The effect of wild radish (*Raphanus raphanistrum* L.) flower morphology on the diversity, abundance, plant-pollinator interactions, and foraging behaviour of floral visitors in Lesotho.

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DECLARATION

I hereby declare that except where otherwise indicated and acknowledged in the text, footnotes, tables and figures, the thesis is based on my original work. I also acknowledge that to my knowledge it has not been previously or concurrently submitted for any other degree at NUL or other institutions.

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General Abstract

Wild radish (*Raphanus raphanistrum*), a prevalent weed in Lesotho, is known for its varied flower morphology and reliance on insect pollination. This study explores the diversity, abundance, and foraging behavior of insect pollinators in two agroecological zones of Lesotho: Nyakosoba research station and the National University of Lesotho (NUL) farm. The study further investigated the influence of flower morphology on pollinator-mediated selection, pollen removal, pollen production and comparison of seven floral traits between white and yellow color morphs. The Shannon-Wiener Diversity Index was used to measure species diversity, while cross-tabulation and chi-square tests compared pollinator abundance and visitation patterns. Descriptive statistics and ANOVA analyzed visitation rate, visitation frequency, and stay time, associating these behaviors with pollen deposition using ANCOVA. Descriptive statistics and linear regression model assessed pollen production. ANOVA was used to compare pollen removal from short and long stamens. Mean differences and standard errors of yellow and white flower morphs were compared using independent sample t-test. A total of eighteen insect species visited wild radish at NUL farm, and twenty-four species were documented at Nyakosoba, with Hymenoptera and Diptera being the most represented orders. *Apis mellifera* (honeybee) and *Eristalis tenax* (a syrphid fly) were the most abundant and effective pollinators. Visitation frequencies and stay times varied, with *Stizus sp.* and *Apis mellifera* showing the highest visitation rates. Floral visitors' preferences for pollen or nectar were also recorded, with *Apis mellifera* being the highest pollen forager. Yellow flowers with dark veins were predominantly visited by *Eristalis tenax* and *Apis mellifera*. Long stamens produced more pollen grains (mean = 6110.56 ± 384.87) than short stamens (mean = 4768.00 ± 389.99). More pollen was removed from dimorphic stamens (mean = 1.39 ± 0.08) than from long stamens (1.25 ± 0.05) and short stamens (mean = 0.91 ± 0.02). Morphological comparisons revealed significant differences in pedicel length ($p = 0.01$), anther length ($p = 0.03$), and pistil length ($p = 0.05$) between white and yellow flowers, though other traits showed no significant differences. These findings highlight that wild radish exhibits significant variation in flower morphology, which influences pollinator behavior and efficiency. *Apis mellifera* and *Eristalis tenax* were the most effective pollinators. Yellow flowers and long stamens were particularly favored, because of higher pollen production resulting in higher pollen removal. Future research should focus on investigating the genetic basis of floral trait variations in wild radish, explore further the impact of environmental factors on plant-pollinator interactions and development of strategies to enhance pollinator services in agricultural systems by promoting floral diversity.

Key words: *Floral morphology, plant-pollinator interactions, diversity, abundance, visitation frequency, pollen produced, pollen removed, dimorphic stamen lengths.*

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Chapter 1- General Introduction

1.1 Background

Wild radish is (*Raphanus raphanistrum* L.) is a pervasive weed found in agricultural and natural ecosystems worldwide, including Lesotho. It is characterized by its diverse flower morphology, with variations in color, size, and shape, making it a significant subject for studying plant-pollinator interactions (Warwick & Francis, 2005). Despite being a weed, wild radish can have significant ecological interactions with pollinators (Warwick & Francis, 2005). Pollinators are essential to the health of ecosystem and agriculture in Lesotho. They facilitate the reproduction of many crops and wild plants, contributing to biodiversity and food security (Klein *et al*., 2007). The decline of pollinator populations poses a significant thread to both natural and agricultural systems (Ollerton *et al*., 2011; Potts *et al*., 2010). Regardless of its status as a weed, wild radish's flowers serve as an important resource for pollinators by providing nectar and pollen. Pollinators, such as bees, butterflies, and flies play a crucial role in the reproduction of many crops and wild plants, thus contributing significantly to biodiversity and food security (Ollerton *et al*., 2011).

The complex relationship between wild radish flower morphology and insect pollination is a fascinating subject of scientific investigation with far-reaching suggestions for the successful reproduction and ecological dynamics of this plant. *Raphanus raphanistrum* L. (Wild radish), shows remarkable diversity in floral traits, including flower color, anther exsertion, anther pollen count, and anther height dimorphism, within its populations (Sapir e*t al*., 2017). These traits, honed by the forces of natural selection, have evolved over time to enhance the plant's reproductive success (Sapir e*t al*., 2017). The variation in these morphological traits has reflective consequences for the types of insects that visit wild radish flowers, which significantly influence the plant's reproductive fitness (Stanton *et al*., 1989; Lee *et al*., 1998).

Insects, particularly bees and butterflies, serve as vital agents in the pollination of wild radish and numerous other flowering plants, thus facilitating the reproduction and genetic diversity of these species (Conner *et al*., 2001). Tetradynamy (four long and two short stamens) is one of the distinctive features of wild radish, which further exemplifies the interesting interplay between floral morphology and pollinator behavior (Sapir e*t al*., 2017). Positioning of anthers within the flower can act as a visual signal, influencing pollinator visitation (Sapir e*t al*., 2017). Prominently exserted anthers can serve as an attractive display, signaling the availability of pollen rewards, while conversely, they may pose challenges to pollinators seeking nectar, potentially prolonging visit durations (Sapir e*t al*., 2017; Conner *et al*., 1995).

Moreover, the interplay of floral traits has profound ecological and evolutionary significance, shaping the interactions between wild radish and its pollinators (Conner e*t al*., 1995). A diverse community of pollinators is crucial for wild radish populations, safeguarding against dependence on a single pollinator species. Abundant and efficient pollinator populations are vital for maximizing the success of pollination and seed production (Zou *et al*., 2017; Zych *et al*., 2013). The foraging behaviors of insects, such as their pollen-gathering efficiency and their ability to access nectar from different flower types, further impact wild radish pollination dynamics (Esposito *et al*., 2021; Conner *et al*., 1995). Understanding these factors is pivotal for effective wild radish population management.

In addition to floral traits and pollinator behaviors, external factors like plant density, flowering timing, the availability of pollinator habitat, and the impact of pesticide use also contribute to the complexity of wild radish pollination dynamics (Conner *et al*., 1997). The morphology of wild radish flowers can influence which pollinators visit and how they behave, affecting both pollinator abundance and the overall health of ecosystems (Conner *et al*., 1996). By studying these interactions, we can gain valuable insights into plant-pollinator dynamics and develop strategies to manage wild radish more effectively. This study aims to investigate the impact of wild radish flower morphology on pollinator abundance and diversity, the nature of plant-pollinator interactions, and the foraging behaviour of pollinators in Lesotho. Through this research, we can enhance our understanding of how wild radish influences local pollinator communities and inform practices that support both agricultural productivity and pollinator conservation. Moreover, understanding the dual role of wild radish as a competitive weed and a nectar and pollen resource for pollinators can help develop strategies that balance agricultural productivity with ecological conservation. This research is crucial for enhancing food security and maintaining environmental health in Lesotho, highlighting the need for integrated approaches to weed and pollinator management.

1.2. Problem statement

Increasing agricultural intensification and habitat fragmentation have raised concerns about the decline of pollinator populations, impacting ecosystem stability and crop productivity. Despite the crucial role of pollinators in wild radish (*Raphanus raphanistrum*) reproduction, little is known about the diversity, abundance, and foraging behavior of their pollinators. Understanding these aspects is essential for effective conservation and management strategies to ensure the long-term viability of wild radish populations and associated ecosystems. Moreover, the reproductive success of wild radish is influenced by various factors, including pollen production, pollen removal and flower morphological traits. However, there is limited understanding of how these factors differ between yellow and white wild radish flower morphs within wild radish populations. Such knowledge is essential for comprehensively understanding the reproductive biology of wild radish and its implications for plant-pollinator interactions, genetic diversity, and population dynamics.

1.3. Justification

Studying wild radish and its interactions with pollinators in Lesotho is essential for advancing ecological knowledge, improving agricultural practices, and supporting biodiversity conservation. This research will provide valuable insights that can inform strategies to manage wild radish effectively while promoting the health and diversity of pollinator populations.

1.4. General Objective

- To assess the diversity, abundance, and foraging behavior of floral visitors in wild radish (*Raphanus raphanistrum)* in agroecological zones of Lesotho.
- To compare wild radish flower morphs and determine their effect on plant-pollinator interactions.

1.4.1 Specific Objectives

- To assess the impact of wild radish flower-morphs on the diversity of floral visitors in lowlands and foothills of Lesotho.
- To compare the impact of wild radish flower morphs on abundance of floral visitors in lowlands and foothills of Lesotho.

- To assess the foraging behaviour of pollinators wild radish in the foothills of Lesotho.
- To compare the morphological traits between yellow and white wild radish flowers.
- To assess pollen removal between long and short stamens.
- To quantify pollen production between short and long stamens in wild radish.

1.5. Hypotheses

1.5.1. Null hypothesis:

- There is no difference in the diversity of floral visitors to wild radish between the foothills and lowlands of Lesotho.
- There is no difference in the abundance of floral visitors to wild radish between the foothills and lowlands of Lesotho.
- The foraging behavior of floral visitors does not vary significantly among species in the foothills of Lesotho.
- There is no significant difference in pollen produced between the short and long stamens of wild radish.
- There is no significant difference in pollen removed between short and long stamens.
- There is no significant difference in flower morphological traits between yellow and white wild radish flowers.

1.3.2. Alternative hypothesis:

- There is a significant difference in the diversity of floral visitors to wild radish between the foothills and lowlands of Lesotho.
- There is a significant difference in the abundance of floral visitors to wild radish between the foothills and lowlands of Lesotho.
- The foraging behavior of floral visitors varies significantly among species in the foothills of Lesotho.
- There is a significant difference in flower morphs between white and yellow wild radish flowers.

- There is no significant difference in pollen produced between the short and long stamens of wild radish.
- There is no significant difference in pollen removed between short and long stamens.

Chapter 2 - Literature review

This chapter discusses the experimental literature on diversity, abundance, and foraging behavior of wild radish pollinators. It further discusses the relationship of insect pollinators with various wild radish flower characteristics. The topics covered are diversity and abundance of floral visitors, visitation rate, visitation frequency, stay time, different visitation times, nectar and pollen feeding, pollen deposition, pollen removal, flower morphology and anther exsertion and anther height dimorphism.

2.1 Introduction

The Brassicaceae, inclusive of wild radish (*Raphanus raphanistrum*) are dicot angiosperms. Wild radish flowers are normally 1.02-1.27cm widespread and have four petals which range from pale yellow to cream yellow, pink or white colored usually with distinguishing purple veins (Conner *et al*., 1996). It has upright flower stalks that are 0.64cm long, it also has green and fleshy seedpods initially cylindrical forming below the flowers, which are 2.03-7.62cm long with a 1.02-5.08cm long beak and contain two to 10 seeds (Malik, 2009).

The morphology of wild radish flowers has been studied extensively in relation to its pollination ecology. Wild radish is a generalist plant that is pollinated by numerous insect species, comprising honeybees, butterflies, and syrphid flies (Conner *et al*., 2009; Sapir *et al*., 2017). The shape and position of the flower parts, such as petals, anthers, and stigma, are all important factors that influence pollination efficiency (Conner *et al*., 2009; Conner *et al*., 2017). For instance, the anthers of wild radish flowers are exserted, meaning that they are held above the level of the petals. This allows for pollen to be easily transferred to the pollinators as they visit the flowers. The stigma of the flower is also exserted, and it is positioned so that it is receptive to pollen when the pollinators visit the flower (Conner *et al*., 2009).

Wild radish flower morphology has also been studied in relation to its evolution. It is a highly variable species, and there is a great deal of variation in flower morphology among different populations (Conner *et al*., 2003). These variations may be due to multiple factors such as natural selection, genetic drift, and hybridization with other Brassicaceae species (Conner *et al*., 2003).

Pollinators determine agricultural productivity as they provide essential ecological services (Divija *et al*., 2022; Robacker *et al*., 1988). Wild radish is a popularly grown vegetable in the temperate and tropical regions, it however has self-incompatible flowers and depends completely on insect pollination for effective reproduction (Divija *et al*., 2022; Conner *et al.*, 1996). Besides the phenomenon of self-incompatibility, reproductive success may also be affected by steps in the pollination process (Zameer *et al*., 2017). Plants which are in terrible need for insect pollination have advanced to depend on specific anthophilous insects.

2.2. Diversity of floral visitors

The diversity of flower visitors to wild radish plays a fundamental part in influencing the plant's pollination success and variety of pollinators is essential for wild radish's reproductive process (Conner *et al*., 2007). The diversity of floral visitors can vary by location, as evidenced by a study in India, which documented 15 different insect species visiting wild radish flowers, while a study in the United States found only 10 insect species (Conner *et al*., 2007).

Research indicates that the pollinator community of wild radish is diverse, encompassing various anthophilous species such as honey bees (*Apis dorsata* F. and *Apis florae* L.), mining bees (*Andrena sp.*), and syrphid flies (*Episyrphus balteatus, Eristalinus aeneus,* and *Episyrphus laetus*) (Zameer *et al*., 2017; Divija *et al*., 2022). Additionally, supplementary pollination by honeybees (*A. mellifera* and *A. cerana*) has been shown to increase radish seed yield (Zameer *et al*., 2017).
Furthermore, Divija *et al*. (2022), identified fifteen insect pollinator species belonging to three major orders. Among these, hymenopterans from families Apidae, Halictidae, Vespidae, and Scoliidae constituted the majority, with Lepidoptera represented by four species from Pieridae and Nymphalidae families. Other visitors included dipterans with three species from Syrphidae, Calliphoridae, and Rhiniidae families. However, only six species were frequent visitors to radish flowers: *Apis dorsata* F. (Apidae), *Apis cerana* F. (Apidae), *Apis florea* L. (Apidae), *Tetragonula iridipennis* (Smith) (Apidae), *Lassioglossum sp.* (Halictidae), and *Ischiodon scutellaris* (F.) (Syrphidae).

2.3. Abundance of floral visitors

According to Divija *et al*., (2022), the most abundant species of floral visitors in descending order are *Apis florea*, *A. dorsata*, *Tetragonula iridipennis*, *A. cerana*, *Lassioglossum sp*., and *Ischiodon scutellaris*. In contrast, Adedoja *et al*. (2017) reported a total of 820 insects from four orders, 15 families, 16 genera, and 20 insect species, with butterflies having the highest percentage composition (25.80%), followed by bees (22.80%), while Scarabid beetles had the least percentage composition (0.73%). Dao *et al*. (2014), reported varying activity patterns for different visitors, with bumblebees and Apis mellifera being most active at certain times.

Sithole and Mawdsley (2010), observed that beetles (Coleoptera) were the most abundant floral visitors for some plant species, while Hymenoptera species predominated over others. Lepidoptera, such as the moth *Arniocera auriguttata* (Hopffer), were the dominant floral visitors for *Flueggea virosa* (Mawdsley *et al*., 2009). Within Coleoptera, Scarabaeidae and Chrysomelidae species were the most abundant floral visitors, with Scarabaeidae mainly belonging to the subfamily Cetoniinae. Other beetle families encountered frequently included Lycidae, Curculionidae, Buprestidae, Cleridae, and Meloidae (Mawdsley and Sithole, 2010). In the Hymenoptera order, Formicidae, Apidae, and Halictidae species were the most abundant floral visitors of *Acacia nilotica, Acacia tortilis* and *Acacia exuvialis* while Chalcidae, Colletidae, Megachilidae, Scoliidae, Sphecidae, Tiphiidae, and Vespidae species were also present (Mawdsley *et al*., 2010). The activity patterns of solitary species were influenced by seasonal rains, except for the honeybee *A. mellifera* L. (Mawdsley *et al*., 2010).

In a study by Ali *et al*. (2022), syrphid flies were more abundant than bee species, with *A. dorsata* being the third most abundant insect pollinator visiting carrot flowers. *A. dorsata* was reported as the most abundant and efficient pollinator for onions and other crops in Southern Punjab (Saeed *et al*., 2008; Zameer *et al*., 2017; Saeed *et al*., 2012; Ali *et al*., 2011).

However, some studies reported a low abundance of honeybees on carrot flowers due to limited foraging resources like nectar and pollen (Ali *et al*., 2022; Topitzhofer *et a*l., 2019).

2.3.1. Visitation frequency

Visitation frequency is an essential factor that influences the pollination dynamics of wild radish. Research has shown that the number of flowers and flower size increase visitation by syrphid flies, while only the flower number enhances visitation by small bees (Conner *et al.*, 1996; Sapir *et al.*, 2017). According to Johnson *et al.* (2000), wild radish's most common pollinators are syrphid flies, bumble bees, and cabbage butterflies, with syrphid flies being the most frequent visitors, particularly favoring wild plants. Bumble bees exhibit no preference for wild or hybrid plants, while cabbage butterflies exhibit a strong preference for wild plants. Various factors such as flower size, number and color influence visitation frequency of pollinators in wild radish (Stanton, 1987). The foraging behavior of wild radish pollinators varies depending on the insect species. For example, honeybees (*A. mellifera*) are known for their systematic foraging behavior where they visit flowers in a straight line, pollinating each flower as they go (Dag *et al.*, 2001). Bumblebees (*Bombus* sp.), on the other hand, have a more erratic foraging behavior, they fly from flower to flower in a random pattern (Dag *et al.*, 2001). Moreover, *A. florea* visit flowers frequently, however, the percentage of visits result in less stigma contact for this species (Divija *et al.*, 2022).

