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Pollination ecology of Madras Pea Pumpkin, *Mukia maderaspatana* (L.) Roem. (Cucurbitaceae), a valuable herbaceous climber in inland and coastal wetlands

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ABSTRACT

Mukia maderaspatana is an annual herbaceous climber and grows under varied ecological conditions in inland and coastal wetland habitats. The flowering occurs seasonally but extends its flowering duration depending on the dampness of the habitat. It is strictly

monoecious with male and female flowers produced in different fascicles on the same plant. Both flower sexes offer nectar as a reward but male flowers additionally offer pollen as a reward. The plant is entomophilous involving melittophily and psychophily. Fruits are indehiscent berries and dispersed by gravity. The plant is a potential candidate as a vegetable, in folklore medicine for treating diabetes in humans and for treating hygroma in cattle, and also in restoration programs for coastal habitats.

Keywords: *Mukia maderaspatana*, herbaceous climber, monoecy, melittophily, psychophily.

1. INTRODUCTION

Members of Cucurbitaceae exhibit different sexual systems such as monoecy, andromonoecy, gynoecy and hermaphroditism (Bomfim et al., 2016). They are visited by a wide range of insect pollinators which include bees, wasps, ants, butterflies, flies and beetles (McGregor, 1976; Free, 1993; Delaplane and Mayer, 2000). But, pollen foraging bees are the predominant pollinators of this family (Renner and Schaefer, 2010). Cross-pollination by insects is most likely in most of the species because of the functionality of different sexual systems but little is known about the pollination biology of most species (Vogel, 1990; Renner and Schaefer, 2010).

The genus *Mukia* is a constituent of the sub-tribe Melothiirnae of the tribe Melothriae in Cucurbitaceae family. Pitkin and Jenkins (2004) reported that *Mukia* is a monotypic genus and often referred to as a genus of moths of Crambidae. These authors also noted that the flowers of this genus are tubular, sweetly scented and possibly pollinated by sphingidae or other nocturnal moths. de Wilde and Duyfjes (2006) reported that *Mukia* consists of about nine species and distributed in the tropical parts of the Old World representing the Sub-Saharan Africa, Yemen, Asia, Australia and New Zealand. These authors noted that *M. gracilis, M. javanica, M. leiosperma, M. maderaspatana, M. ritchiei* and *M. rhumphiana* occur in Asia. Lemmens and Bunyapraphatsara (2003) stated that *Mukia* genus can be distinguished by its small monoecious flowers which are fascicled in the leaf axils, its small sub-sessile baccate fruits produced in clusters and its tumid seeds. Of these species, *M. maderaspatana* is the most commonly described and the taxonomic validity of other species is still debated. This species is distributed throughout tropical Africa, the Indian Ocean Islands, tropical and subtropical Asia and tropical Australia. It shows considerable variation over its range into local variants by displaying variation in certain traits such as habit and size of plant, flower number and petal length, and seed number, size and shape (Lemmens and Bunyapraphatsara, 2003). It is a salt tolerant species that grows in sand dune, sandy beach and encroached mangrove lands in the coastal areas indicating that it can grow under varied ecological conditions as halophyte or glycophyte (Gokhale et al., 2011). Different authors described the uses of *M. maderaspatana* across its distribution range. The leaves, stems, fruits and roots are used as a vegetable by different communities (Bettencourt and Konopka, 1990; Seidemann, 2005; Petrus et al. 2011). In South India, its leaves and tender shoots are used to prepare crispy savoury pancakes (Petrus et al. 2011). In Andhra Pradesh, the leaves, pounded with garlic, pepper and cumin are used for treating hygroma in livestock (Reddy and Sudarsanam, 1987).

In India, *M. maderaspatana* is extensively used in folklore medicine as an anti-diabetic plant (Srilath and Ananda, 2014). These various reports indicate that *M. maderaspatana* is highly valued as a vegetable and medicine for humans and cattle. Despite its wide uses, this plant species has not been studied for its pollination ecology to understand its sexual system, mating system, pollination syndrome, fruiting and seed dispersal. Keeping this in view, the present study was contemplated to provide information on all these aspects of *M. maderaspatana* as this information is important for its conservation and management in wild habitats and for its commercial cultivation in suitable habitats for use as food.

