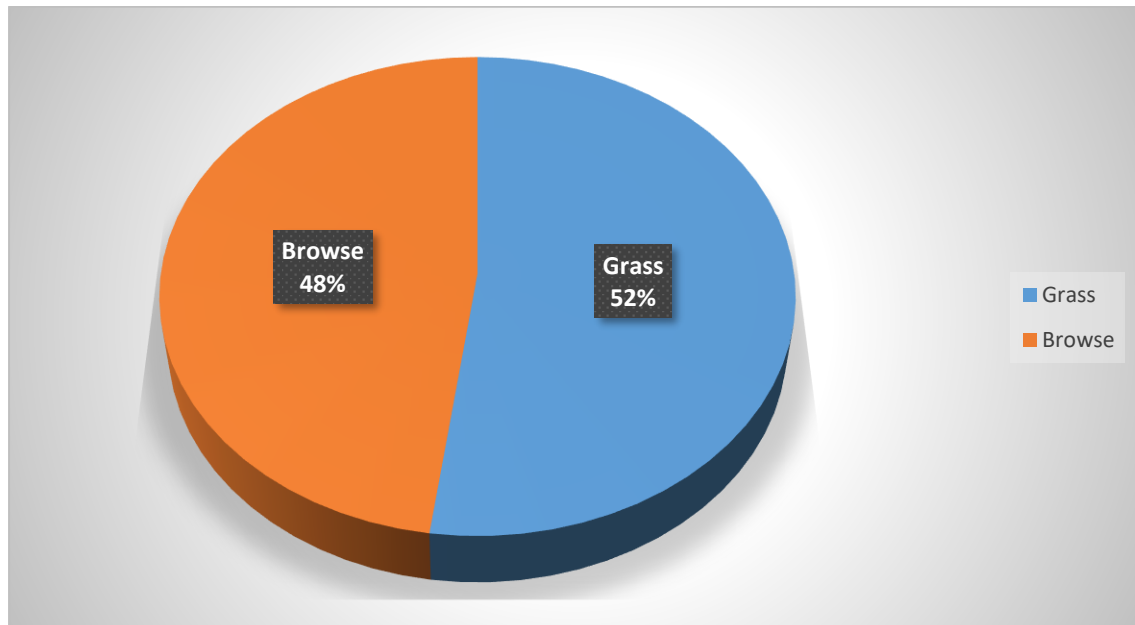


Figure 6. Pie-chart showing percentage of grass and browse consumed by Rhino in BNP, Nepal.

5. DISCUSSION

The study reported that Kans (*S. spontaneum*) was the most preferred grass species among Rhino, exhibiting a higher relative frequency compared to other available food sources. This was supported by the study performed by Pradhan et al. (2008), Dinerstein (1979), Lehmkuhl (1990) which showed *S. spontaneum* the major diet of Rhino. It might be due to its unusual ability to sprout all year long (Dinerstein 1979; Lehmkuhl 1990), as well as its large standing biomass (Jnawali 1995). A decline in the quality of their diet may have contributed to their decision to eat more grass. The initial rain of the pre-monsoon season encourages the growth of new grass, and monocots' intercalary meristem growth provides more nutrient-rich food than browsing plants' apical growth (Jarman 1974). The consumption of browse species than grasses was found to be higher in the winter season (Ghosh and Das 2007). It may be due to the high availability of *Trewia nudiflora* commonly known as Rhino apple during that season (Jnawali 1995).

Grass species were found to be the more dominant food source of rhino according to our results. Similarly, the study conducted by Jarman (1974), also found the grass species were the primary food source for Rhino. This indicates that Rhino heavily relies on grass species for their nutritional needs. The abundance of grass species can be attributed to the availability of rain, which facilitates the growth of grass. In addition, significant concentration of grasses near riverbanks and grasslands serves as major sources of food for Rhino Hazarika and Saikia (2012). Three grass species (*S. spontaneum*, *Eulaliopsis binata*, *I. cylindrica*) and four browse species (*T. nudiflora*, *Justicia* sp., *Ficus semicordata* and *Eugenia* sp.) are the major diets contributors of total diet consumed. This shows the staple food list of Rhino in Babai Valley of BNP. Similar study in Chitwan and Bardiya by Jnawali (1995) documented grass as the key food sources for Rhino in the area. This might be due to the high palatability of those species (Jarman 1974).