2.3.2. Visitation rate

Pollination dynamics of various plants species are influenced by visitation rates of floral visitors (Latif *et al.*, 2019). Visitation rate, typically measured as the number of visits per flower per unit time, provides insights into the efficiency and effectiveness of pollinators. It directly effects pollen deposition, pollination success, and consequently, plant fitness and fruit set. High visitation rates generally enhance cross-pollination and genetic diversity, while low rates may indicate insufficient pollinator services, potentially threatening plant reproduction (Waser and Ollerton 2006). The visitation rate of insects is high for *A. florea* visiting more flowers per minute than *Amegilla sp.* (Latif *et al.*, 2019). Additionally, bees tend to visit a higher number of flowers per visit compared to other insects, and they are more effective at pollen transfer between flowers (Divija *et al.*, 2022). Flies, on the other hand, tend to visit fewer flowers per visit and are less effective at pollen transmission (Divija *et al.*, 2022).

The number of flowers visited per minute tends to be highest for *A. florea*, followed by *A. dorsata*, *T. iridipennis*, and *A. cerana*, whereas *Lassioglossum sp*. and *I. scutellaris* have lower visitation frequency values per minute (Divija *et al.*, 2022). Alternatively, Zameer *et al.* (2017), reported that

E. balteatus had the highest visitation rate, being the most frequent floral visitor per flower per 60 seconds, followed by *Andrena sp.* and *A. florea*. *Apis dorsata* and *E. aeneus* were not as frequent visitors, with *E. laetus* having the lowest visitation rate. In addition, Apidae accounted for the highest total visits, followed by Nymphalidae, Syrphidae, and Sphecidae (Dao *et al.*, 2014).

2.3.3. Stay time

Pollinators' stay time on wild radish flowers is influenced by various factors. Fenster *et al*. (2004), found that stay time significantly increases when flower density is high, as pollinators are more likely to encounter multiple flowers to visit. There is also an observable average stay time of about 10 seconds for pollinators on wild radish (Pyke, 2015). However, stay time varies among pollinator species, with bumblebees tending to stay longer compared to honeybees and bumblebees work more slowly when accumulating pollen than when scavenging for nectar (Adjaloo *et al*., 2003). Maximum stay time is observed in the case of *E. aeneus* and *E. laetus*, followed by *Andrena sp*., while the minimum stay times are recorded for *A. dorsata*, *E. balteatus*, and *A. florea* (Zameer *et al*., 2017).

In the study of Divija *et al*. (2022), maximum time spent on an individual flower was recorded for *Lassioglossum sp.*, followed by *T. iridipennis*, *I. scutellaris*, and *A. florea*, whereas *A. dorsata* and *A. cerana* had the minimum stay time. Additionally, *A. florea* spent the most time per umbel and visited the highest number of umbullets per umbel, followed by *E. aeneus* and *Ischiodon scutellaris* (Ali *et al*., 2022). In contrast, *Lassioglossum sp.* had the lowest stay time per umbel, and *A. dorsata* had the lowest number of umbullets visited per umbel (Ali *et al*., 2022).

2.3.4. Different visitation times

Under favorable weather conditions, insects initiate their visits in the early morning hours and reach their peak between 11 am and 3 pm, with Apidae being more active during the early hours (Dao *et al*., 2014). The movement array of flies is most pronounced in the morning and early afternoon. However, the activity of all potential pollinators decrease after 2 pm and cease at dusk hours (Dao *et al*., 2014). Even though, some visitors had a low percentage of total visits, sequential overlapping of potential pollinators' activity during their observation period was observed (Dao *et al*., 2014).

2.3.5. Nectar and pollen feeding

The most important pollinators of wild radish are bees (Zameer *et al*., 2017). Except for *Apis florea*, which predominantly feeds on nectar during most of its visits, all other pollinators, including bees, show a preference for consuming both nectar and pollen, in contrast, all flies show a preference for feeding exclusively on pollen (Zameer *et al*., 2017).

On the other hand, Conner *et al*. (1995), found that flies and butterflies are attracted to the flowers' nectar, while moths are attracted to their pollen. Some pollinators, like little bee *Apis florea* and mason wasp *Delta conoideum*, are observed engaging in nectar robbing activities, bypassing the natural floral openings and making holes in the calyx to feed on nectar, this behavior did not facilitate plant pollination (Divija *et al*., 2022).

2.3.6. Pollen deposition

Wild radish flowers are pollinated by insects, such as bees, butterflies, and flies (Bernauer *et al*., 2022; Conner *et al*., 1995). The bright yellow colour of flowers and the nectar attract insects and when the insects visit the flowers, they pick up pollen on their bodies and transfers it to the stigma of other flowers when the insects visit them (Bernauer *et al*., 2022; Conner *et al*., 1995).

The critical aspect of pollination is the amount of pollen which insects transfer to the stigmas. Howlett *et al*. (2017) used two approaches to measure pollen deposition: the static approach, where the flower head remains attached to the plant and researchers wait for an insect visit, and the active approach, where flowers are detached from the plant and placed near to where target pollinators visit flowers. *A. mellifera* deposits the most pollen grains, followed by *L. huakiwi*, *B. terrestris*, *E. tenax*, and *C. stygia*, with *L. sericata* depositing the least (Howlett *et al*., 2017).

In the research conducted by Mawdsley *et al*. (2010), they found that bees (Hymenoptera: Apidae) have heavy pollen loads, this included *A. mellifera L*., *Xylocopa caffra (L.)*, Halictidae, and Megachilidae species. Additionally, some beetles, particularly larger scarabs, have pollen adhering to their bodies, mainly on ventral setal patches, setal fringes on the legs, and surfaces of the head or pronotum (Mawdsley and Sithole, 2010). Moreover, scarabs like *Leucocelis amethystina* and *Mausoleopsis amabilis* have their various body parts attached with pollen (Mawdsley and Sithole, 2010).

Bees, both Apidae and Megachilidae families, gather pollen by scouring it with their appendages and assembling it hooked on the corbicula or pollen basket found on their hind tibia. Flies, like *Eristalis tenax*, collect pollen on various body parts, including the thorax, abdomen, and legs (Kusuma and Windriyanti, 2022). Scarab beetles, although likely functioning as pollinators, were also observed to feed on nectar and pollen, making them potential pollen predators. However, *Plaesiorrhinella trivittata* found on flowers lack pollen loads (Mawdsley and Sithole, 2010).

In contrast, Zameer *et al*. (2017), reported that *A. dorsata* deposits the highest number of pollen grains, followed by *Andrena sp.*, *E. laetus*., *A. florea* and *E. aeneus*, with the lowest number of pollen grains deposited by *E. balteatus*. Moreover, Divija *et al.* (2022), noted varying pollen deposition among different species. *A. dorsata* and *A. cerana* deposited the most pollen grains per visit, while *I. scutellaris* deposited the least.

2.3.7 Pollen removal

The proportion of pollen removed in single visits to tristylous species is greater from long level anthers than intermediate and short anther heights (Wolfe e*t al*., 1987; Harder *et al*., 1993). Also, in non-heterostylous species, pollen removal in single visits by bumblebees increases with increasing anther exsertion (Murcia, 1990). The presence of short stamens increases the duration of visits by nectar-foraging captive bumblebees, which in turn increase pollen deposition on stigmas (Conner *et al*., 2009). However, the presence or absence of short stamens had no effect on pollen removal from long stamens in studies of (Conner *et al*., 2009).

The intermediate anther exsertion leads to maximum pollen removal (Conner *et al*., 2009; Conner *et al*., 1993), this arises from the observation that the visits of all pollinators are similar, that is, they land on the open part of the corolla and feed on pollen or nectar, or both (Conner *et al*., 1997). Pollen is primarily placed on the head and thorax of pollinators during visitation, as a result, anthers that are inserted into the tube do not effectively contact pollinators' body and highly exserted anthers do not contact bodies of small pollinators (Conner *et al*., 1995).

2.4. Flower morphology

Wild radish is an annual weed that is native to Europe and Asia, but has been introduced to many other parts of the world, including North America, South America, Australia, and Africa (http://cals.Cornell.edu/weed-science/weed-profiles/wild-radish). Wild radish flowers are perfect, meaning that they have both male and female reproductive parts (http://cals.Cornell.edu/weed-science/weed-profiles/wild-radish). They are arranged in racemes, which are clusters of flowers on a single stalk. The flowers are typically yellow or pale yellow in color and have four petals. The petals are arranged in a cross-shape, and are each about 1 cm long and 0.5 cm wide (http://cals.Cornell.edu/weed-science/weed-profiles/wild-radish).

The male reproductive parts of the wild radish flower consist of six stamens, which are arranged in two pairs, the stamens produce pollen, which is transferred to the female reproductive parts of the flower by pollinators, such as insects (Holm *et al*., 1979). The female reproductive parts of the wild radish flower consist of a single pistil, which is located in the center of the flower. The pistil has a stigma, which is a sticky surface that receives pollen, and an ovary, which contains the ovules (Holm *et al*., 1979). When pollen is transferred to the stigma, it germinates and grows down the style, which is the tube that connects the stigma to the ovary (Jahed *et al*., 2017). Once the pollen reaches the ovary, it fertilizes the ovules, and seeds are produced (Jahed *et al*., 2017). The sepals of the wild radish flower are small and green, and they are located at the base of the flower. The sepals protect the flower buds from damage (Holm *et al*., 1979).

Flower morphology in plants often experiences natural selection driven by insect pollinators (Campbell, 1989; Morgan *et al*., 2005). This type of selection, influenced by pollinators, operates on the variation in physical traits among individuals within a population (Morgan *et al*., 2005). The strength of selection and the evolutionary response to it are compromised when there is more variability within individual flowers than among different plants (Morgan *et al*., 2005). This is particularly relevant because many plant species produce multiple flowers, and if each flower on a plant exhibits a wide range of floral traits, pollinators may have difficulty distinguishing between flowers from different plants (Campbell, 1989; Morgan *et al*., 2005). However, if different plants consistently produce flowers with varying traits, pollinators may preferentially visit one plant over another, resulting in selection for specific flower traits (Morgan *et al*., 2005).

This variation in flower traits can arise from spatial differences within a plant, leading to variations among flowers that bloom simultaneously on the same plant, differences among inflorescences, variations among individual flowers within inflorescences, and variations within flowers for traits that have multiple copies, such as petals and stamens (Morgan *et al*., 2005). Conversely, temporal variation can stem from variations among flowers that open at different times throughout an individual plant's flowering period, as well as from ontogenetic changes and environmental fluctuations over time (Morgan *et al*., 2005).

The impact of selection on floral traits can differ depending on whether the variation is due to spatial or temporal factors. If there are temporal changes in flower size, but individual plants maintain their relative ranking over time, then pollinators will consistently select the same plants each day, and selection will remain effective. Conversely, if flower size varies more within individual plants than among different plants, or if there is a strong interaction between time and individual traits, pollinators may not consistently prefer one plant, leading to weakened selection (Morgan *et al*., 2005).

Pollinators play a pivotal role in driving the selection of the floral traits and this type of selection operates on the variability of physical characteristics within a population of individual plants (Hopkins *et al*., 2012). The strength of selection and the subsequent evolutionary response are compromised when there is greater variation within individual flowers as opposed to among different plants (Conner *et al*., 1996; Conner, 1997). This is especially relevant because many plant species produce multiple flowers, and when each flower on a plant displays a wide range of floral traits, pollinators may encounter challenges in distinguishing between flowers from distinct plants (Conner *et al*., 1996; Conner, 1997). However, if different plants consistently produce flowers with varying traits, pollinators may develop preferences for specific plants over others, ultimately leading to the selection of flower traits (Conner *et al*., 1996).

This variability in flower traits can arise due to spatial distinctions within a single plant, resulting in differences among flowers that bloom simultaneously on the same plant, variations among inflorescences, differences among individual flowers within inflorescences, and variations within flowers for traits with multiple copies, such as petals and stamens (Morgan *et al*., 2005). Conversely, temporal variation can occur due to differences among flowers that open at various times during an individual plant's flowering period, as well as owing to ontogenetic changes and environmental fluctuations over time (Morgan *et al*., 2005).

The impact of selection on floral traits can vary depending on whether the variation is due to spatial or temporal factors (Conner *et al*., 1996). If there are temporal changes in flower size, but individual plants maintain their relative ranking over time, then pollinators will consistently select the same plants each day, and selection will remain effective (Conner *et al*., 1996). In contrast, if flower size varies more within individual plants than among different plants, or if there is a strong interaction between time and individual traits, pollinators may not consistently prefer one plant, leading to weakened selection (Morgan *et al*., 2005).

2.5. Anther exsertion and Anther height dimorphism

Anther Position as a Visual Signal: Anther position in a flower has been proposed to play a crucial role in visual displays. Prominent anthers can serve as a direct signal to pollen foragers, indicating the reward they can expect to receive (Lunau, 2000). This visual cue can guide potential pollinators to the source of pollen (Song *et al*., 2015).

On the other hand, the prominence or exsertion of anthers may have contrasting effects on flower visitors seeking nectar. Such anther positions could potentially interfere with nectar foragers, leading to longer visits, which, in turn, may increase the chances of pollen removal or deposition (Young and Stanton, 1990; Conner *et al*., 1995;). These longer visits could be due to the need for pollinators to navigate around the prominent anthers to access nectar (Conner *et al*., 1995).

In some plant groups, particularly within angiosperms, dimorphic or polymorphic positions of anthers are observed (Conner *et al*., 1995). This means that anthers are situated at varying heights or in distinct whorls within the same species or flower (Conner *et al*., 1995). This phenomenon is common in certain angiosperm families, including the Brassicaceae (Conner *et al*., 1995).

Among the various angiosperm families, the Brassicaceae stands out due to its characteristic feature called tetradynamy. This feature is defined by the presence of four long stamens and two short stamens in each flower (Conner e*t al*., 2003; Conner *et al*., 2023). Species like *Brassica rapa* and *Raphanus raphanistrum* have a significant genetic variation in the differences in anther heights

(Conner *et al.*, 2023). Additionally, *Brassica rapa* is widely cultivated for its edible parts and economic value, it has a diverse genetic background due to extensive cultivation and breeding. It includes several subspecies and varieties with distinct characteristics, such as Chinese cabbages and turnips. While *Raphanus raphanistrum* is typically regarded as a weed with occasional uses in soil improvement and traditional medicine.it is considered more genetically uniform as it is primarily a wild species, known to hybridize with cultivated radish *Raphanus sativus*, which can lead to genetic mixing and variation (Harder and Barrett 2006).

In some cases, tetradynamy and variations in anther positions have been linked to stabilizing selection, a process that favors intermediate traits over extreme ones. Such traits may impact pollinator efficiency and have ecological consequences (Conner *et al.*, 2003). A study by Leege *et al.* (2002), explored the relationship between anther position and pollen removal. Their findings highlighted the importance of anther exsertion, showing that maximum pollen removal occurs when anthers are positioned at their most exserted point. Murcia (1990) further supported the idea that anther position plays a crucial role in pollination efficiency. The study provided empirical evidence for the positive correlation between anther exsertion and pollen removal.

In their study, Harder and Barrett (1993), investigated the influence of anther position relative to the corolla tube opening on pollen removal. Their results added to the growing body of evidence suggesting that anther exsertion is a critical factor in maximizing pollen removal. Conner *et al.* (1995), conducted research that aligned with the findings of previous studies. Their work reinforced the notion that maximum pollen removal is associated with maximum anther exsertion. This consistency across studies highlights the robustness of the relationship between anther position and pollination.

Conner *et al.* (1993) and Conner *et al.* (1995), proposed that intermediate levels of exsertion for both anthers and stigmas might be favored by natural selection, indicating that extreme variations in exsertion levels could be less advantageous in terms of pollination. This hypothesis adds complexity to the understanding of anther position's role in plant reproductive success.

However, another position in wild radish is the important factor because pollinators can visualize pollen from a distance and that helps in pollination as insects will go to the blooms and initiate pollination process by having contact with anthers and stigmas of the plant.

Chapter 3 -Assessment of the Diversity, Abundance, and Foraging Behaviour of Floral Visitors of Wild Radish (*Raphanus raphanistrum* L.) in the Foothills and lowland Agroecological zones of Lesotho.

3.0 . Abstract

Wild radish (*Raphanus raphanistrum*) a popular weed in agricultural fields, depends completely on insect pollination. The advancement of ecological knowledge, improvement of agricultural practices, and support of biodiversity conservation depends on interactions between wild radish and its pollinators. The present study was conducted at Nyakosoba research station and National University of Lesotho farm to understand the diversity, abundance, and foraging behavior of insect pollinators to wild radish in two agroecological zones of Lesotho. Shannon-Wiener Diversity index (H') was used to measure diversity of species in both areas. Cross tabulation was used to compare pollinators abundance and to determine pollinators abundance based on their different visitation times. Descriptive statistics was used to give a clear summary of the data in foraging behavior and ANOVA was used to compare the means between visitation rate, visitation frequency and stay time by the various floral visitors. Cross tabulation was further used to assess species according to their pollen or nectar feeding habits. ANCOVA was used to associate the mean amount of pollen deposited with insect species and pollinators' stay time on the flower.

A total of eighteen insect species that belong to four insect orders (Hymenoptera, Diptera, Lepidoptera, and Coleoptera) were found to visit wild radish at NUL farm. The most abundant species was *Apis mellifera* (Apidae), Morpho-species 2 (Apidae), *Musca domestica* (Muscidae), *Eristalis tenax* (Syrphidae), *Eristalinus sp.* (Syrphidae) and Morpho-species (1) (Apidae). Similarly, twenty-four insect species were documented at Nyakosoba, with Diptera and Hymenoptera, Coleoptera and Lepidoptera being the represented orders. The most abundant species were *Apis mellifera* (Apidae), *Eristalis tenax* (Syrphidae), *Allograpta fuscotibialis* (Syrphidae), *Anthelia* (Tenthredinidae), *Musca domestica* (Muscidae), *Pontia helice* (Hesperiidae), *Anthophora* (Apidae), *Vanessa cardui* (Nymphalidae) and *Alcimus* (Asilidae). Among the observed species in NUL farm, *Apis mellifera* (Hymenoptera) had the highest relative abundance (26%) during both morning and afternoon periods, 16 visits in the morning and 10 visits in the afternoon. *Musca domestica* (order Diptera) also had a relatively high relative abundance (22%) during both time periods with 13 visits in the morning and 9 visits in the afternoon. While *Eristalis tenax* (Diptera), made 12 visits in the morning and 6 visits in the afternoon also presenting high relative abundance (18%). Whereas at Nyakosoba, *Apis mellifera* (honeybee) of order Hymenoptera had the highest relative abundance (18.5%) making 12 visits in the morning, 16 visits at 1PM, and 9 visits at 4PM. *Eristalis tenax* (order Diptera) had high relative abundance (12.3%) with 9 visits in the morning, 11 visits at 1PM, and 4 visits at 4PM. *Locris arithmetica* (order Hemiptera) also had a high relative abundance (10.8%) at all time periods with 7 visits made at each time. *Musca domestica* (order Diptera), showed an increase in visits from 2 visits in the morning to 9 visits at 1PM and 7 visits at 4PM, also presenting high relative abundance (9.2%). Floral visitors differed significantly in terms of their visitation frequency with *Stizus sp.* with (2.25±3.20 visits/flower/minute) being the most frequent, followed by *Apis mellifera* (1.63±2.09 visits/flower/minute). Stay time was highest for *Musca domestica* (300.00 ± 0 seconds), followed by *Stizus sp.* (152.50±170.35 seconds), *Anthophora sp.*, (95.00±120.21 seconds) and *Eristalis tenax* (94.25±104.65 seconds) (Table 3.5). while shortest time was spent by *Simulium sp.* (1.50 ±0 seconds) followed by *Phytomia sp.* (5.00 ±0 seconds) and *Apis mellifera* (14.04±35.30 seconds). Number of flowers visited per minute was high for *Stizus sp.* (3.25±3.86) and *Apis mellifera* (7.19±3.47). *Apis mellifera* (100%), *Anthophora sp.* (100%), *Simulium spp.* (100%), and *Musca domestica* (75%) were the highest pollen foragers. *Phytomia sp.* (100%), *Apis mellifera* (62.5%), *Eristalis tenax* (62.5%) and *Stizus sp.* (25%) showed preference for both pollen and nectar (Table 3.6.). Maximum number of pollen grains was deposited by *Apis mellifera* (128.75±31.65), followed by *Eristalis tenax* (24.13±4.31). Based on the overall pollinator effectiveness per day, honeybees and syrphid flies were found to be the most effective pollinator species.

Key words: *Diversity, abundance, visitation frequency, stay time, visitation rate, relative abundance, pollen or nectar feeding, pollen deposited.*

3.1. Introduction

Lesotho, a small mountainous country in Southern Africa, is characterized by its diverse agroecological zones, which include the foothills and lowlands. These zones support a variety of plant species, among which wild radish plays a significant ecological role. The foraging behavior and diversity of floral visitors to wild radish are crucial aspects of the plant’s ecology, impacting pollination, plant reproduction, and the overall biodiversity of the region (Potts *et al.*, 2010). Wild radish, a common herbaceous plant, serves as an essential component of the agricultural and natural landscapes in Lesotho. It not only contributes to soil health and ecosystem functioning but also supports a numerous insect species that rely on its flowers for nectar and pollen (Altieri, 1995). These floral visitors include bees, butterflies, beetles, flies and other pollinators, which play a pivotal role in the pollination process, affecting plant reproductive success and genetic diversity (Eardley, Gikungu, & Schwarz, 2009). However, some pollinators are more efficient at pollinating wild radish than others. For example, bees are more efficient at pollinating wild radish than flies. If bees are abundant in an area, they may outcompete other pollinators for access to wild radish flowers (Conner *et al.*, 1995).

A diverse community of pollinators is important for wild radish populations because it helps to ensure that pollination is not dependent on a single species of pollinator. If a single species of pollinator is lost or declines in abundance, the wild radish population may suffer (Katumo *et al.*, 2022; Manlik *et al.*, 2023). Abundant pollinator populations are also important for wild radish populations because they help ensure that pollination is efficient. When there are more pollinators, they can visit more flowers and transfer more pollen, which leads to more seeds being produced (Zou *et al.*, 2017; Zych *et al.*, 2013).

Moreover, pesticide use can also affect pollinator diversity and abundance. Pesticides can kill pollinators directly, or they can reduce the availability of nectar and pollen by killing or damaging the plants that pollinators rely on (Sponsler *et al.*, 2019). Plants that are in sunny, open areas may be more attractive to pollinators than plants that are located in shady wooded areas (Gorden, 2013) and larger plants may produce more nectar and pollen, which can attract more pollinators (Conner *et al.*, 1997; Conner *et al.*, 1995). The presence of other environmental stressors, such as drought or heat can weaken plants and make them less attractive to pollinators (Mareri *et al.*, 2022).

Lesotho’s foothills and lowlands exhibit distinct environmental conditions that influence the floral diversity and foraging patterns of insects (Department of Agricultural Research, Lesotho, 2017). The foothills, with their moderate climate and varied topography, contrast with the more uniform and often intensive agricultural landscapes of the lowlands. Understanding how these different zones impact the interactions between wild radish and its floral visitors is critical for ecological conservation and agricultural productivity (Donaldson, 2002).

Despite the recognized importance of pollinators in agroecosystems, there is significant lack of comprehensive studies focusing on the diversity and behavior of floral visitors in Lesotho (FAO, 2018). Current knowledge is fragmented, with most research being generalized across broader regions or specific to other plant species. Controversies arise regarding the extent to which agricultural practices, habitat fragmentation, and climate change are affecting pollinator populations and their foraging behavior (Ricketts *et al*., 2008). Additionally, the potential impacts of these factors on wild radish pollination in different agroecological zones remain poorly understood (Potts *et al*., 2010).

This study aims to fill the existing knowledge gaps by providing detailed insights into the diversity and foraging behavior of floral visitors to wild radish in the foothills and lowlands of Lesotho. By comparing these two distinct zones, the research will elucidate how environmental factors and anthropogenic influences shape pollinator communities and their interactions with wild radish. The findings will not only contribute to the conservation of pollinators and sustainable agricultural practices but also enhance our understanding of ecological dynamics within Lesotho’s diverse land scapes.

Therefore, this study assesses the diversity and abundance of floral visitors of wild radish in the foothills and lowlands of Lesotho. It further analyzes the foraging behavior of these visitors and investigates the impacts of agroecological differences on pollinator visitation rates. To achieve these objectives, the study tests the hypotheses that diversity of floral visitors of wild radish is higher in the foothills compared to the lowlands and that foraging behavior of floral visitors varies significantly between the foothills and lowlands.

3.2. Materials and Methods

3.2.1. Study sites

The study was carried out in foothills and lowland agroecological zones of Maseru district in Lesotho. The first site was Nyakosoba located in the foothills agro ecological zone, at an elevation of 2034.78 meters above sea level (masl). During the summer season from December to January, temperatures in Nyakosoba averages between a high of 25.5˚C in December and a peak of 26.1˚C in January to a low of 13.8˚C and 14.8˚C respectively. The highest rainfall average from 51mm in February to 71mm in December, hence making it the wettest period throughout the year. The second study site was National University of Lesotho faculty of Agriculture farm, Roma campus, in Lesotho, located in the lowlands agroecological zones at an elevation of 1610 masl. From December to January, temperatures in Roma averages between 26.1˚C in December and a peak of 27˚C in January to a low of 16˚C and 15˚C respectively. Roma typically receives about 83.47mm total rainfall. Nyakosoba had abandoned wheat in the field and a small area planted maize. Apple and peach trees were numerous. The environment in NUL farm comprised of farm animals and maize fields nearer to the experimental site.

3.2.2. Study design

Wild radish population naturally growing in a field that was lying fallow was used at Nyakosoba. During the growing season in October 2022, various areas within Agriculture research station were surveyed to identify potential study sites. Among these, a 100m^2 field was selected due to the high density of wild radish plants observed. This field was chosen based on the visible abundance of wild radish compared to other areas. In November 2022 we returned to the field to check the flowering status of the wild radish plants and observed that the majority of the plants had begun to flower, displaying their characteristic four-petaled flowers in shades of white, yellow, and sometimes light purple. In December we marked out four 1m x 1m quadrats within the 100m^2 field where wild radish plants were present. Due to uneven distribution of wild radish plants, the quadrats were placed in areas where these plants were found, ensuring that a sufficient number of plants were included for data collection (Figure 3.1). (Flower visitors were assessed using the methodology used by (Zameer *et al*., 2017). The flower visitors inside the quadrats were counted for 1 minute using a stopwatch. If an insect left the quadrat and came back in, they were counted again whereas the pollinators which visit different flowers inside the quadrat were counted once, and thus a total number of flower visitors present in 1 m^2 area for 1 min was assessed (Kumar *et al.*, 2019; Zameer *et al.*, 2017; Kumar *et al.*, 2020; Stanley *et al.*, 2017). To determine amount of pollen deposited, we used a total of twelve flowers, six being visited by *Apis mellifera* and the other six by *Eristalis tenax.* Only this 12 flowers were collected because bad weather disrupted the experiments which were meant to give more data the following day. The trial in Nyakosoba intended to understand ecological and agricultural aspects in Nyakosoba by looking at species diversity and abundance, foraging behavior, pollen dynamics, plant and pollinator interactions and adaptations, and to inform conservation and sustainable agricultural practices.

Figure 3.2: Widely spaced wild radish flowers in 1m^2 quadrat.

The trial at NUL farm was conducted using the seeds which were collected on 20th January 2023 at Nyakosoba from the previous season plants. The field from NUL farm was ploughed on the 26th January and disked on the 5th February. 16 Study plots were measured and marked within the field, each measuring 1m by 2m. Planting stations were placed and planting took place on the 9th February 2023 with 10cm intra-row and 50 cm inter-row spacing. Thinning was not done because the target was to attract pollinators and all germinated plants were used for data collection.

Flower density was estimated by counting all heads with open florets on daily basis before data collection. All open florets were used as a target to attract insects. Insects foraging on wild radish plants were collected using standard aerial net on the 6th March 2023, for identification, However, butterflies and flies were most often escaping the catch. Data were collected from 09:00 – 13:00 pm and afternoon time from 2:00-5:00 pm during every 2 hours (9 am, 11 am, 1 pm, 3 pm, and 5 pm) to accommodate different insects' visitation times during peak flowering period with 3 to 5 scoops directed by the present insects' population.

3.2.3. Data Collection

3.2.3.1 Diversity of insect pollinators visiting wild radish

Diversity of wild radish pollinators refers to number of different species of insects that visit wild radish flowers for pollen and nectar and in the process help with pollination. During peak flowering of wild radish, the insect floral visitors were observed visually for 30 minutes from the quadrats and were collected using a sweep net and kept in ethyl acetate-filled tubes for storage. These were later pinned and identified with the help of goggle, insect field guide, and taxonomic literature specific to the insect group (Picker *et al*., 2019; Scholtz *et al*., 2008). Photographs of specimens were taken using Dino Capture 2.0 (Digital microscope) to show several characters. Insects were further identified by comparing their morphological features, looking at their body length and size, color patterns, wing venation, abdomen and thorax, structure of legs, and eyes characteristics. Where specimens could not be identified to species level, they were named by morpho-species and classified under the order and family which they showed similar characteristics to

3.2.3.2 Abundance of pollinators visiting wild radish

Abundance of pollinators refers to the large quantity and variety of pollinating insects in a given area. Abundance of the insect pollinators was recorded on wild radish plants found in Nyakosoba during walks between quadrats and from the sixteen experimental plots at the National University of Lesotho farm site.

Relative abundance was calculated as the numbers of individuals of a particular species captured relative to the total number of species in the area. It was used to show species composition of pollinator insects.

Relative abundance (%) = $(n/N) * 100$

Where,

n = Number of each individual species.

N = Total number of individuals captured

3.2.3.3 Pollen deposition per single visit

The pollen transfer potential was measured as the amount of pollen grains dropped on the stigma per single visit, irrespective of pollination attainment. To protect the stigma from predation by insects before data collection and experimentation, floral buds were covered with nylon mesh bags each evening, weather permitting, by covering single branch (Divija *et al.*, 2022). This was not done throughout the study period because animals and unpredictable weather were a thread to the experiments often. The confined buds were carefully unveiled individually throughout the highest pollinators action period between 0900hr and 1400hr. Then the stigmas from unveiled flowers were carefully removed using tweezers after a single insect visit and fixed in 70% Ethanol and taken to the lab for further analysis (Divija *et al.*, 2022; Zameer *et al.*, 2017; Dafni, 1992). The insects which landed on the flower for the first single visit were closely observed for the duration of visits, visitor identity and all information was recorded by help of photographs and stopwatch.

3.2.3.4 Foraging Behavior

Scavenging activities of pollinators on the flowers remained documented based on the stay time, visitation rate, visitation frequency plus nectar or pollen searching pattern (Ali *et al.*, 2011; Ali *et al.*, 2022). The stay time was estimated using a stopwatch as per the period spent by a single insect on each bloom, it was timed focused on the honeybees (Apidae) and Syrphid flies (Syrphidae) as they were frequent visitors in the present study similar to studies of Young *et al.*, 2007 and Sharma *et al.*, 2012. Maximum observation period for stay time was 5mins per quadrat (Sharma *et al.*, 2016). Visitation rate was recorded as the average number of visits made by a pollinator to a flower in one quadrat or the plot for every minute. While visitation frequency was recorded as the number of visits made by pollinators to the flowers inside the quadrat or the plot. Visual observations were made carefully between 9:00 AM and 5:00 PM to understand if pollinators visited to collect nectar, pollen or both (Ali *et al.*, 2011; Ali *et al.*, 2022; Divija *et al.*, 2022; Younas *et al.*, 2022). The visitor behavior on the flower, that is the nature of pollen or nectar feeding: legitimate (via the corolla mouth) or robbing (chewing a hole through the corolla) was also assessed.

3.2.6. Pollen counting and analysis.

The hemocytometer was used to tally the pollen particles by firstly suspending pollen particles attached on the stigma, anther and insects' bodies in 3ml of 70 % ethanol and vortex to guarantee uniform fraternization. At that point a sample of pollen sediment was taken by a pipette then placed on the hemocytometer. The stereoscopic microscope with 40 × and 100 × magnification was then used to sum pollen particles (Divija *et al*., 2022; Sharma *et al*., 2016; Dafni, 1992; Delaplane *et al*., 2015). To count the number of pollen grains, each grid was counted systematically using a hand tally counter from a corner of the counting chamber. Only fully visible pollen grains were counted.

3.2.7. Data analysis

Shannon-Wiener Diversity index (H') was used to measure diversity of species in Nyakosoba and NUL farm (Ali *et al*., 2022; Divija *et al*., 2022). Cross tabulation was used to compare pollinators abundance and to determine pollinators abundance based on their different visitation times. Descriptive statistics was used to give a clear summary of the data and ANOVA was used to compare the means between visitation rate, visitation frequency and time spent on flowers by the various floral visitors. Post hoc tests were not performed because at least one group had fewer than two cases. Cross tabulation was further used to assess species according to their pollen or nectar feeding habits. ANCOVA was used to associate the mean amount of pollen deposited with insect species and pollinators' stay time on the flower, enhancing the accuracy and interpretability of statistical analyses by accounting for covariate of effects. All arithmetical analysis was done by using SPSS (v 20) (Younas *et al*., 2022).

3.3. Results

3.3.1. Diversity and Abundance of floral visitors

The diversity and abundance of wild radish pollinators in NUL farm (Table 3.1) show that eighteen species of insect pollinators were identified and characterized to species level during the study. They belonged to four major orders of Hymenoptera, Diptera, Lepidoptera and Coleoptera. Out of the eighteen species, only six were recorded frequently during the observations. These were *Apis mellifera* (Apidae), Morpho-species 2 (Apidae), *Musca domestica* (Muscidae), *Eristalis tenax* (Syrphidae), *Eristalinus sp.* (Syrphidae), Morpho-species (1) (Apidae).

The remaining species, which were rare during systematic observation included nine morpho-species of order Diptera (Muscidae), Nine *Stomoxys calcitrans* species (Muscidae),four morpho-species of order Diptera (Muscidae), six morpho-species of order Diptera (Syrphidae), four *Astylus atromaculatus* species of order Coleoptera (Melyridae), three *Leucilia* spp. of order Diptera (Calliphoridae), three *Anthophora* species of order Hymenoptera (Apidae), two *Osmia* species of order Hymenoptera (Megachilidae), one *Systoechus sp.* of order Diptera (Bombyliidae), one *Neolophonotus* species of order Diptera (Asilidae), one morpho-specie of order Hymenoptera (Colletidae),one morpho-specie of order Hymenoptera (Apidae), one *Anthelia* specie of order Hymenoptera (Tenthredinidae), one morpho-specie of order Hymenoptera (Colletidae), one morpho-specie of order Coleoptera, one *Vanessa cardui* species of order Lepidoptera (Nymphalidae), and one *Colias electo* specie of order Lepidoptera (Pieridae).