This study reports that Rhino mostly feeds on Gramineae family than others in general. This is particularly due to samples collected during just after monsoon and monsoon grasses contain more nutrients than grasses in other seasons (Sukumar 1992), whereas other seasons' grasses tend to become extremely rough and low in nutrients. However, it contradicts with Ghosh and Das (1970) in which out of 57 plants, the majority of species come from the Poaceae family, indicating that this gigantic herbivore favors grasses in

general. This might be because Rhino relies on a significant concentration of fermentable fibers in their diet to meet its nutritional needs. The prolonged retention time results in a highly effective fermentation process of cellulose. As a result, grasses are good fodder for Rhinos (Owen-Smith 1988).

A total of three invasive species were recorded in the diet of Rhino. *Ageratum* sp., *Eupatorium adenophorum* and *A. conyzoides* were the consumed invasive species fodder by Rhino in Babai Valley. This occurrence could be attributed to the relatively lower invasion of foreign plant species in western Nepal when compared to the eastern region Murphy et al. (2013). However, in certain areas, it has been observed that invasive species like *Mikania* make up a significant portion of the Rhino's diet. In riverine forest, these invasive species can get adequate amount of moisture, sunlight, and support for climbing which explains its high presence (Baidar et al. 2017). Similar findings were observed in Sal community forest of east where some percentage of the study area was invaded by *Mikania* (Zou and Greenberg 2019). It was also observed that areas with low or sparse understory plants had low *Mikania* presence because high shrub population provides sufficient environment for its establishment and growth (Shrestha and Dangol 2014).

The value of Standardized Levin's Measure of Niche Breadth showed the diet resource used by Rhino were generalist or near about similarly preferred across the environment where they live presently. This may be due to low food availability and poor quality of forage as well as the interplay between body mass and the extent of the individual home range (Huang et al. 2021).

The ratio of browse to grass revealed a strong inclination towards grass plants. This might be because of the growth of new grasses due to monsoon rain which provides more nutrient-rich food than browsing plants and also due to high palatable grass species (Jarman 1974). A similar study by Chetri (2006) showed the browse to grass ratio peaked in January and gradually declined, reaching its lowest point in June. This can be attributed to two main factors: Firstly, the scarcity of moisture in the substrate during January restricts the growth of nutrient-rich grass until the pre-monsoon rains. Secondly, after the pre-monsoon rains, fresh and succulent shoots of *I. cylindrical*, *V. zizanoides*, *P.karka*, and *Themeda* sp. become available for consumption (Chetri 2006).

According to the study, a certain percentage of the plant fragments were unidentified which could be due to almost complete degradation of ingested plant parts. A similar study by

Chetri (2006) shows a relatively low percentage of the fragments in this investigation were unidentified. According to Jarman (1974), animals were observed nibbling on fresh shoots since they digest more easily than mature plants. The accuracy of microhistological analysis can be affected by several factors, including the way samples are prepared, the level of training of the technicians involved, and the varying digestibility of different components of the diet. (Berwick 1974; Vavra and Holecek 1980; Holecek et al. 1982; Sharma et al. 2005; Baskaran et al. 2011).