A total of one hundred and fifty individuals (Table 3.1) of all insect families were recorded, of which 39.33% were Apidae, 32% Syrphidae, 17.33% Muscidae, and 2.67% Melyridae, 2% Calliphoridae, and Colletidae, 1.33% percent Megachilidae with the remaining 0.67% comprising of Asilidae, Bombyliidae, Tenthredinidae, Nymphalidae and Pieridae. The most abundant family was Apidae *(*59 individuals) followed by Syrphidae (32 individuals), and Muscidae with (26 individuals); comprising 39.33, 32, and 17.33 percent of overall pollinator abundance.

Table 3.2: Diversity and abundance of insect pollinators in NUL Farm.

Order	Family	Genus/ Species	Key distinguishing features	No. of specimens.	References.
Diptera	Muscidae.		*Anal vein of Muscidae never reaches the wing margin. *The under surface of the scutellum is usually without hairs.	26	*Picker et al., 2019 *www.diptera.info,wikimediaCommons *Scholtz et al., 2008

			*The fold of the second anal vein does not curve around the apex of the anal vein.		
		Musca domestica.	*The head of the adult fly has reddish-eyes and sponging mouthparts. *The thorax bears four narrow black stripes *There is a sharp upward bend in the fourth longitudinal wing vein. *The abdomen is grey or yellowish with dark midline and irregular dark markings on the sides. *A1 never reaching wing margin. *R4+5 and M1 parallel-sided or M1 bent forward to meet R4+5 apically.	21	*Picker et al., 2019 
		Stomoxys calcitrans	*They have a hard black proboscis. *Has a grey abdomen with the black dorsal patches.	5	*Picker et al., 2019 

	Anthomyiidae.	*Anthomyia parapluvialis sp.*	*Transverse postsutural band separate into three spots or narrowly joined. *Presutural acrostichal setae closer. Anterior katepisternal seta longer than in *tempestatum*. *Long ovate abdomen, tapering from 4th to 3rd tergite. *t3 with 1 av, 5 ad, 2 pd and only 3-4 short p setae. *5th sternite with the membranious lobe on processes apically placed, projecting caudally.	9	 *Ackland, 2001
		Morph species 2	*	4	
	Syrphidae.		*Syrphidae are medium to large in size and are often rather colorful black and yellow. *The head and body are usually without bristles and males are usually holoptic.	48	*Scholtz et al., 2008

			*A very characteristic spurious vein occurs between the radial and medial sectors, the r5 cell being closed. *Many syrphids bear a strong mimetic resemblance to wasps and bees.		
		Eristalinus	*Prosoma bears a pair of antennae, which are divided into the scape, the pedicle and the flagellum from base to end. *The upper part of compound eye has dense dark brown short fluff and lower part nearly naked. *At the top of the head are three ocelli arranged in triangle *Five yellow-grey stripes extend from the front end to the posterior end, with a thin one in the middle and a transverse stripe at the posterior end. *The scutellum is pale yellow or bright brown, sometimes with metallic gloss, with shorter black hair in the middle and long yellow hair on the sites and the end.	12	*Picker et al., 2019 *Cao et al., 2022 

		Eristalis	*Fore wings have a floating or false vein (SV-s). *Black eyes and an hour glass-shaped black pattern on the first section of abdomen. *A broad, dark stripe running down the centre of the face. *Has a distinctively enlarged and curved hind tibia. *The ground colour is darkish brown and the body covered in fine hairs.	24	*Picker et al., 2019 *https://petehillmansnaturephotography.wordpress.com/drone-fly-eristalis-tenax 
		Eupeodos sp.	* Has light brown antennae *Scutellum is yellow to light brown. *Abdomen is oval in shape. *Abdominal area is interspersed with black stripes beginning from the Scutellar in a shape of a letter Y forming two yellow spots on both sides of abdomen.	2	 Sahar et al., 2024
		Morphospecies 3	*	4	

	Calliphoridae.		*They are mostly stoutly built and of medium size. *Many are metallic blue or green in colour. *The outer post-humeral seta is situated laterally with respect to the pre-sutural seta and the erista is plumose. *The eyes of the male are holoptic or closely approximated, eyes of the female are separated. *The hypo pleural setae are present.	3	*Scholtz et al., 2008
		Leucilia	*Posterior slope of humeral callus with 6-8 hairs. *Surface of noto-pleuron between last noto-pleural seta and edge of noto-pleuron with 8-16 hairs. *Central occipital area with 2-8 setulae below each inner vertical seta. *Uniformly green, with bronze cast on thorax. *Leucilia species have 3 pairs of acrostichal bristles between the scutellum and the transverse sutuae of mesonotum.	3	*Lutz et al., 2017 *Picker et al., 2019 

			*Leucilia body is slender, the head is small and the cheeks are silvery and rather smooth.		
	Asilidae		*The top of the head is hollowed out between the eyes. *There are three ocelli. *The third antennal segment is usually elongate and often bears a short terminal arista or style. *The body varies considerably from very hairy to bare, but the face is covered with well-developed setae.	1	*Scholtz et al., 2008
		Neolophonotus	*Thorax often very humped and with black median stripe. *Abdomen elongate and banded in stripes of black or grey. *Legs virtually originating at same level to capture and hold preys. *Antenna positioned in dorsal ½ of head. *Fore- and mid coxa positioned close together. * All ocelli circular, placed on single ocellar triangle.	1	*Picker et al., 2019 *Dikow et al., 2023 

			*Proepisternum fused to lateral postpronotum.		
	Bombyliidae	*Systoechus sp.*	*They are medium to large insects. *They are stout bodied and hairy, *The third antennal segment is variable is shape. *Vein M1 ends behind the wing tip. *There are three or four posterior cells. *The discal cell is present. *The anal cell may be open or closed.	1	*Scholtz et al., 2008 
Hymenoptera.	Tenthredinidae		*Fairly small symphyte wasps 5-8mm long. *Stout bodied and slow moving. *The head and thorax are most often black and shiny. *The legs are yellow. *The legs have dark bands at the joints of the segments. *The wings are distinctly infused. *The antennae usually have 10-13 (rarely 9nine) subclavate segments.	1	*Scholtz et al., 2008

		Athalia/ Anthelia	*Stout-bodied, and lack wasp waist between thorax and abdomen. *Black with yellow abdomen and legs. *Antennae have 10-13 segments.	1	*Picker et al., 2019 
	Megachilidae .		*Megachilidae are small to large (3-22mm) bees. *Their labrum is longer than broad. *The sub antennal sutures are directed towards the outer margins of the antennal sockets. *There are two sub-marginal cells in the fore wing. *The scopa when present occurs on the metasomal sterna.	2	*Scholtz et al., 2008 *Picker et al., 2019
		Osmia sp.	*The scutellum hides the metanotum when seen from above. *The scutellum posterior margin reaches the same level as the metanotum or even overhangs it when seen in profile.	2	*Muller, (2018). 

	Colletidae		*Colletid bees are usually either very hairy or almost smooth. *They are short tongued bees that can be recognized by shape of the apex of the glossa. *The glossa is rounded, truncate, bilobed or bifid and never acute as in all other bees. *They are mostly black or dark-coloured and have a wasp like appearance. *Hair on the abdomen are often arranged in conspicuous bands.	2	*Picker et al., 2019 *Scholtz et al., 2008
		Morphospecies 1	*	2	
	Apidae		*The prepalpal portion of the galea is either as long as, or longer than, the postpalpal portion. *The mentum and submentum are virtually absent. *They have only one subantennal suture directed towards the mesal margin of each antennal socket.	59	*Picker et al., 2019 *Scholtz et al., 2008

			*They have strongly curved basal vein in the fore wing. *The epistomal suture is either complete or incomplete.		
		Morphospecies 1	*	1	
		Morphospecies 2	*	12	
		Apis mellifera.	**Apis mellifera* have airs on head and thorax including long hairs coming out of eyes. *The integument on the abdomen has stripes with colour variations ranging from light orange to dark brown or black. *Female workers have hind legs with wide, flattened plates for the corbiculae.	43	*Picker et al., 2019 *Scholtz et al., 2008 *Mason et al., 2022 

			*Basal segments of abdomen more or less banded in reddish-brown.		
		Anthophora sp.	*A black or yellowish furry bee. *Head and thorax densely covered with grey hairs sometimes brownish on thorax. *Legs fringed with grey hairs. *Antennae black. *Abdomen with bands of grey hairs. *First recurrent vein joining posterior margin of SM2 near midpoint, never interstitial with vein between SM2 and SM3. *Anterior and posterior margins of sub marginal cell 3 more nearly equal in length. *Marginal cell is shorter than combined length of sub marginal cells along their posterior margins.	3	*Picker et al., 2019 *Brooks (1986) 
Lepidoptera.	Nymphalidae.		*The fore tarsi of the adult are brush like and reduced to a single elongate segment in the male, but with five segments in the female. *Claws may be present in either sex. *The antennae are always scaled.	1	*Scholtz et al., 2008

			*The labial palpi are ascending and the maxillay palpi are one-segmented. *The discal cell of the fore wing is usually not closed, and that of hind wing is never closed by a tubular vein, although sometimes by a weak vestigial or non-tubular vein. *The terminal margins of the wings are often scalloped, crenulated or denate.		
		Vanessa cardui	**Vanessa cardui* are medium sized. *Mostly orange with black markings. *Tip of fore wings black, with several white markings and a broken black bar across orange area of wing. *Hind wings with 3 rows of black spots parallel to outer margins. *Underside of wings cryptically coloured, with a row of eyes spots parallel to outer margins.	1	*Picker et al., 2019 
	Pieridae		*Most butterflies of Pieridae family usually have white or yellow black-margined wings and sometimes with red and yellow patterns beneath. *The hind wings are never tailed.	1	*Scholtz et al., 2008

			*They do not have an epiphysis on the fore tibia. *They have bifid tarsal claws. *There are two anal veins in the hind wing.		
		Colias electo	*Male orange, with broad brownish-black border to both wings. *Males have a black spot at the end of the fore wing cell. *In females, orange areas paler, sometimes almost white, and suffused with brown on basal areas of wings. *Wing borders are black with a few yellow or white markings. *Underside of wings green or greenish-yellow with a row of brown spots near the margins and a white central spot.	1	*Picker et al., 2019 
Coleoptera	Melyridae		*The melyrids are small, mostly soft-bodied, elongate ovoid and dorsally flattened beetles. *The body is covered with long, erect setae. *The antennae are ten or 11 segmented, inserted anteriorly on the frontal production, distant from the eyes.	4	*Scholtz et al., 2008

			*Antennae are filiform, serrate or flabellate, the basal segments are modified in males. *The pronotum has distinct side edges. *The tarsi are five-segmented, with segment 4 simple or bilobed. *The tarsal claws are simple or appendiculate and are often each with a fleshy appendage beneath. *The elytra are smooth, never striate and sometimes shortened exposing several of the apical abdominal tergites.		
		Astylus atromaculatus	*Head and antennae black. *Pronotum with flat-lying white hairs and two black patches. *Body covered with black scattered, erect black hairs. *Elytra yellow, with large black patches.	4	*Picker et al., 2019 

A total of twenty-four species of wild radish pollinators were recorded for the duration of the study in Nyakosoba (Table 3.2.). Nine species were frequently recorded during the observations and these were: *Apis mellifera* (Apidae), *Eristalis tenax* (Syrphidae), *Allograpta fuscotibialis* (Syrphidae), *Anthelia* (Tenthredinidae), *Musca domestica* (Muscidae), *Pontia helice* (Hesperiidae), *Anthophora* (Apidae), *Vanessa cardui* (Nymphalidae) and *Alcimus* (Asilidae).

The remaining (15) species which were not frequent were: 4 species of *Stomoxys calcitrans*, 4 Morpho-species of order Diptera (Muscidae),4 *Melanostoma spp.* Diptera (Syrphidae), 4 *Anthomyia parapluvialis sp.* order Diptera (Calliphoridae), 2 species of *Chrysomya* (Calliphoridae), 3 morpho-species of order Diptera (Muscidae), 2 morpho-species of order Hymenoptera (Halictidae), and 2 morpho-species of order Lepidoptera (Hesperiidae), 1 *Leucilia* species of order Diptera (Calliphoridae), 1 morpho-specie of order Diptera (Bombyliidae), 1 *Xylocopa caffra* specie of order Hymenoptera (Apidae), and 1 morpho-specie of order Hymenoptera (Apidae).

A total of 229 individuals (Table 3.2.) of all the insect species were recorded, of which were *Apis mellifera* (Order: Hymenoptera) 99 (43.23%) and *Eristalis tenax* (Order: Diptera: Syrphidae) 50 (21.83%), followed by *Anthelia* (Order: Hymenoptera: Tenthredinidae) 10 (4.37%), *Musca domestica* (Order: Diptera: Muscidae) 10 (4.37%), *Pontia helice* (Order: Lepidoptera: Hesperiidae) 10 (4.37%), and *Allograpta fuscotibialis* (Order: Diptera, Family: Syrphidae) 8 (3.49%) were observed.

The other species which recorded the lowest abundance were *Vanessa cardui* (Order: Lepidoptera: Nymphalidae) 5 (2.18%), *Anthophora* (Order: Hymenoptera, Family: Apidae) 5 (2.18%), *Alcimus sp.* (Order: Diptera) 5 (2.18%), *Stomoxys calcitrans* (Order: Diptera: Muscidae) 4 (1.75%), *Anthomyia parapluvialis sp.* (Order: Diptera: Anthomyiidae) 4 (1.75%), Morpho-species 1 (Order: Diptera: Asilidae) 2 (0.87%), Morpho-species 1 (Order: Hymenoptera: Halictidae) 2 (0.87%), *Melanostoma mellinum* (Order: Diptera: Syrphidae) 2 (0.87%), *Melanostoma sp.* (Order: Diptera: Syrphidae) 2 (0.87%), *Chrysomya sp.* (Order: Diptera: Calliphoridae) 2 (0.87%), *Systoechus sp.* (Order: Diptera: Bombyliidae) 1 (0.44%), Morpho-species 1 (Order: Hymenoptera: Apidae) 1 (0.44%), *Xylocopa caffra* (Order: Hymenoptera: Apidae) 1 (0.44%), Morpho-species 1 (Order:

Lepidoptera: Hesperiidae) 1 (0.44%), Morpho-species 2 (Order: Lepidoptera: Hesperiidae) 1 (0.44%), and *Leucilia sp.* (Order: Diptera: Calliphoridae) 1 (0.44%).

The most abundant species (Table 3.2.), were *Apis mellifera* (99 individuals) followed by *Eristalis tenax* (50), *Anthelia* (10), *Pontia helice* (10), and *Allograpta fuscotibialis* with (8 individuals), encompassing 43.23, 21.83 and 4.37 percent of total pollinator abundance, respectively.

Table 3.2: Diversity and Abundance of insect pollinators in Nyakosoba.

Order	Family	Genus/ Species	Key distinguishing features	No. of specimens.	References.

Diptera	Muscidae		*Anal vein of Muscidae never reaches the wing margin. *The under surface of the scutellum is usually without hairs *The fold of the second anal vein does not curve around the apex of the anal vein.	20	*Picker et al., 2019 *www.diptera.info,wikimediaCommons *Scholtz et al., 2008

		Musca domestica.	*The head of the adult fly has reddish-eyes and sponging mouthparts. *The thorax bears four narrow black stripes *There is a sharp upward bend in the fourth longitudinal wing vein. *The abdomen is grey or yellowish with dark midline and irregular dark markings on the sides. *A1 never reaching wing margin. *R4+5 and M1 parallel-sided or M1 bent forward to meet R4+5 apically.	10	*Picker et al., 2019 
		Stomoxys calcitrans	*They have a hard black proboscis. *Has a grey abdomen with the black patches.	4	*Picker et al., 2019 

	Anthomyiidae.	*Anthomyia parapluvialis sp.*	*Transverse postsutural band separate into three spots or narrowly joined. *Presutural acrostichal setae closer. Anterior katepisternal seta longer than in *tempestatum*. *Long ovate abdomen, tapering from 4th to 3rd tergite. *t3 with 1 av, 5 ad, 2 pd and only 3-4 short p setae. *5th sternite with the membranious lobe on processes apically placed, projecting caudally.	4	 Ackland et al., 2001
		Morphospecies 3	*	2	

	Anthomyiidae	*Anthomyia parapluvialis sp.*	*Transverse postsutural band separate into three spots or narrowly joined. *Presutural acrostichal setae closer. Anterior katepisternal seta longer than in *tempestatum*. *Long ovate abdomen, tapering from 4th to 3rd tergite. *t3 with 1 av, 5 ad, 2 pd and only 3-4 short p setae. *5th sternite with the membranious lobe on processes apically placed, projecting caudally.	9	 *Ackland, 2001

	Syrphidae.		*Syrphidae are medium to large in size and are often rather colourful black and yellow. *The head and body are usually without bristles and males are usually holoptic. *A very characteristic spurious vein occurs between the radial and medial sectors, the r5 cell being closed. *Many syrphids bear a strong mimetic resemblance to wasps and bees.	62	*Scholtz et al., 2008
		Allograpta fuscotibialis	*Abdomen parallel-sided with yellow bands. *Thinner than those of *Asarkina*. *First band is divided. *Thorax largely shiny black.	8	*Picker et al., 2019 

		Melanostoma mellinum.	*Small hover fly with yellow and black markings. *Scutellum and face are dark. *Males have square yellow abdominal markings with tergite 2 and 3 being as long as wide.	2	 *Ball *et al.*, 2023
		Melanostoma sp.	*Face with a distinct tubercle. *Abdominal terga with large yellow abdominal markings. *Male surstylus curved towards ventral part. *Male abdomen almost entirely orange with apical margins narrowly black and genitalia segments black.	2	 *Ramage *et al.*, 2018

		Eristalis	*Fore wings have a floating or false vein (SV-s). *Black eyes and an hour glass-shaped black pattern on the first section of abdomen. *A broad, dark stripe running down the centre of the face. *Has a distinctively enlarged and curved hind tibia. *The ground colour is darkish brown and the body covered in fine hairs.	50	*Picker et al., 2019 *https://petehillmansnaturephotography.wordpress.com/drone-fly-eristalis-tenax 

	Calliphoridae.		*They are mostly stoutly built and of medium size. *Many are metallic blue or green in colour. *The outer posthumeral seta is situated laterally with respect to the presutural seta and the erista is plumose. *The eyes of the male are holoptic or closely approximated, eyes of the female are separated. *The hypopleural setae are present.	6	*Scholtz et al., 2008

		Calliphora sp.	* They are mostly stoutly built and of medium size. *They are metallic blue in colour. *The outer posthumeral seta is situated laterally with respect to the presutural seta and the erista is plumose. *The eyes of the male are holoptic or closely approximated, eyes of the female are separated. *The hypopleural setae are present.	3	 *Scholtz et al., 2008

		Leucilia	*Posterior slope of humeral callus with 6-8 hairs. *Surface of notopleuron between last notopleural seta and edge of notopleuron with 8-16 hairs. *Central occipital area with 2-8 setulae below each inner vertical seta. *Uniformly green, with bronze cast on thorax. *_Leucilia_ species have 3 pairs of acrostichal bristles between the scutellum and the transverse sutuae of mesonotum. *_Leucilia_ body is slender, the head is small and the cheeks are silvery and rather smooth.	1	*Lutz et al., 2018 *Picker et al., 2019 

		Chrysomya	*A central cluster of sensilla is surrounded by a ring of several semicircular pro-tuberance. *A number of orbital are usually present in front of the prevertical seta. *There is a bread and strongly white-dusted vitta that extends very far forwards along the midline, almost to the front end of the mesonotum where it ends in a blunt point. *Hairs on general dilation and postgena orange, anterior spiracle brownish-black. *Frons of female with fronto-orbital plate dark. *Lower calypter brownish with dark rim. *Have single pair of acrostichid bristles. *The body is stout, head is large and the cheeks are yellowish and conspicuously hairy.	2	*Rognes et al., 2005 *Lutz et al., 2018 

	Asilidae	*Neolophonotus sp.*	*The top of the head is hollowed out between the eyes. *There are three ocelli. *The third antennal segment is usually elongate and often bears a short terminal arista or style. *The body varies considerably from very hairy to bare, but the face is covered with well-developed setae.	5	*Scholtz et al., 2008 
	Bombyliidae	*Systoechus*	*They are medium to large insects. *They are stout bodied and hairy, *The third antennal segment is variable is shape. *Vein M1 ends behind the wing tip. *There are three or four posterior cells. *The discal cell is present. *The anal cell may be open or closed.	1	*Scholtz et al., 2008 

Hymenoptera.	Tenthredinidae.		*Fairly small symphyte wasps 5-8mm long. *Stout bodied and slow moving. *The head and thorax are most often black and shiny. *The legs are yellow. *The legs have dark bands at the joints of the segments. *The wings are distinctly infused. *The antennae usually have 10-13 (rarely 9nine) subclavate segments.	10	*Scholtz et al., 2008
		Athalia/ Anthelia	*Stout-bodied, and lack wasp waist between thorax and abdomen. *Black with yellow abdomen and legs. *Antennae have 10-13 segments.	10	*Picker et al., 2019 

	Halictidae	Morphospecies 1.	*The prepalpal portion of the galea is either as long as, or longer than, the postpalpal portion. *The mentum and submentum are virtually absent. *They have only one subantennal suture directed towards the mesal margin of each antennal socket. *They have strongly curved basal vein in the fore wing. *The epistomal suture is either complete or incomplete.	2	*Picker et al., 2019 *Scholtz et al., 2008 

	Apidae.		*The prepalpal portion of the galea is either as long as, or longer than, the postpalpal portion. *The mentum and submentum are virtually absent. *They have only one subantennal suture directed towards the mesal margin of each antennal socket. *They have strongly curved basal vein in the fore wing. *The epistomal suture is either complete or incomplete.	106	*Picker et al., 2019 *Scholtz et al., 2008

		Apis mellifera.	*_Apis mellifera_ have airs on head and thorax including long hairs coming out of eyes. *The integument on the abdomen has stripes with colour variations ranging from light orange to dark brown or black. *Female workers have hind legs with wide, flattened plates for the corbiculae. *Basal segments of abdomen more or less banded in reddish-brown.	99	*Picker et al., 2019 *Scholtz et al., 2008 
		Xylocopa caffra	*Female black and hairy with two bands of yellow hairs on last part of the thorax and first segment of the abdomen.	1	*Picker et al., 2019 

		Anthophora	*A black or yellowish furry bee. *Head and thorax densely covered with grey hairs sometimes brownish on thorax. *Legs fringed with grey hairs. *Antennae black. *Abdomen with bands of grey hairs. *First recurrent vein joining posterior margin of SM2 near midpoint, never interstitial with vein between SM2 and SM3. *Anterior and posterior margins of submarginal cell 3 more nearly equal in length. *Marginal cell is shorter than combined length of submarginal cells along their posterior margins.	5	*Picker et al., 2019 *Brooks, (2017) 
		Morphospecies 1	*	1	

Lepidoptera.	Nymphalidae		*The fore tarsi of the adult are brush like and reduced to a single elongate segment in the male, but with five segments in the female. *Claws may be present in either sex. *The antennae are always scaled. *The labial palpi are ascending and the maxillay palpi are one-segmented. *The discal cell of the fore wing is usually not closed, and that of hind wing is never closed by a tubular vein, although sometimes by a weak vestigial or non-tubular vein. *The terminal margins of the wings are often scalloped, crenulated or denate.	5	*Scholtz et al., 2008

		Vanessa cardui	**Vanessa cardui* are medium sized. *Mostly orange with black markings. *Tip of fore wings black, with several white markings and a broken black bar across orange area of wing. *Hind wings with 3 rows of black spots parallel to outer margins. *Underside of wings cryptically colored, with a row of eyes spots parallel to outer margins.	5	*Picker et al., 2019 

	Hesperiidae	Morpho-species	*It has variable mottled grey color *Adults have all three pairs of legs developed for walking. *The tarsi have normal five segments in both sexes and apical claws. *The inner side of the fore tibia bears the epiphysis. *The head is very broad with prominent smooth eyes. *Antennae are widely separated at the base and they are gradually thickened to form a club that is usually hooked. *The wing patterns are usually sombre. *The fore wing is distinctly triangular in shape. *Hind wings are may be rounded, sometimes with the anal angle slightly produced. *Wing venation is remarkably constant throughout the family and relatively simple, all peripheral veins	1	*Scholtz et al., 2008 

			arising separately from the base of discal cell from both wings.		
		Pontia helice	**Pontia helice* male have upper side white, with squarish brown or black markings at the tip of the fore wing, another at the end of fore wing cell. *Small dark markings on outer margin of the hind wings. *Female has more extensive black markings and a broad dark outer margin of the hind wings, enclosing white spots. *Undersides of hind wings has yellow and white markings and the veins are outlined in brown.	10	*Picker et al., 2019 
		Morphospecies 1	*		

3.3.2. Relative abundance and daily visitation times of pollinators

The relative abundance and visitation times of various insect pollinators at NUL farm is presented in (Table 3.3). Among the observed species, *Apis mellifera* (Hymenoptera) had the highest relative abundance (26%) during both morning and afternoon periods, 16 visits in the morning and 10 visits in the afternoon. *Musca domestica* (order Diptera) also had a relatively high relative abundance (22%) during both time periods with13 visits in the morning and 9 visits in the afternoon. *Eristalis tenax* (Diptera), made 12 visits in the morning and 6 visits in the afternoon also presenting high relative abundance (18%). *Astylus atromaculatus* had 2 visits in the morning and 4 in the afternoon while *Anthophora* (Hymenoptera) did not visit any flowers in the morning and only visited twice in the afternoon. *Simoides crassipes* (Diptera) had consistent number (3) of visits throughout the day and *Ischiodon scuttelaris* (Diptera) also had consistent number (4) of visits both in the morning and afternoon. Species such as *Colias electo* (Lepidoptera), *Eristalinus* (Diptera), and *Vanessa cardui* (Lepidoptera) showed low relative abundance (2%) with 1 visits during both time periods.

Table 3.3: Visitors at different times in NUL.

Species		Number of flower visitors at various times			
	Order	0900-1300	1400-1700	Total (species)	Relative abundance (%)
Musca domestica	Diptera	13	9	22	22
Apis mellifera	Hymenoptera	16	10	26	26
Astylus atromaculatus	Coleoptera	2	4	6	6
Simoides crassipes	Diptera	3	3	6	6
Ischiodon scuttelaris	Diptera	4	4	8	8
Eristalis tenax	Diptera	12	6	18	18
Colias electo	Lepidoptera	1	1	2	2
Hilarempis sp.	Diptera	1	1	2	2
Vanessa cardui	Lepidoptera	1	1	2	2

Chrysomya sp.	Diptera	1	3	4	4
Anthophora	Hymenoptera	0	2	2	2
Eristalinus	Diptera	1	1	2	2
Total		50	50	100	100

As observed from (Table 3.4.). *Alcimus sp.*, *Peridroma sp.,* and *Chrysomya* species of Diptera had relatively consistent presence throughout the day, making 3 visits at each time period. *Eristalinus sp.* (order Diptera) had a low relative abundance (6.2%) making 4 visits at each period. *Eristalis tenax* (order Diptera) showed an increase in relative abundance (12.3%) from 9 visits in the morning, 11 visits at 1PM, and 4 visits at 4PM. *Leucilia sp.* (order Diptera) made 2 visits in the morning and 1 visit in the afternoon. *Locris arithmetica* (order Hemiptera) had a relatively high relative abundance (10.8%) at all time periods with 7 visits made at each time. *Musca domestica* (order Diptera), showed an increase in visits from 2 visits in the morning to 9 visits at 1PM and 7 visits at 4PM, also presenting high relative abundance (9.2%). *Apis mellifera* (honeybee) of order Hymenoptera had the highest relative abundance (18.5%) making 12 visits in the morning, 16 visits at 1PM, and 9 visits at 4PM.

Athalia and *Anthophora sp.* (Hymenoptera) had a consistent presence with 2 visits throughout the day. *Amegilla cingulata* and Morpho-species 1 (Apidae), *Seladonia sp.,* Morpho-species 2, and *Delta sp.,* (order hymenoptera) had a consistent presence of 1 visit at each time of the day. *Xylocopa caffra* (hymenoptera) made 1 visit in the morning, 2 visits at 1PM and no visits at 4 PM. Moreover, *Systoechus* of order Diptera (Bombyliidae), did not have consistent visits as it did not visit flowers in the morning, and had 2 appearances at 13PM, while only 1 individual visited at 4PM. *Vanessa cardui* (order Lepidoptera) showed relatively high relative abundance (6.2%) with 6 visits in the morning, 2 visits at 1PM and 4 visits at 4PM. *Pontia helice* (order Lepidoptera), had a consistent presence with 1 visit at each period.

Table 3.4: Visitors at different times in Nyakosoba.

Species	Order	Number of flower visitors at various times	Total from 16	Relative abundance(%)

		9AM	13PM	4PM		
Alcimus sp.	Diptera	3	3	3	9	4.6
Amegilla cingulata	Hymenoptera	1	1	1	3	1.5
Athalia	Hymenoptera	2	2	2	6	3.1
Anthophora sp.	Hymenoptera	2	2	2	6	3.1
Apis mellifera	Hymenoptera	12	16	9	37	18.5
Peridroma sp.	Diptera	3	3	3	9	4.6
Chrysomya sp.	Diptera	3	3	3	9	4.6
Morpho-species 2	Hymenoptera	1	1	1	3	1.5
Eristalinus sp.	Diptera	4	4	4	12	6.2
Eristalis tenax	Diptera	9	11	4	24	12.3
Leucilia sp.	Diptera	2	0	1	3	1.5
Locris arithmetica	Hemiptera	7	6	7	20	10.8
Musca domestica	Diptera	2	9	7	18	9.2
Pontia helice	Lepidoptera	1	1	1	3	1.5
Seladonia sp.	Hymenoptera	1	1	1	3	1.5
Systochus sp.	Diptera	0	2	1	3	1.5
Stomoxys calcitrans	Diptera	1	1	1	3	1.5
Syrphus sp.	Diptera	1	1	1	3	1.5
Vanessa cardui	Lepidoptera	6	2	4	12	6.2
Delta sp.	Hymenoptera	1	1	1	3	1.5
Morpho-species-1	Hymenoptera	1	1	1	3	1.5
Xylocopa caffra	Hymenoptera	1	2	0	3	1.5
Total		64	73	58	195	100

3.3.3 Foraging behavior.

The foraging behavior of all groups of pollinators was recorded in terms of flowers visited at varying frequencies, different visitation rates, and time spend on the bloom (Table 3.6.).

3.3.3.1 Visitation frequency.

The analysis of variance shows non-significant difference (P = 0.64) in visitation frequency among the seven pollinators (Table 3.6). The trend showed that the most frequent visitor was *Stizus sp.* with (2.25±3.20) visits/flower/minute, followed by *Apis mellifera* with an average of (1.63±2.09) visits/flower/minute. The minimum visitation frequency was recorded for *Musca domestica, Phytomia sp., Simulium sp., Anthophora sp.* with (1.00±0.00) visits/flower/minute respectively. The smallest visitation frequency was recorded for *Eristalis tenax* with (0.63±0.50) visits/flower/minute (Table 3.5).

3.3.3.2 Stay time

The findings of analysis of variance (Table 3.6) shows the P-value of 0.02 suggesting that there are significant differences in stay times amongst insect species on wild radish flowers, the standard deviation of zero indicates that there is no variability in stay time among insect species. Time taken on a single bloom was highest for *Musca domestica* (300.00 ± 0 seconds), followed by *Stizus sp.* (152.50±170.35 seconds), *Anthophora sp.*, (95.00±120.21 seconds) and *Eristalis tenax* (94.25±104.65 seconds) (Table 3.5). The shortest time on each flower was spent by *Simulium sp.* (1.50 ±0 seconds) followed by *Phytomia sp.* (5.00 ±0 seconds) and *Apis mellifera* (14.04±35.30 seconds).

3.3.3.3 Visitation rate

The analysis of variance (Table 3.6) (*p*= 0.00) shows a highly significant difference in visitation rates among insect species highlighting variations in the overall foraging activity and effectiveness of flower visits among insects' species. Number of flowers visited per minute by *Stizus sp.* was around (3.25±3.86) and *Apis mellifera* visited around (7.19±3.47) flowers per minute, making it the only pollinator which visited many flowers in a single visit. The larger standard deviation further suggests that there is more variability in visitation rates of *Apis mellifera* across the observations. *Anthophora sp.* visited around (3.00 ±0.00) flowers per minute, though there were only two observations while *Eristalis tenax* visited around (2.56±1.63) flowers per minute.

Table 3.5: Foraging behavior (Visitation rate, Stay time and Visitation frequency) of different pollinator species on flowers of Wild radish.

Species	Abundance	Visitation frequency (Visits/flower/mins)	Stay time (Seconds)	Visitation rate (Visits/flower/mins)
Eristalis tenax	16	0.63±0.50	94.25±104.65	2.56±1.63
Anthophora sp.	2	1.00±0.00	95.00±120.21	3.00±0.00
Apis mellifera	16	1.63±2.09	14.03±35.30	7.19±3.47
Stizus sp.	4	2.25±3.20	152.50±170.35	3.25±3.86
Musca domestica	1	1.00±0	300.00±0	4.00±0
Phytomia sp.	1	1.00±0	5.00±0	2.00±0
Simulium sp.	1	1.00±0	1.50±0	1.00±0

Table 3.6 ANOVA table for foraging behaviour of pollinator species on flowers of wild radish.

		Sum of Squares	Df	Mean Square	F	Sig.
No. of Flowers Visited/Mins	Between Groups	204.97	6	34.16	4.38	0.00
	Within Groups	265.13	34	7.79		
	Total	470.09	40			
Number of visits/flower/mins	Between Groups	12.77	6	2.13	0.72	0.64
	Within Groups	100.25	34	2.95		
	Total	113.02	40			
Time spend/flower/sec	Between Groups	149797.83	6	24966.30	2.98	0.02
	Within Groups	284461.23	34	8366.51		
	Total	434259.06	40			

3.3.3.4 Nectar and pollen feeding on flowers of *R. raphanistrum.*

The foraging behavior of different pollinator species associated with nectar and pollen feeding on blooms of wild radish (*Raphanus raphanistrum*) is presented (Table 3.7.). Mainstream of pollinators visited wild radish blossoms to accumulate pollen as, *Apis mellifera* (100%), *Anthophora sp.* (100%), *Simulium spp.* (100%), and *Musca domestica* (75%) were the highest pollen foragers, followed by *Stizus sp.* (31.2%) and *Eristalis tenax* (25%) respectively. Some of the pollinators showed preference for both nectar and pollen, this included *Phytomia sp.* (100%), *Apis mellifera* (62.5%), *Eristalis tenax* (62.5%) and *Stizus sp.* (25%). While at times, some insects only came to collect nectar alone, *Musca domestica* (100%), *Eristalis tenax* (12.5%) and *Apis mellifera* (6.2%) (Table 3.6.).