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The Rhino in Bardiya National Park, Nepal, consumed 21 plant species from 15 different families to supplement their nutrition. They are selective in their feeding, choosing a mixture of different plant categories in varying proportions. The Rhino primarily feed on grass plant types that are rich in nutrients, with trees being their preferred food followed by shrubs forbs and herbs. The Rhino adopts a generalized feeding strategy to fulfill their nutritional requirements, as evidenced by their wider niche breadth of food items in their diets. Kans (*S. spontaneum*) was the most frequently consumed plants by the rhino which accounts the highest percentage of Relative frequency followed by Khar (*Eulaliopsis binata*), Siru (*Imperata cylindrica*), Vellar (*Trewia nudiflora*), while Kadam (*Adina cordifolia*), Rudilo (*Pogostemon benghalensis*) and Gandhe jhar (*A. conyzoides*) shows least RFO respectively. The Gramineae family was found to be the dominant component of the diet, comprising a significant proportion, while the browse to grass ratio indicated a preference for grazing over browsing. These findings highlight the Rhino's ability to adapt to different plant sources and emphasize the importance of grasses, in their diets.

6.2 Recommendations

The following recommendations are made in consideration of this investigation:

1. Bardiya National Park and other landscapes should also host year-long study on the species dietary habits with sufficient sample sizes.
2. Concerning its effect on the feeding ecologies of large herbivores like Rhino, the ongoing phatas (grassland) management to expand grassland area should be evaluated.
3. Consistent management of grassland should be done in order to reduce the negative effect of invasive species and increase forage availability.

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8. ANNEXES

Annex. I. Plants collected from Babai Valley of Bardiya National Park for reference library preparation.

S.N	Local name	Scientific name	Family	Functional category	Broad category
1	Kans	<i>Saccharum spontaneum</i>	Gramineae	Grass	Monocots
2	Siru	<i>Imperata cylindrica</i>	Gramineae	Grass	Monocots
3	Khar	<i>Eulaliopsis binata</i>	Gramineae	Grass	Monocots
4	Dhaddi	<i>Themeda triandra</i>	Gramineae	Grass	Monocots
5	-	<i>Cyperus</i> sp.	Cyperaceae	Grass	Monocots
6	-	<i>Themeda</i> sp.	Poaceae	Grass	Monocots
7	Bhellar	<i>Trewia nudiflora</i>	Euphorbiaceae	Trees	Dicots
8	Sadan	<i>Eugenia</i> sp.	Myrtaceae	Trees	Dicots
9	Khanyeu	<i>Ficus semicordata</i>	Moraceae	Trees	Dicots
10	Kadam	<i>Adina cordifolia</i>	Rubiaceae	Trees	Dicots
11	Sal	<i>Shorea robusta</i>	Dipterocarpaceae	Trees	Dicots
12	Jamun	<i>Syzygium cumini</i>	Myrtaceae	Trees	Dicots
13	Dhairo	<i>Woobfordia fruticose</i>	Lythraceae	Shrubs	Dicots
14	Rudilo	<i>Pogostemon benghalensis</i>	Labiatae	Shrubs	Dicots
15	Banmara	<i>Eupatorium adenophorum</i>	Oleaceae	Shrubs	Dicots
16	Bhati	<i>Clerodendrum</i>	Verbenaceae	Shrubs	Dicots
17	Gandhejhar	<i>Ageratum conyzoides</i>	Asteraceae	Forbs	Dicots
18	Ilamejhar	<i>Ageratum</i> sp.	Asteraceae	Forbs	Dicots
19	-	<i>Justicia</i> sp.	Acanthaceae	Forbs	Dicots