Table 3.7: Foraging behavior of different pollinator species associated with nectar and pollen feeding on flowers of *R. raphanistrum*.

		POLLEN/NECTAR FORAGER		
Species	Stigma Contact(%) N=41	PF(%) N=41	NF(%) N=41	NF/PF(%) N=41
Eristalis tenax	100	25.0	12.5	62.5
Anthophora sp.	100	100.0	0.0	0.0
Apis mellifera	100	100.0	6.2	62.5
Stizus sp.	43	31.2	0.0	25.0
Musca domestica	100	75.0	100.0	0.0
Phytomia sp.	100	0.0	0.0	100.0
Simulium spp.	100	100.0	0.0	0.0

Key: PF = Pollen Forager, NF = Nectar Forager, NF/PF = Nectar and Pollen Forager.

3.3.3.5. Pollen deposited.

The mean pollen deposition as presented in (Figure 3.2) shows that maximum number of pollen grains was deposited by *Apis mellifera* (128.75±31.65), plus or minus the standard error followed by *Eristalis tenax* (24.13±4.31). The overall model fit F (2,13) = 5.16, p = 0.02, is statistically significant, suggesting that stay time and species collectively explain variability in pollen deposition. Stay time does not significantly (F (1,13) = 0.19, p = 0.66) affect pollen deposition, while species significantly (F (1,13) = 9.93, p = 0.01) indicate varying pollen deposition by *Apis mellifera* and *Eristalis tenax*. The model explains 44.2% of the variance in pollen grains deposited (R Squared = 0.442, Adjusted R Squared = 0.357) (Table 3.7). These findings highlight the significant influence of pollinator species on pollen deposition, while the duration of flower visit does not play a significant role in pollen deposition.

Table 3.7: Results of ANCOVA of the average amount of pollen grains deposited by insect species and amount of pollen deposited per individual insect's stay time on the flower.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	44646.71[a]	2	22323.36	5.16	0.02
Intercept	48959.95	1	48959.95	11.31	0.01
Stay Time/ flower/sec	861.15	1	861.15	0.19	0.66
Species	42973.86	1	42973.86	9.93	0.01
Error	56273.22	13	4328.71		
Total	194403.00	16			
Corrected Total	100919.94	15			

a. R Squared = 0.442 (Adjusted R Squared = 0.357)

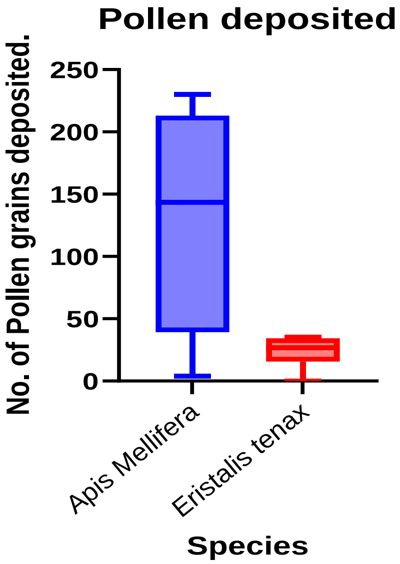

Figure 3.2: Foraging behavior in terms number of pollen grains deposited. Box and whisker plots show medians (lines in boxes), quartiles (box ends), 95th percentiles (whiskers).

3.3. Discussions

3.3.1. Diversity and Abundance

The information provided (Table 3.1 and Table 3.2) reveals some similarities in species abundance and composition between the two study areas as *Apis mellifera* (Apidae), *Eristalis tenax* (Syrphidae) and *Musca domestica* (Muscidae) were recorded as frequent visitors. The similarities in species abundance between the two areas (Nyakosoba & NUL Farm) can be caused by the slight similarities in yearly temperature which is 17.61˚C in Nyakosoba and 18.37˚C in Roma (http://tcktcktck.org or https://www.weather-atlas.com), flora and fauna are to some extent similar in these two areas. Similarly, the variation in abundance with other study areas is mainly due to differences in climate, topography, fauna and flora. Even so, honeybees and flies were found as the abundant species in both locations. They were widely distributed because of their generalist feeding habits and adaptability to diverse environments. They are also known to play a major role in pollination of cruciferous crops, especially wild radish (Goodwin *et al*., 2011; Sharma *et al*., 2016).

On the other hand, the differences in species composition may be due to specific ecological challenges, the environment in NUL farm comprised of farm animals and maize fields nearer to the experimental site which are thought to have brought more composition of flies, particularly house flies (*Musca domestica*). Nyakosoba had abandoned wheat in the field and a small area planted maize. Apple and peach trees were numerous, this also resulted in most of the insects in the area being honeybees and flies which collected nectar from the fruit trees.

Our results are dissimilar to the findings of Divija *et al*. (2022), from their study conducted in ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India, in which *Apis cerana, Apis dorsata, Apis florea, T. iridipennis, Lassioglossum sp. and Ischiodon scutellaris* were the most abundant floral visitors however, they were all honeybees and flies. Additionally, Zameer *et al*. (2017), also found *Episyrphus balteatus* followed by *Andrena sp.* as the most abundant floral visitors of *R. raphanistrum* in Pakistan contrasting to the current findings. Sharma *et al*. (2016), found *Apis cerana* as the most abundant floral visitors while *Apis mellifera* recorded the lowest abundance in India. Saeed *et al*. (2012), reported *A. dorsata* and *A. florea* as the most abundant among 15 pollinator species on bitter gourd. In another study on *Brassica napus*, *A. dorsata* and *A. florea* were ranked second and third in terms of abundance (Akhtar *et al*., 2018).

3.3.2 Different Visitation times and Relative abundance

The data from Tables 3.3. and Table 3.4. highlight the varying presence and activity levels of different insect species across different time periods. *Apis mellifera* (honeybee) and *Musca domestica* (housefly) emerged as consistent visiting species, while other species exhibited fluctuations in their relative abundance, possibly influenced by their foraging and activity patterns. Which can be credited to factors such as floral resource accessibility, fluctuations in temperature and humidity, or specific preferences of the pollinators (Conner *et al*., 1995; Lawson *et al*., 2019). This implies that specific pollinator species can show a more important role in wild radish pollination during certain times of the day, while others may be less active or absent (Zoller *et al*., 2020).

Moreover, Kumar *et al*. (2019), established that the peak foraging action of *A. cerana* (honeybee) was at 1 PM, these findings are similar to current study as *Apis mellifera* (honeybee) was observed in abundance at 1PM from the two study areas. Verma and Partap (1994), further found that bees started foraging on cauliflower and cabbage at 07:00h and 06:30h, in that order and stopped flying action at 18:00h and 18:30h. Foraging on cauliflower started at ambient temperatures of 7 degrees Celsius, highest foraging movement of bees was in the middle of 11:00h and 13:00h for each crop, this foraging movement was also observable during our study period (Verma *et al*.,1994). Thus, insect activity could be limited by cold morning temperatures and increased by high temperature in the late morning and afternoon in Nyakosoba and NUL farm.

3.3.3 Visitation frequency, Visitation rate and Stay time

In the current study (Table 3.5.), the most frequent visitors were *Stizus sp.* with (2.25±3.20) visits/flower/minute, and *Apis mellifera* with an average of (1.63±2.09) visits/flower/minute. These findings agree with the known fact that honeybees are known for their potential role as pollinators predicting pollination effectiveness, while *Stizus sp.* (predatory wasp) are attracted to the nectar produced by flowers and potentially preying on other insects found on the flowers. Generally, these two species are attracted to the nectar produced from the flowers while they perform the pollinating activities. Divija *et al*. (2022), found that visitation frequency values were high for *A. florea* (honeybees*)* followed by *A. dorsata, T. iridipennis,* and *A. cerana*.

Frequent visitors generally pollinate a greater number of flowers, while other visitors may provide higher quality pollen (Rogers *et al*., 2013; Hoehn *et al*., 2008). To confirm this hypothesis in the context of flower visiting community of wild radish (*Raphanus raphanistrum*), future studies should assess individual plant reproductive success (seed production) associated with single visits to untouched flowers. *Stizus sp.* spent more time on the flower as they have smaller bodies and were potentially gathering more resources.

The frequency of flower visitation is often considered more essential than the effectiveness of each visit when assessing a species' involvement to pollination (Zych *et al*., 2013). However, honeybee pollination can have unfavorable effects on plant reproductive accomplishment, as it tends to promote self-pollination rather than outcrossing (Magrach *et al*., 2017).

In divergence to the current results, Divija *et al*. (2022), recorded maximum stay time for *Lassioglossum sp*., *T. iridipennis, I. scutellaris* and *A. florea*, they found the minimum stay time for *A. dorsata and A. cerana* (honeybee), which is similar to the current findings where minimum stay time was recorded for *Apis mellifera* (honeybee). *Apis mellifera* spent the shorted time on the flowers of wild radish because they are efficient pollinators that are able to quickly collect pollen and nectar and move on to the next flower. They have evolved to maximize their foraging efficiency, visiting numerous flowers in short amount of time to gather resources for their hive.
Competition and interactions with other insects had the influence in foraging behavior as *Apis mellifera* and *Eristalis tenax* could not feed on one flower at the same time. This influenced the number of flowers visited per minute by *Apis mellifera* because it was always foraging away from *Eristalis tenax*.
Temperature and humidity also played a big role in insect activity and foraging behavior because more flies, wasps and bees were observed during the hot sunny days, meaning that high temperatures around the day lead to high insect activity leading to high visitation rates. Additionally, spare populations of wild radish played negative role in the visitation of honeybees and other pollinators, as it was evidenced by low populations from all the pollinators taxa. *Apis mellifera* required more visits to gather enough pollen leading to higher visitation rates and sometimes focused more on resources collection and efficient foraging, they also spend less time on the flowers.

Generally, *A. mellifera* had higher visitation rates and visited more inflorescences in each plant than other insects' groups. These findings agree with preceding studies of (Divija *et al.*, 2022; Esposito *et al.*, 2021; Jaca *et al.*, 2019) on flower visitation, where bees were found to be most efficient visitors since they moved quickly between flowers and presented lower flower handling time.

Flower visitation for large bees is different to other insects because of their behavioral and anatomical distinctions as well as differences in resource utilization. For instance, small bees and hover flies visit flowers solely for pollen, leading to longer flower handling times (Esposito *et al*., 2021). Butterflies also exhibit low visitation rates due to extended probing and feeding periods on select inflorescence (Esposito *et al*., 2021). Moreover, Jauker *et al.* (2012), found that hover flies are less efficient pollinators than bees due to their extended flower handling times and visitation rates unaffected by flower density. Moreover, in all the observed species, visitation rates declined in the progression of the flowering season and happened together with the decrease in open flowers from the plants.

3.3.4. Nectar or pollen feeding.

As presented from (Table 3.6.), most of the insects visiting wild radish came to collect pollen, *Apis mellifera*, *Anthophora sp.*, *Simulium spp.*, and *Musca domestica* were the highest pollen foragers, followed by *Stizus sp.* and *Eristalis tenax*. They landed on petal of the flower and the flower opening allowed the insects to contact anthers during their visits. Some insects would sit on sepals where the assumption is that those insects came to accumulate nectar from the cylindrical base of the flower petal using their piercing mouth parts. The foraging behaviors determine the efficiency of pollen transfer on the anthers to the stigmas of wild radish flowers. Some species such as *Musca domestica* and *Eristalis tenax*, primarily collected nectar.

The presence of multiple insect species from different orders and their varying foraging behaviors suggests complex ecological interactions within the wild radish community. Conner *et al.* (2007), established that small bees and syrphid flies in contrast to larger pollinators, primarily collected pollen on wild radish flowers, he further found that large bees and Lepidoptera are primarily nectar feeders. Their findings are similar to the current findings where syrphid flies, flies and honey bees (*Apis mellifera*) collected both pollen and nectar.

Pollinators that scavenge on pollen are more effective than those which feed on nectar because they enter the flower from above anther and stigma (Zameer *et al*., 2017; Divija *et al*., 2019). Sihag and Saini (2023) recorded the main pollinators as *Apis dorsata, A. mellifera, A. florea,* and *Sarcophaga sp.,* they found that the honeybee species of *A. dorsata, A. mellifera* and *A. florea* searched for nectar and pollen on the Aonla crop and intentionally gathered pollen by brushing their limbs on the dehisced anthers due to which their body got profoundly covered with pollen.

3.3.5 Pollen deposition.

The results (Table 3.7.: Figure 3.3.) indicated that *Apis mellifera and Eristalis tenax* are effective in transferring pollen from anthers to stigmas, enhancing the fertilization success and potentially leading to higher fruit or seed set (Conner *et al.,* 2007; Conner *et al*., 1996; Conner *et al*., 1995). This further proves that honeybees collect pollen during most of their visits more than they collected nectar.

The variations in pollen deposition are also attributed to pollinator preferences, which may be influenced by factors such as flower color, scent, and nectar rewards (Conner *et al*., 2009). Conner *et al*. (2007), found that large bees and butterflies deposited a greater number of pollen grains while they look to gather nectar from the base of the flower (Esposito *et al*., 2021). The observed variations in pollen deposition highlight the importance of different pollinator species in the reproductive success of plants (Sihag *et al*., 2023; Esposito *et al*., 2021). Effective pollen transfer is crucial for achieving fertilization and subsequent seed or fruit set (Divija *et al*., 2022).

3.4. Conclusion

Honeybees and flies were most abundant in both locations due to their generalist feeding habits. However, specific ecological challenges in each area led to differences in species composition. Further analysis revealed varying visitation times and relative abundance among insect species, influenced by factors like floral resource accessibility and temperature fluctuations. *Apis mellifera* and *Musca domestica* were consistent visitors, with honeybees showing peak foraging activity at specific times. Visit frequency, rate, and stay time varied among species, with honeybees exhibiting higher visitation rates. Temperature, humidity, and flower density influenced insect activity and foraging behavior. Most insects visited wild radish flowers for pollen, affecting pollen transfer efficiency, with honeybees and some flies being effective pollinators.

Chapter 4- The Effect of Wild Radish Flower Morphology on Plant -Pollinator Interaction and the differences between wild radish flower morphs.

4.0 Abstract

Wild radish (*Raphanus raphanistrum*) is a common weed in Lesotho, known for its varied flower morphology. The plant produce flowers in multiple colors, yellow, white, purple and pink, which exhibit differences in morphological traits. There variations influence plant-pollinator interactions, which are crucial for the plant's reproductive success and genetic diversity. In this study, we investigated the morphological variation in seven floral traits of wild radish and the influence of these flower traits on pollinator-mediated selection, pollen removal and pollen production. Gross-tabulation and chi-square test were used to determine pollinators visitation based on flower color. Pollen production was assessed using descriptive statistics and Linear regression model. Descriptive statistics and the least-square linear model was further used to compare amount of pollen grains removed from short and long stamens. ANOVA results were obtained from the regression out-put and it was used to determine if there was statistically significant relationship between pollen removal, natural dimorphism and long stamen. The mean differences and standard error of the means, of Yellow and White flower morphs were compared using an independent sample t-test. Observations revealed that various pollinator species exhibited distinct preferences for flower color morphs. Yellow flowers with dark brown distinct veins were visited by *Eristalis tenax* (10 visits), followed by *Apis mellifera* (7 visits), *Stizus sp.* (3 visits), *Anthophora sp.* (2 visits) and *Simulium sp.* (1 visit). *Apis mellifera* and *Eristalis tenax* visited all the four flower colors. Long stamens produced more pollen grains (mean = 6110.56 ± 384.87) than short stamens (mean = 4768.00 ± 389.99) (Table 4.2). Additionally, results on pollen removal indicates that more pollen was removed from naturally dimorphic stamens (mean =1.39 ± 0.08) than it was removed from long stamens (mean = 1.25 ± 0.05) and short stamens (mean = 0.91 ± 0.02) (Table 4.5). Sepal lengths (mm) of white flowers was slightly greater (mean 1.05, n = 16) than sepal length (mm) of yellow flowers (mean 0.96, n = 25). There is no significant difference (p = 0.06) in sepal lengths (mm) between yellow and white flowers. Corolla tube length (mm) of white flowers (mean 0.95, n =16) was greater than corolla tube length (mm) of yellow flowers (mean 0.94, n= 25), (p = 0.84). Petal length (mm) of white flowers was longer than (mean 0.91, n = 16) petal length (mm) of yellow flowers (mean 0.89, n= 25), however, there were no significant differences (p = 0.83). Petal width (mm) of yellow flowers (mean 0.74, n = 25) was wider than petal width (mm) of white flowers (mean 0.66, n = 16), and there were no significant differences (P >0.05) in petal width between yellow and white flowers. The pedicel length (mm) of white flowers (mean 1.35, n =16) was longer than of yellow flowers (mean 1.05, n = 25). Showing a highly significant difference (p = 0.01) between the two groups (Table 4.7). Anther length (in mm) of white flowers was longer (mean 0.29, n =16) compared to anther length (mm) of yellow flowers (mean 0.25, n = 25) and there was a significant difference (p = 0.03). The filament length (mm) of white flowers was a little longer (mean 0.80, n =16) than filament length (mm) of yellow flowers (mean 0.77, n = 25) but there were no significant differences (p = 0.27) in filament length. Stigma diameter (mm) of both flower morphs was equal (mean 0.05). Also, pistil length (mm) of white flowers was longer (mean 1.24, n = 16) than pistil length (mm) of yellow flowers (mean 1.11, n = 25) (p = 0.05). These results contribute to our understanding of the complex interplay between floral morphology, pollinator preferences, and reproductive success in wild radish populations.