20	Dubo	<i>Cynodondactylon</i>	Gramineae	Forbs	Monocots
21	Unyo	<i>Drynaria mollis</i>	Polypodiaceae	Hetbs	-
22	Datiwan	<i>Achyranthes aspera</i>	Amaranthaceae	Herb	Dicots
23	Titepati	<i>Artemisia dubia</i>	Asteraceae	Shrub	Dicots
24	Gayo	<i>Brideliasp.</i>	Phyllanthaceae	Tree	Dicots
25	Kemona	<i>Cleistocalyx operculatus</i>	Myrtaceae	Tree	Dicots
26	Mathe	<i>Cyperus sp.</i>	Cyperaceae	Grass	Monocots
27	Banso	<i>Digitaria sp.</i>	Poaceae	Grass	Monocots
28	Tatari	<i>Dilenia pentagyna</i>	Dilleniaceae	Tree	Dicots
29	Kush	<i>Desmostachya bipinnata</i>	Poaceae	Grass	Monocots
30	Farsa	<i>Grewia sapida</i>	Malvaceae	Tree	Dicots
31	Ghodedubo	<i>Hemarthria compressa</i>	Poaceae	Forb	Monocots
32	Galgale/Galeni	<i>Leea macrophylla</i>	Vitaceae	Shrub	Dicots
33	Karauti ghaas	<i>Lindernia ciliate</i>	Scrophulariaceae	Shrub	Dicots
34	Belauni	<i>Maesa chisia</i>	Primulaceae	Shrub	Monocots
35	Sindure	<i>Mallotus philippensis</i>	Euphorbiaceae	Tree	Dicots
36	Lajjawati	<i>Mimosa pudica</i>	Fabaceae	Shrub	Dicots
37	Kalikath	<i>Myrsine semiserrata</i>	Primulaceae	Tree	Dicots
38	Pati Jhar	<i>Parthenium hysterophorus</i>	Asteraceae	Shrub	Monocots
39	Narkat	<i>Phragmites karka</i>	Poaceae	Grass	Monocots
40	Pipla	<i>Piper longum</i>	Piperaceae	Shrub	-
41	Bhalayo	<i>Semecarpus anacardium</i>	Anacardiaceae	Tree	Dicots

42	Khareto/Jhadu	<i>Sida rhombifolia</i>	Malvaceae	Shrub	Dicots
43	Bayar	<i>Ziziphus mauritiana</i>	Rhamnaceae	Tree	Dicots
44	Amala	<i>Phyllanthus emblica</i>	Euphorbiaceae	Tree	Dicots
45	Asare	<i>Murraya koenigii</i>	Rutaceae	Tree	Dicots
46	Vede kuro	<i>Xanthium strumarium</i>	Asteraceae	Shrub	Dicots
47	Aank	<i>Calotropis gigantea</i>	Asclepiadaceae	Shrub	Dicots
48	Sisoo	<i>Dalbergia sissoo</i>	Leguminosae	Tree	Dicots
49	Unidentified	-	-	Tree	-
50	Unidentified	-	-	Tree	-
51	Unidentified	-	-	Shrub	-
52	Unidentified	-	-	Shrub	-
53	Unidentified	-	-	Grass	-
54	Unidentified	-	-	Grass	-
55	Unidentified	-	-	Grass	-
56	Unidentified	-	-	Tree	-
57	Unidentified	-	-	Tree	-
58	Unidentified	-	-	Tree	-
59	Unidentified	-	-	Forb	-
60	Unidentified	-	-	Forb	-
61	Unidentified	-	-	Forb	-

Annex. II. Key Diagnostic Features for identified reference plant specimens

Category	Diagnostic Features
Grasses and sedges	<p>Elongated rectangular shape of epidermal cells arranged parallel to veins.</p> <p>Epidermal cells aligned in longitudinal strips.</p> <p>Parallel venation.</p> <p>Present “short” cells (alone or in pairs) and “long” epidermal cells (except family <i>Juncaceae</i>).</p> <p>Parallel arrangement of stomata.</p> <p>Presence of dumbbell-shaped guard cells of stomata (Gramineous type) exception of the family <i>Juncaceae</i>, which have kidney-shaped of stomata (Amaryllis type).</p> <p>Presence of macrohairs, microhairs, prickle hairs, and papillae, except family <i>Juncaceae</i> which have the sporadic presence of papillae and trichomes.</p>
Herbaceous plants and leaves of deciduous trees	<p>Epidermal cells of variable, mostly irregular-shape (hexagonal, round, elongated), Cells of elongated organs have elongated shape.</p> <p>Epidermal cells of leaves arranged diffusely, rarely parallel to the veins.</p> <p>Usually, reticulated venation. Kidney-shaped guard cells of stomata (Amaryllis type) arranged irregularly or/and randomly.</p> <p>Great morphological variability of trichomes, which occur in different taxa.</p> <p>Presence of different types of crystalline inclusions found in almost all taxa.</p>
Ferns and mosses	<p>Fine structure fragments.</p> <p>Cells of ferns have puzzle-like, and mosses have labyrinth-like pattern.</p> <p>Mosses single-layered fronds (leaves of mosses) have no developed epidermis and is not differ.</p> <p>A thick layer of photosynthetic cells on a leaf surface of mosses forms lamellae; cells are like furrows or ridges that run parallel to each other. Ferns have open venation and all cells of the lower and upper epidermis contain chloroplasts.</p> <p>Right (true) vascular tissue contains phloem and xylem absent in mosses.</p> <p>Exclusively presence of Mmium type of stomata.</p> <p>Presence of sporangia on the abaxial side of the fern leaves form clusters, sometimes covered by sharps.</p>

Source: Diagnostic features adopted from (Veselovská et al., 2021)

Annex. III. Number of plants found per sample in Microscopic Examination of Microhistological Slide

S.N	Sample number	Number of plant species found per sample
1	R-1	8
2	R-2	6
3	R-3	10
4	R-4	6
5	R-5	11
6	R-6	7
7	R-7	9
8	R-8	14
9	R-9	9
10	R-10	11
11	R-11	10
12	R-12	12
13	R-13	9
14	R-14	6
15	R-15	8
16	R-16	10
17	R-17	9
18	R-18	11
19	R-19	9
20	R-20	10
21	R-21	9
22	R-22	11
23	R-23	10
24	R-24	10
25	R-25	11
26	R-26	7
27	R-27	10
28	R-28	11
29	R-29	10
30	R-30	9
31	R-31	10
32	R-32	13
33	R-33	12
34	R-34	9
35	R-35	11
36	R-36	11
37	R-37	10
38	R-38	14
39	R-39	7
40	R-40	9
41	R-41	8
	Mean	9.68
	Standard deviation	1.93

8. Photo plates

1. Field visit and data collection



Greater One-horned Rhino



Fresh dung sample



Rhino sample collection



Reference sample



Quadrant drawing



Protection by patrolling team

2. Lab work photos



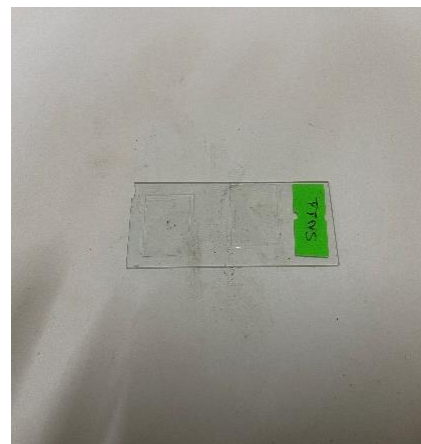
Grinding of sample



Washing of soaked sample



Staining the sample



Slide prepared to examine

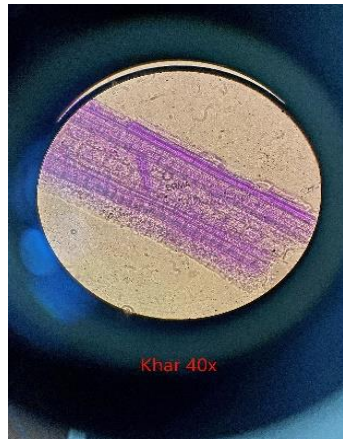


Microscopic Examination

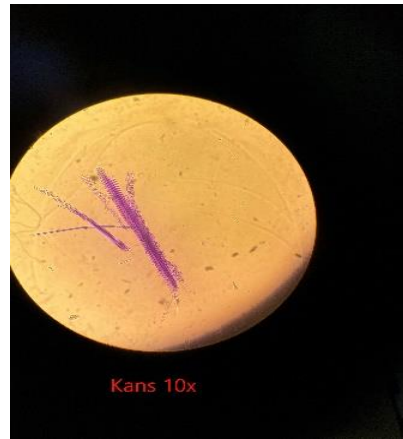
3. Selected histological photographs of principal food plants of Rhino in BNP, Nepal.