Key words: *Floral morphology, Plant pollinator interactions, pollen produced, pollen removed, dimorphic stamen lengths.*

4.1. Introduction

Pollination is a critical ecological process that affects plant reproduction, biodiversity, and ecosystem stability. Among various flowering plants, the wild radish serves as an excellent model for studying the complex dynamics between floral traits and pollinator behaviour. Flower morphology, encompassing attributes such as colour, size, and shape plays pivotal role in attracting pollinators and facilitating successful pollination (Conner *et al.*, 1996a; Stanton & Preston, 1988). Flower colour significantly impact pollinator visitation rates, with bees showing a marked preference for white and yellow flowers over other colors (Stanton *et al.*, 1988).

Wild radish flower morphology plays a crucial role in influencing insect pollination, which is an essential procedure in the reproductive success and ecological dynamics of this plant species. The intricate relationship between wild radish flower morphology and insect pollination has been a subject of scientific investigation with significant implications. The diversity in flower color, anther exsertion, anther pollen count, and anther height dimorphism within wild radish populations reflects the remarkable adaptability of this species to various ecological niches (Conner *et al.*, 1995). These traits have evolved over time, shaped by natural selection to enhance the reproductive accomplishment of the plant (Conner *et al.*, 1993).

Studies have reported the variation in morphological traits such as flower color, stamen exsertion, stigma exsertion, petal size and corolla tube length have a profound influence on the types of insects that visit wild radish flowers, the regularity of visitation, as well as the efficiency of pollen transfer, thereby affecting the plant's reproductive fitness (Stanton *et al.*, 1989; Lee *et al.*, 1998). Insects, particularly bees and butterflies, play a key role in the pollination of wild radish and many other flowering plants, facilitating the reproduction and genetic diversity of these species (Morgan *et al.*, 2005).

Wild radish, a fellow of the Brassicaceae family, exhibits a remarkable feature known as tetradynamy, characterized by the presence of four long and two short stamens in each flower (Conner *et al.*, 2017). These are mechanisms through which anther position within the flower may exert an influence on pollinator visitation behavior, for example, prominent anthers especially when they are exserted, can serve as a visual display, signaling the reward available for pollen foragers (Conner *et al.*, 2017).

Conversely, exserted anthers may pose challenges to pollinators foraging for nectar, potentially leading to longer visit durations (Conner *et al.*, 1995; Conner *et al.*, 2017). Extended visitation times, in turn, can enhance the efficiency of pollen removal or deposition (Young *et al.*, 1990; Conner *et al.*, 1995; Kudo, 2003). Dimorphic or polymorphic positions of anthers, where anthers are situated at varying heights or in whorls within the flower, is common in various species within the Brassicaceae family, including wild radish (Conner *et al.*, 1995; Conner *et al.*, 2017). This feature offers an opportunity to investigate the role of anther position in pollinator behavior.

The study of Stanton *et al.*, 1988, did not delve deeply into the synergistic effects of colour and other morphological traits, such as petal size and shape. Additionally, the influence of environmental factors and seasonal variations on pollinator behaviour was not extensively examined, leaving a critical gap in our understanding of how these variations interact in natural settings. There is a trade-off between flower size and number, with larger flowers attracting more pollinators. However, the study of Conner *et al.* (1996a), did not look into how bees versus butterflies might respond differently to flower size and number. The assumption is that, there is no difference in pollen produced and pollen removed between the short and long stamens of wild radish, and that flower morphological traits between yellow and white wild radish flowers are not different. The study provides an understanding of the effect of wild radish flower morphology on plant-pollinator interactions and look at the differences in wild radish flower morphs. Therefore, this can enhance understanding of flower morphology and pollinator preferences as we can identify which floral traits are most effective in attracting pollinators and ensuring successful pollination. Further, knowledge about how flower morphs respond to different pollinators and environmental conditions can help predict how these interactions might shift in response to climate change and habitat alterations.

4.2. Materials and Methods

4.2.1. Study site

The study was carried out in foothills and lowland agroecological zones of Maseru district in Lesotho. The first site was Nyakosoba located in the foothills agro ecological zone, at an elevation of 2034.78 meters above sea level (masl). During the summer season from December to January, temperatures in Nyakosoba averages between a high of 25.5˚C in December and a peak of 26.1˚C in January to a low of 13.8˚C and 14.8˚C respectively. The highest rainfall average from 51mm in February to 71mm in December, hence making it the wettest period throughout the year. The second study site was National University of Lesotho faculty of Agriculture farm, Roma campus, in Lesotho, located in the lowlands agroecological zones at an elevation of 1610 masl. From December to January, temperatures in Roma averages between 26.1˚C in December and a peak of 27˚C in January to a low of 16˚C and 15˚C respectively. Roma typically receives about 83.47mm total rainfall.

4.2.2. Study design

Wild radish population naturally growing in a field that was lying fallow was used at Nyakosoba. During the growing season in October 2022, various areas within Agriculture research station were surveyed to identify potential study sites. Among these, a 100m^2 field was selected due to the high density of wild radish plants observed. This field was chosen based on the visible abundance of wild radish compared to other areas. In November 2022 we returned to the field to check the flowering status of the wild radish plants and observed that the majority of the plants had begun to flower, displaying their characteristic four-petaled flowers in shades of white, yellow, and sometimes light purple. In December we marked out four 1m x 1m quadrats within the 100m^2 field where wild radish plants were present. Due to uneven distribution of wild radish plants, the quadrats were placed in areas where these plants were found, ensuring that a sufficient number of plants were included for data collection (Kumar *et al*., 2019).

The field from NUL farm was ploughed on the 26th January and disked on the 5th February. 16 Study plots were measured and marked within the field, each measuring 1m by 2m. Planting stations were placed and planting took place on the 9th February 2023 with 10cm intra-row and 50 cm inter-row spacing. Thinning was not done because of non-100% germination, instead, all germinated plants were used for data collection.

To compare floral traits of white and yellow wild radish flowers, samples were collected randomly from the sixteen plots at NUL farm. There were yellow and white flower morphs and the yellow flower morphs dominated the white resulting in picking uneven numbers of flower color morphs from all the experimental plots.

4.2.3. Effect of flower colour morphs on flower visitation by insect pollinators

To assess pollinators behavior in relation to visitation of various flower color morphs at Nyakosoba, the insects were closely observed and recorded to see which insect visited which flower color (Conner *et al*., 1995). The flowers were taken to the lab to accurately identify the colors and veins of the flowers prior data collection. Direct observation was used where petals were looked at closely to note the predominant color. Natural light was also used to view flowers for the accurate color representation. Randomized block design was used to ensure each quadrat had four flower morphs each represented by eight flowers per quadrat. Plastic bottles were used to collect flowers which were visited by insects and these were labeled on the basis of visiting insects.

4.2.4. Flower morphology.

To study differences in flower morphs, plants in NUL farm were used. Only two flower colors (yellow and white) were observed from the offsprings in NUL farm. Forty-one flowers were collected from all plants that had flowers at the time of collection on the 18th April 2023. Sixteen were white and twenty-five were yellow. These flowers were dissected, and nine flower traits were measured using Vernier caliper (Conner *et al*., 1993). The length and width of the distal part of the petal, outside the corolla tube, the length of the corolla tube the proximal part of the petal, the length of the pistil, the lengths of the filaments (four long and two short), anther length, stigma diameter, stalk length, and sepal length were measured following methods by (Conner *et al.,* 1993).

4.2.5. Pollen production.

To measuring pollen production, thirty-six flowers were randomly selected from different plants within the quadrats at Nyakosoba, which were at similar developmental stage (it was done to minimize variability). The eighteen long and eighteen short stamens were carefully removed from the flowers without disturbing the pollen. Then placed anthers on a microscope slide under the microscope and added a drop of aniline blue which stained pollen grains in blue for easy observation. Number of pollen grains per stamen anther from all six stamens separately (4 long and 2 short) were counted using compound light microscope under 40× magnification and pollen grains were multiplied by the total number of flowers in the population to estimate the total pollen production of both stamen lengths.

4.2.6. Pollen removal.

Three individual experimental flowers from the four quadrats were covered with nylon mesh to prevent insect visitation. The flowers were open during peak pollinator activity and the visited flowers were detached immediately after the insect left and placed in a bottle containing 70% ethanol to preserve the anthers to the lab where pollen counting was done. Flowers which we not visited were used as the control. The species of visiting pollinators that contacted anthers were closely observed and recorded.

4.2.7. Pollen counting.

The hemocytometer slide was used to count the pollen grains removed by firstly suspending pollen grains in a known volume of 70 % ethanol and vortex to assure homogenous mixing. Then remove a sample of pollen sediment with a pipette and place on the hemocytometer. The stereoscopic microscope with 40 × and 100 × magnification was then used to count pollen grains (Divija *et al*., 2022; Sharma *et al*., 2016; Dafni, 1992; Delaplane *et al*., 2015). To count the number of pollen grains, we counted each grid systematically from a corner of the counting chamber, we used a hand tally counter to track the counts accurately and only counted fully visible pollen grains and excluded debris to ensure consistency in counting. We recorded the number of pollen grains counted in each grid along with the corresponding grid location. The counting process was repeated for multiple grids within the counting chamber to obtain representative sample (Smith *et al.*, 2019).

4.2.8. Data analysis.

To determine effect of flower color on flower visitation by insect pollinators, Gross-tabulation and chi-square test were used. Descriptive statistics was used to assess which stamens produced more pollen and Linear regression model was used to explore the relationship between two stamen types and the amount of pollen produced. ANOVA tables were obtained from the regression out-put and regression was used to determine if there was statistically significant relationship between pollen production and long and short stamens (Conner *et al.*, 2009; Conner *et al.*, 1995). Descriptive statistics was used to compare amount of pollen grains removed from short and long stamens. The least-square linear model was used to relate the amount of pollen removed to natural dimorphism and long stamens. The natural dimorphism ratio for each plant was calculated by dividing the long stamen length by the short stamen length because larger ratio equals greater natural dimorphism, the data was handled following the methods used by (Conner *et al.*, 2003). ANOVA results were obtained from the regression out-put and it was used to determine if there was statistically significant relationship between pollen removal, natural dimorphism and long stamen. The mean differences and standard error of the means, of Yellow and White flower morphs were compared using an independent sample t-test. All statistical analysis was carried out by using SPSS (v 20) (Smith *et al.*, 2019; Younas *et al.*, 2022).

4.3. Results

4.3.1. Effect of flower colour on flower visitation by insect pollinators

The results from table 4.1 displays the observed frequencies of insects' visits to various flower colors. The flowers ranged from white flowers with light brown distinct veins Pale yellow flowers with lilac veins, yellow flowers with dark brown distinct veins and yellow flowers with light brown veins. It was observed that yellow flowers with dark brown distinct veins were visited by *Eristalis tenax* (10 visits), followed by *Apis mellifera* (honey bees) (7 visits), *Stizus sp.* (3 visits), *Anthophora sp.* (2 visits) and *Simulium sp.* (1 visit). *Apis mellifera* and *Eristalis tenax* visited all the four flower colors. Even though, *Stizus sp.* made one visit to yellow flowers with dark brown distinct veins and pale-yellow flowers with lilac veins (Table 4.1; Fig 4.2b, 4.2c). *Phytomia sp.* completed one visit to yellow flowers with light distinct veins and *Musca domestica* made one visit to white flowers with light distinct veins.

The chi-square statistic results suggested that there was no association between insects' visitation and flower color morphs, further, p-value indicates that there is no significant association between the two variables (Chi-square = 19.48, p = 0.36, df = 18). Which could mean that insects visited flowers randomly, either influenced by the presence of pollen or nectar and flower morphology or scent, instead of flower color. Additionally, since the yellow flower color was more abundant than other flower colors, insects visited those yellow-colored flowers more than they visited other flowers.

Table 4.1: Insect visitation based on flower color morphs.

Species	Flower Colour				Total
	Yellow dark brown distinct veins	Pale yellow lilac veins	Yellow light brown distinct veins	White light brown distinct veins	
Eristalis tenax	10	1	2	3	16
Apis mellifera	7	3	5	1	16
Musca domestica	0	0	0	1	1
Stizus sp.	3	1	0	0	4
Phytomia sp.	0	0	1	0	1
Simulium sp.	1	0	0	0	1
Anthophora sp.	2	0	0	0	1
Total	23	5	8	5	41

Chi-Square = 19.48, df = 18, p = 0.36

Figure 4.2a: White flower with light brown distinct veins.

Figure 4.2b: Pale-yellow flower with lilac veins.

Figure 4.2c: Yellow flowers with light brown and dark brown veins.

4.3.2. Stamen exsertion in relation to pollen production.

Long stamens produced more pollen grains (mean = 6110.56 ± 384.87) than short stamens (mean = 4768.00 ± 389.99) (Table 4.2). The type of stamen significantly influenced pollen production *F* (1,34) = (6.34, P = 0.02), suggesting that there are observable differences in pollen produced depending on the type of stamen (Table 4.3). The outcome size, as indicated by R Squared (0.157), signifies that approximately 15.7% of the variability in pollen production is attributed to the variation in stamen types. These findings highlight the importance of considering the type of stamen when assessing pollen production, as it significantly contributes to the seen variability.

Table 4.2: Mean amount of pollen grains produced.

	Mean	N	Std. Deviation	Std. Error of Mean	Sum
Long stamens	6110.56	18	1632.807	384.856	109990
Short stamens	4768.00	18	1654.627	389.999	85824

Table 4.3: Stamen type and amount of pollen produced in wild radish flowers.

Source	Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	15298528.44[a]	1	15298528.44	6.34	.02
Intercept	1057507040.44	1	1057507040.44	437.99	.00
Stamen type	15298528.44	1	15298528.44	6.34	.02
Error	82092449.11	34	2414483.79		
Total	1154898018.00	36			
Corrected Total	97390977.56	35			
a.R^2=0.157	(AdjustedR^2=0.132)				

The overall regression model for number of pollen grains produced by short and long stamens is explained 17.7% of the variance in the amount of pollen grains produced by anthers of both stamens. The stamen length had a moderate positive effect on the amount of pollen produced by both stamens. For example, the standardized regression coefficient of 0.42 for anther length shows that an increase in 1 SD in anther length resulted in a 42% increase in relative pollen produced. Specifically, for every unit increase in stamen length, the amount of pollen produced was expected to increase by 2039.30 units, on average. This was supported by a t-value of 2.71, indicating that the relationship between stamen length and pollen produced is statistically significant at the 0.01 level.

Despite the significant difference observed in pollen produced when short stamens and long stamens were combined, pollen production was further determined by the length of the stamens separately (Table 4.4). However, the results indicate that there was no significant relationship between short stamen and amount of pollen produced, *F* (1,16) = 0.16, P = 0.69. Similarly, there was no significant relationship between long stamen and amount of pollen produced, *F* (1,16) = 3.99, P = 0.06. Which could be due to the small variability in the actual lengths of the anthers amongst the groups of both short stamens and long stamens Fig (4.3).

Table 4.4: Regression analysis table of stamen lengths and amount of pollen produced per stamen exsertion.

Variables	Unstandardized Coefficients		Standardized Coefficients	T	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Stamen length	2039.30	753.63	0.42	2.71	0.01	507.74	3570.87
Long stamen	1510.04	756.08	0.45	1.99	0.06	-92.79	3112.86
Short stamen	-3345.99	8289.02	-0.10	-0.40	0.69	-20917.93	14225.94
R squared =0.177							

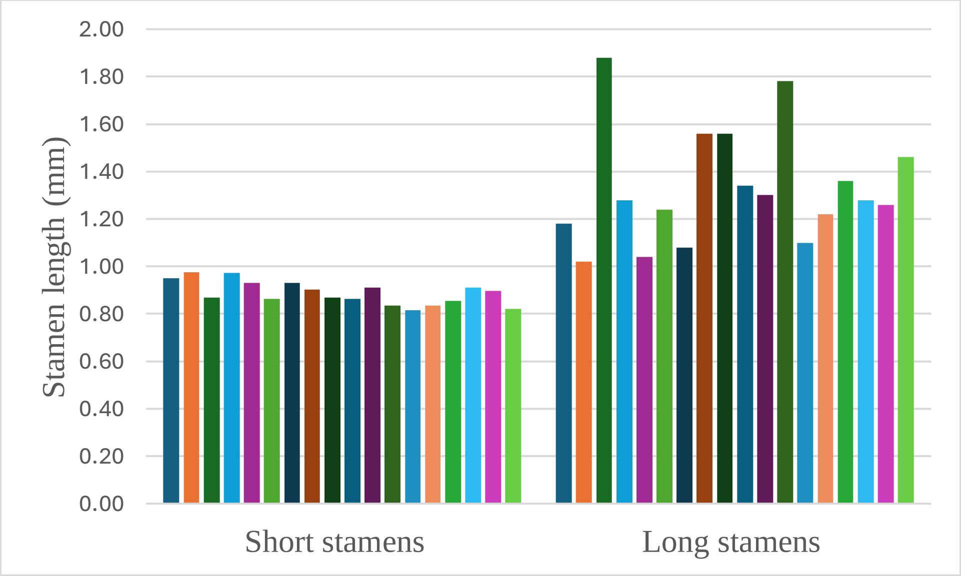

Fig 4.3: Variability in lengths amongst short stamens and long stamens

4.3.3. Pollen removal

The results on pollen removal indicates that more pollen was removed from naturally dimorphic stamens (mean =1.39 ± 0.08) than it was removed from long stamens (mean = 1.25 ± 0.05) and short stamens (mean = 0.91 ± 0.02) (Table 4.5). The overall regression model for pollen removal was not highly significant F (1,5) = 1.95, p = 0.24), with three predictor variables explaining 66% of variance in number of pollen grains removed by single insect visit (Table 4.6). There was a strong positive relationship (R = 0.81) between long stamen, natural dimorphism, number of pollen grains on the control flower and number of pollen grains removed. The results suggest that, for one unit change in long stamen, amount of pollen removed was expected to increase by 45884.19 units and an F-value also indicates that long stamen had a notable influence on the variability observed in the number of pollen grains removed, but it was not statistically significant (F =52611.22, p = 0.45). Additionally, the negative slope in natural dimorphism (-32120.64) implies that an increase in natural dimorphism could lead to a decrease in the amount of pollen removed and an F-value also indicates that natural dimorphism has a notable influence on the variability observed in the number of pollen grains removed, but it was also not statistically significant (F= 30068.74, P = 0.33).