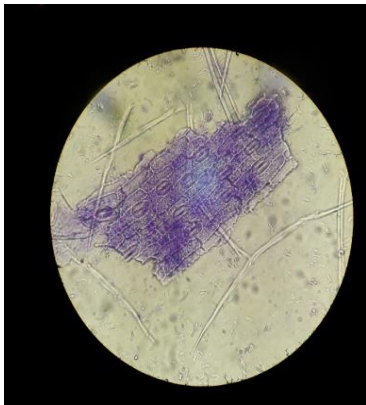
Imperata cylindrica 10x (Siru)



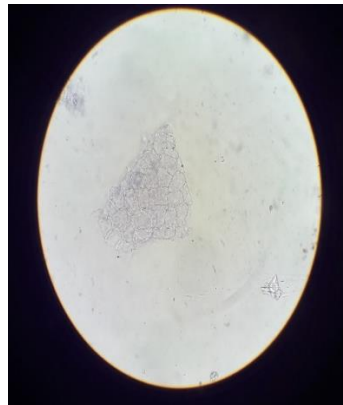
Eulaliopsis binata 40x (khar)



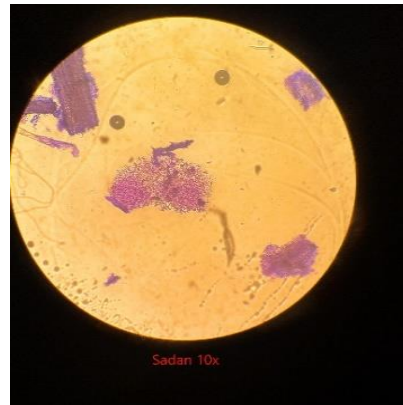
Saccharum spontaneum 10x (Kans)



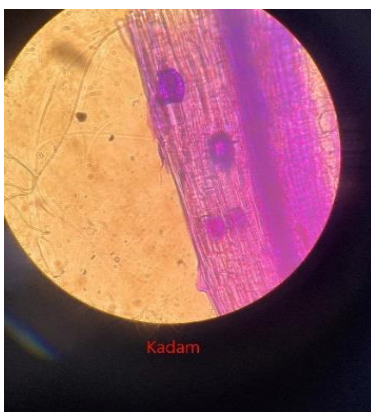
Themeda arundinacea 40x (Dhaddi)



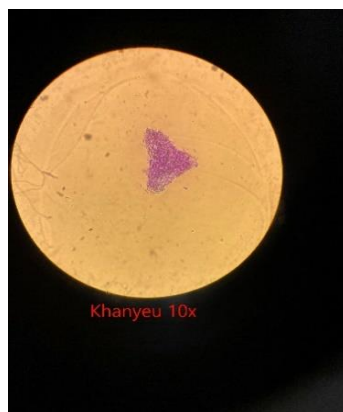
Trewia nudiflora 40x (Bhellar)



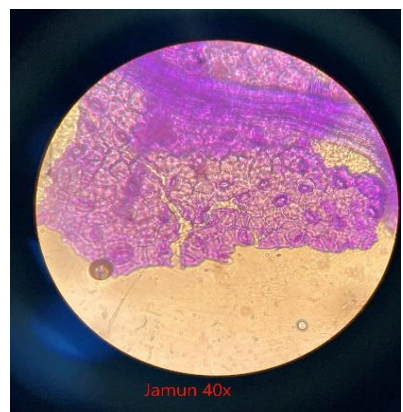
Eugenia sp. 10x (Sadari)



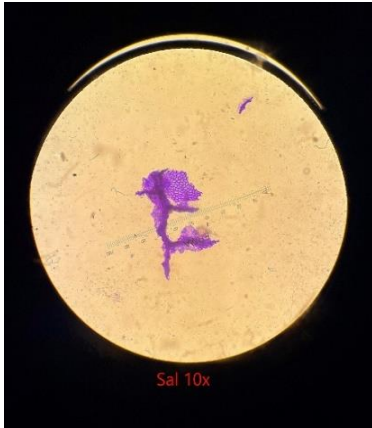
Adina cordifolia 40x (Kadam)



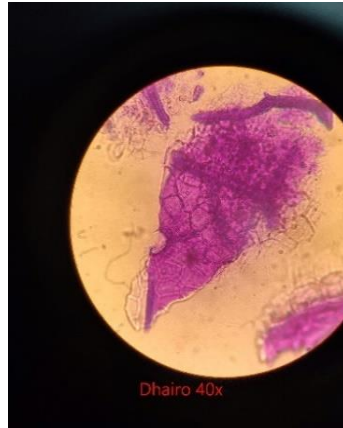
Ficus semicordata 10x (Khanyeu)



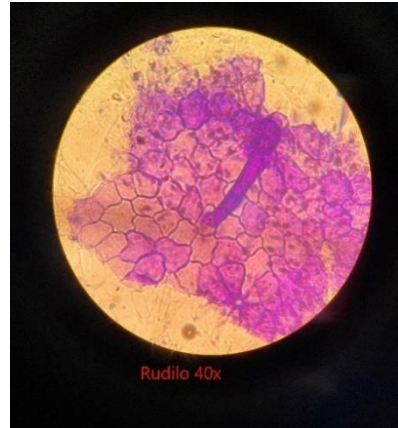
Syzygium cumini 40x (Jamun)



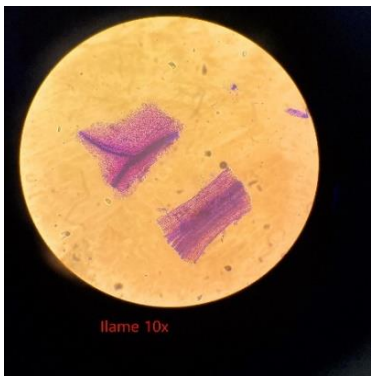
Shorea robusta 10x (Sal)



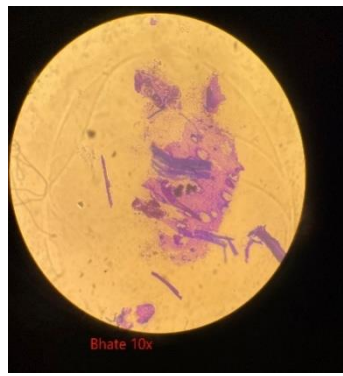
Woodfordia fruticosa 40x
(Dhairo)



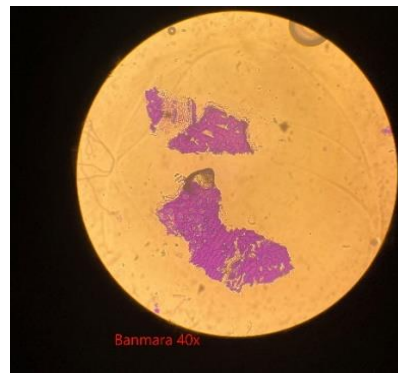
Pogostemon benghalensis
40x (Rudilo)



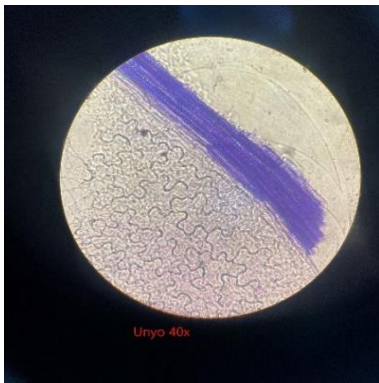
Ageratum sp.10x (Ilame)



Clerodendrum sp. 10x
(Bhate)



Eupatorium adenophorum
10x (Banmara)



*Drynaria mollis*10x (Unyo)