Table 4.5 Mean amount of pollen grains removed.

	N	Mean	Std. Deviation	Std. Error	95% Confidence interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Long stamen	11	1.25	0.17	0.05	1.14	1.36	1.02	1.56
Natural dimorphism	11	1.39	0.25	0.08	1.22	1.56	1.04	1.79
Short stamen	11	0.91	0.05	0.02	0.87	0.94	0.82	0.98

Table 4.6: Least-square linear model analysis of number of pollen grains removed.

	Df	Sum of squares or slope	*F* or SE	*P*
No. of pollen in control flower	1	-.35	.16	.08
Natural dimorphism	1	-32120.64	30068.74	.33
(Natural dimorphism)^2	1	10124.93	10030.16	.36
Long stamen exsertion	1	45884.19	52611.22	.42
(Long stamen exsertion squared)^2	1	-16411.55	19937.37	.45
ANOVA results:	Df = 5 *F*=1.95 *P* =0.24			

Whole model R^2 = 0.66, P = 0.24

4.3.4. Differences in morphological traits between yellow and white wild radish flowers.

4.3.4.1. General floral traits.

Below are the general floral traits results from an independent samples t-test (Table 4.7) between yellow and white wild radish flowers morphological traits. Sepal lengths (mm) of white flowers was slightly greater (mean 1.05, n = 16) than sepal length (mm) of yellow flowers (mean 0.96, n = 25). There is no significant difference (p = 0.06) (-0.09±0.04) standard error in sepal length (mm) between yellow and white flowers.

Moreover, corolla tube length (mm) of white flowers (mean 0.95, n =16) was a little greater than corolla tube length (mm) of yellow flowers (mean 0.94, n= 25). The corolla tube length (mm) did not show any significant differences (p = 0.84) (-0.01±0.06) standard error between the groups of white and yellow wild radish flowers (Table 4.7). Petal length (mm) of white flowers was longer than (mean 0.91, n = 16) petal length (mm) of yellow flowers (mean 0.89, n= 25). The petal length (mm) did not show any significant differences (p = 0.83) (-0.01±0.05) standard error between the two groups of flowers. Furthermore, the petal width (mm) of yellow flowers was (mean 0.74, n = 25) was wider than petal width (mm) of white flowers (mean 0.66, n = 16), and there were no significant differences (P >0.05) (0.09±0.05) standard error in petal width between yellow and white wild radish flowers.

There was a great difference in pedicel length (mm) of white flowers (mean 1.35, n =16) in comparison to that of yellow flowers (mean 1.05, n = 25). As shown from (Table 4.7), pedicel length (mm), showed a highly significant difference (p = 0.01) (-0.29±0.09) standard error between the two groups of yellow and white flowers with white flowers having longer pedicels.

4.3.4.2. Male floral parts.

The present results (Table 4.7) shows that anther lengths (in mm) of white flowers was longer (mean 0.29, n =16) compared to anther length (mm) of yellow flowers (mean 0.25, n = 25) and there was a significant difference (p = 0.03) (-0.02±0.01) standard error between yellow and white flowers regarding anther length. The mean differences between anther length in yellow and white is approximately -0.02 mm, indicating that anthers are on average 0.02 mm shorter in yellow flowers compared to white flowers.

The filament length (mm) of white flowers was a little longer (mean 0.80, n =16) than filament length (mm) of yellow flowers (mean0.77, n = 25). There was no significant difference (p = 0.27) (0.03±0.02) standard error in filament lengths (mm) between yellow and white flowers (Table 4.7). The mean difference in filament length between yellow and white flowers is approximately 0.03 mm, which is not statistically significant.

4.3.4.3. Female floral parts.

The results (Table 4.7) further compare stigma diameter between yellow and white flowers, Stigma diameter (mm) of white flowers was equal (mean 0.05, n= 16) to the stigma diameter (mm) of yellow flowers (mean 0.05, n =25). There was no statistically significant difference (p = 0.38) (-0.00±0.00) standard error in stigma diameter between the two flower colors. This suggests that stigma diameter does not play a significant role in distinguishing the two flowers. Moreover, pistil length (mm) of white flowers was longer (mean 1.24, n = 16) than pistil length (mm) of yellow flowers (mean 1.11, n = 25). Pistil length (mm), showed a significant difference (p = 0.05) (-0.13±0.06) standard error between the two groups of yellow and white flowers (Table 4.7).

Table 4.7: Independent samples t-test of morphological traits between yellow and white flowers.

Variables	Flower color	N	Mean	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Sepal Length	Yellow	25	0.96	0.06	-0.09	0.04
	White	16	1.05			
Corolla tube length	Yellow	25	0.94	0.84	-0.01	0.06
	White	16	0.95			
Petal width	Yellow	25	0.74[a]	0.10	0.09	0.05
	White	16	0.66			
Petal Length	Yellow	25	0.89	0.83	-0.01	0.05
	White	16	0.91			
Anther Length	Yellow	25	0.25	0.03	-0.02	0.01

	White	16	0.29			
Filament Length	Yellow	25	0.77	0.27	0.03	0.02
	White	16	0.80			
Pistil Length	Yellow	25	1.11	0.05	-0.13	0.06
	White	16	1.24			
Stigma Diameter	Yellow	25	0.05	0.38	-0.00	0.00
	White	16	0.05			
Pedicel Length	Yellow	25	1.05	0.01	-0.29	0.09
	White	16	1.35			

4.4. Discussions

4.4.1 Effect of flower colour on flower visitation by insect pollinators

The current study revealed that yellow flowers with dark brown distinct veins were visited by more insects than white flowers with light brown distinct veins, pale yellow flowers with lilac veins and yellow flowers with light brown distinct veins. Yellow flowers with dark brown distinct veins were mostly visited by *Eristalis tenax, Apis mellifera, Stizus sp.* and *Anthophora sp.* (Table 4.1). Our findings align with those reported by Lee *et al*. (1998), Stanton *et al*. (1989), and Devegili *et al*., (2021). These studies noted that yellow wild radish flowers were visited by more insects compared to other flower color morphs. Lee *et al*. (1998), observed that syrphid flies and halictid bees, made more visits to yellow flowers than white flowers. Stanton *et al*. (1989), found that *Pieris rapae* (butterflies) accounted for more than 90% of the plant visitors received and they visited more yellow flowers than white flowers. They further reported that bumble bees and honeybees overvisited yellow flowers

Even though honeybees were not frequent visitors in the study of Lee *et al*. (1998), they did not discriminate between yellow and white flowers. This may be due to floral signals, and the ecological significance of different flower colors attracting specific pollinator species (Stanton *et al*., 1990).

These suggests that insects visited wild radish flowers not due to color alone but also based on pollen and nectar, as evidenced by majority of insects visiting yellow flowers with dark brown distinct veins which produce more pollen and nectar.

4.4.2 Pollen production

The results from (Table 4.2.) confirms that types of stamen have an impact on the amount of pollen produced. The findings highlight the importance of different types of stamen in pollen production, they mean that for the amount of pollen produced, each stamen type plays a significant role despite it being short or long. This was supported by the regression results (Table 4.3) which further suggests that combination of short and long stamen lengths results in significant amount of pollen produced. However, anther lengths separately did not seem to have any significant impact on the amount of pollen produced. The result was supported by the primary data showing little or no variability amongst long stamens and short stamens (Fig 4.5), meaning that we cannot see any significant change in amount of pollen produced when we look at anthers separately. However, long stamens produced more pollen than the short stamens despite non-significant difference between amount of pollen produced and long stamens. This results are similar to other studies where they found that there is increased pollen production from long anthers which could be due to long anthers having more physical space to accommodate a larger number of pollen producing cells (microsporangia) (Astrand *et al*., 2021). Moreover, anther size affects the plant's ability to release and disperse pollen, larger anthers may produce more pollen grains, potentially increasing the chances of successful pollination and anthers may vary in size due to genetic and environmental factors (Stanton *et al*., 1990). Previous studies suggested that more pollen was produced from short stamen with negatively exerted anthers, which help in fertilization of wild radish flowers after insects removed pollen from the positively exserted anthers (Conner *et al*., 2023). However, more research is required to support this information.

Wild radish anthers produce a substantial amount of pollen as it is an outcrossing species, relying on pollinators for reproduction, however pollen production in wild radish varies depending on factors such as health and age of the plant (Conner *et al*., 2023; Conner *et al*., 2009; Sapir *et al*., 2017).

4.4.3. Pollen removal

The results of our analysis (Table 4.6.) show that there is a strong positive relationship between the long stamen, natural dimorphism, number of pollen grains on the control flower and amount of pollen removed from wild radish anthers. This suggests that flowers with longer stamens are effective at transferring pollen. Longer stamens may enhance the flower's visibility and accessibility to pollinators, thereby increasing the likelihood of pollen removal. Similarly, Conner *et al*. (1996) and Conner *et al*. (1997), found that more pollen was removed from long stamen anthers by bumblebees, honeybees (*Apis mellifera*), lepidopterans (butterflies), and long tongued bees as they hover on top of the anthers and feed for pollen and nectar. Moreover, the presence of natural dimorphism (short and long stamens) played a significant role in pollen removal as more pollen grains were removed from dimorphic stamens (Table 4.5). Short stamens match the feeding and probing structures of a wider variety of insects including honey bees and small bees. This optimize the contact between the pollinator and the reproductive parts of the flower, enhancing pollen transfer (Armbruster *et al.*, 2009a). Flowers with a variety of stamen lengths can appeal to a broader range of pollinators increasing the likelihood of multiples visits, leading to more pollen being removed (Armbruster *et al.*, 2009b). Shorter stamens position the anthers closer to the pollinator's body, ensuring that pollen adheres more effectively. This proximity also facilitates the transfer of pollen the stigmas of other flowers during subsequent visits (Harder *et al.*, 1993). Having stamens of different lengths can result in pollen being placed on different parts of the pollinator's body, reducing chances of self-pollination and increasing cross-pollination, which is often more beneficial for genetic diversity (Barret *et al.*, 1996). Therefore, the natural dimorphism in wild radish flowers enhanced the removal of pollen grains by ensuring better contact and appeal to a diverse range of pollinators.

4.4.4 Morphological traits of yellow and white flowers.

Among the general flower characteristics, sepal length did not show any significant difference between the two flower colors. However, sepal length can influence pollinator attraction and efficiency (Johnson and Dafni, 1998). Longer sepals may serve as landing platforms for pollinators, potentially affecting the type and frequency of visiting insects (Conner *et al.*, 2009).

On the other hand, a longer corolla tube may restrict access to shorter-tongued pollinators favoring those with longer tongues such as bumblebees (Campbell *et al.*, 1996). Additionally, corolla tube length can influence the efficiency of pollen transfer, a match between the tube length and pollinators tongue length can increase the chances of successful pollination, potentially leading to higher seed set and reproductive success (Thompson, 2001). Studies have shown that in some cases, longer corolla tubes may evolve in response to specific pollinator preferences, enhancing the plant's fitness (Schemske *et al*., 1999).

Furthermore, petal length affect the attractiveness of flowers to pollinators. Longer petals may be more visually appealing and attract specific pollinators with longer mouth parts (Pauw, 2022). Petal length influences the accessibility of the reproductive structures (pistils and stamens) to pollinators, potentially affecting the rate of successful pollination and seed production (Guitian *et al*., 1997). Previous studies found that petal length can evolve in response to natural selection and pollinator preferences, it can be subject to adaptive evolution driven by interactions with pollinators (Conner *et al*., 2023). However, yellow flowers produced more pollen, evidenced by majority of the pollen feeding insects visiting yellow flowers more than white flowers this is the sign that larger petals might signal greater pollen and nectar rewards to the pollinators, leading to increased visitation rates (Conner *et al*., 1995; Conner *et al*., 2009).

The significant differences in pedicel length between flower color morphs highlight the potential role of these traits in wild radish reproductive biology. Studies have found that pedicel length can impact the positioning of flowers in relation to the surrounding vegetation where taller stalks may elevate flowers above neighboring plants, potentially increasing their visibility to pollinators and facilitating cross-pollination, which can enhance reproductive success (Galen, 1999). However, in our study white flowers were not attracting more pollinators even though they had long pedicles (Table 4.7), which are assumed to make flowers visible above other flowers in their ecological niche.

The length of the filaments in white and yellow wild radish flowers affects the positioning of the anthers and accessibility of pollen to visiting pollinators (Conner *et al*., 2009). Longer filaments aid the anthers to protrude above the corolla tube allowing visibility of anthers to the pollinators (Conner *et al*., 2009; Conner *et al*., 1795).

The stigma diameter affects chances of successful pollination by various pollinators (Harder *et al*., 2009). A larger stigma provides a larger landing platform for pollen grains, increasing the likelihood of pollen deposition and fertilization (Harder *et al*., 2009). Similarly, plants with larger stigmas might be adapted to receive pollen from a broader range of pollinators, potentially enhancing reproductive flexibility (Ashman *et al*., 2004). The diameter of the stigmas in flowers can be adapted to specific pollinators. This adaptation means that certain flower-visiting insects, such as bees, are more efficient at pollinating flowers that have smaller stigmas, as they are easier for them to contact and pollinate while they collect nectar. Whereas, butterflies often favor larger stigmas, which are more accessible to them due to their long proboscis. These preferences enhance the efficiency of pollination for each type of pollinator based on their physical characteristics and feeding behaviors (Armbruster *et al*., 2009; Armbruster *et al*., 2014).

The differences in pistil length between flower color morphs highlight the potential role of these trait in wild radish reproductive biology. This supports the idea that there are meaningful differences between yellow flowers and white flowers pistil length, where longer pistils in white flowers can promote cross pollination and attract pollinators that have longer mouth parts (Morgan *et al*., 2005). This was evidenced by butterflies visiting more white flowers than they visited yellow flowers in the present study.

4.5. Conclusion

The study explored the relationship between flower color, morphological traits, and pollinator preferences in wild radish (*Raphanus raphanistrum*). Yellow flowers were found to be more appealing to pollinators such as *Eristalis tenax*, *Apis mellifera*, *Stizus sp*., and *Anthophora sp*., aligning with previous research indicating bees' attraction to yellow blooms. Pollen production analysis showed that long stamens and short stamens collectively produced substantial amount of pollen grains, consistent with prior findings. Anther size and position play roles in pollen dispersal and successful pollination, with larger anthers potentially enhancing fertilization chances. However, factors like natural dimorphism, long stamen exsertion and anther pollen count did not significantly predict pollen removal by pollinators, but more pollen was removed from positively exserted stamens and less pollen was removed from dimorphic stamens. Morphological traits such as the length of sepals, corolla tubes, petals, flower stalk, filament and pistils and stigma diameter varied between yellow and white flowers. These influenced pollinator attraction, reproductive structure accessibility, and reproductive success. For instance, longer corolla tubes may limit access to shorter-tongued pollinators, while longer petals may attract pollinators with longer mouthparts. Peduncle length can affect flower visibility and position relative to surrounding vegetation, possibly impacting pollinator visitation rates. Differences in anther color and filament length were also observed, potentially affecting anthers' visibility to pollinators. Larger stigma diameters and pistil lengths could enhance successful pollination by providing larger landing platforms for pollen grains and attracting a broader range of pollinators.

Chapter 5. General Conclusions and Recommendations

4.6. General conclusion.

The study of wild radish (*Raphanus raphanistrum*) revealed significant insights into the influence of floral morphology on plant-pollinator interactions and overall reproductive success of the plant. The diversity and abundance of pollinators varied between the two study sites, with Apis mellifera and Eristalis tenax being the most effective and abundant pollinators. Pollinators exhibited distinct preferences for certain flower color morphs, particularly favoring yellow flowers. Long stamens produced more pollen and were more attractive to pollinators, leading to higher pollen removal rates. Morphological traits such as pedicle length, anther length, and pistil length showed significant differences between white and yellow flowers. These traits influenced pollinator visitation and the efficiency of pollen transfer. However, the study was conducted in only two locations in Lesotho, which may not fully represent the variability in wild radish-pollinator interactions across different regions. It did not consider seasonal variations in pollinator populations and behaviors, and limited observations of pollinator behavior may have overlooked the subtle differences in preferences and efficiency among different pollinator species.

4.7 Recommendations.

The next research can include multiple agroecological zones to understand regional variations in plant-pollinator interactions, investigate how seasonal changes affect pollinator behavior and diversity, explore evolutionary basis of floral trait variations and their ecological importance, develop agricultural practices that promote pollinator diversity and effectiveness by maintaining and enhancing floral diversity in farm lands and implement long-term studies to monitor changes over time and provide more robust data on the dynamics of plant-pollinator interactions.

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