2. MATERIALS AND METHODS

The annual herbaceous climber, *Mukia maderaspatana* growing in inland and coastal wetlands in Visakhapatnam (17°42'N Latitude and 82°18'E Longitude) were selected for the study during June 2018-May 2019. Regular visits to the patches of this climber were made to record flowering and fruiting seasons. Ten inflorescences which were about to initiate flowering on five plants were tagged and followed to record flower-opening schedule and the timing and mode of anther dehiscence. Anther dehiscence timing was confirmed by observing the anthers under a 10x hand lens. Twenty fresh flowers were used to record the floral morphological aspects, flower type, sex, shape, colour, symmetry, calyx, corolla, stamens, ovary, style and stigma. The floral configuration and rewards presentation aspects were observed in relation to the probing and forage collection of activities of insects. Ten mature buds two each on five plants were bagged and tagged to measure nectar volume. The micropipette was inserted into the flower base to extract nectar for measurement. The average nectar of ten flowers was taken as the total volume of nectar/flower and expressed in µl. The stigma receptivity was observed by H_2O_2 test as given in Dafni et al. (2005). The foraging visits of insects were recorded using

1 x 1 m area of flowering patch for 10 min at each hour for the entire day on four different days and the data was tabulated to record the foraging pattern and the percentage of visits made by bees and butterflies. The pollen/nectar collection behaviour of insects was carefully observed to assess their role in transferring pollen and effecting pollination. Ten specimens of each insect species were captured during 0900-1100 h and brought to the laboratory. Each captured specimen was washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present. From this, the average number of pollen grains carried by each insect species was calculated to know the pollen carryover efficiency. Fruit maturation period, the fruit and seed characteristics were recorded. Field observations were made to record fruit/seed dispersal mode.

3. RESULTS

Mukia maderaspatana is an herbaceous much-branched annual climber that grows well in damp inland and coastal habitats. The stem is finely grooved, strongly hispid with spreading bristly hairs and climbing by simple shaggy tendrils. The leaves are petiolate, vary in shape and size; usually broadly ovate to triangular in outline, 5-angled with denticulate margins and acuminate apex. In each leaf axil, a tendril grows off to one side and bears solitary or few-flowered fascicles. The flowering occurs during July-October but it extends depending on the moisture state of soil. The flowers are characteristically unisexual representing either male or female without any residual parts of the opposite sex. Male flowers are pedicellate and usually borne in fascicles on very short peduncles while female flowers are sessile and usually solitary although borne sporadically in 2--3 flowered fascicles which are also sessile. Both male and female flowers are borne in separate fascicles on the same plant. The male to female flower sex ratio is 5:1 at plant level.

Flowers are small, 10 mm long, bright yellow, slightly scented and actinomorphic. In male and female flowers, calyx is narrowly campanulate with five subulate lobes apically, 2-3 mm long, green, villous and erect. Corolla is bright yellow, gamopetalous, tipped with five ovate-oblong lobes, 1.5-4 mm x 1-2 mm and densely clothed with very long, thin, soft hairs. In male flowers, stamens are 3, inserted at the base of calyx tube, anthers ciliate, two anthers are di-thecal and one is mono-thecal (Figure 1b-d). All three anthers are twisted in a complex fashion giving the appearance of fused stamens in the center of the flower. In female flowers (Figure 1f), ovary is globose, bristly with dark forward-pointing hairs, 3 mm long, 3 mm across, mono-locular with many ovules, shortly beaked apically, extended into a 1.3 mm long cylindric style with 3-stigmatic lobes (Figure 1g-k).

Mature buds of both male and female flowers open at the same time during 0700-100 h each day (Figure 1a). In male flowers, anthers dehisce by longitudinal slits shortly before flower-opening. The pollen grains adhere to anther after dehiscence due to their sticky and wet nature. They are spheroidal, tricolporate and granulose (Figure 1e). In female flowers, the stigmatic lobes are receptive during anthesis but more receptive after anthesis and continue receptivity until the noon of the next day. Nectar is secreted in minute volume ranging from 0.8 to 1.2 µl per flower in male flowers and 1.1 to 1.4 µl per flower in female flowers. The male flowers fall after 2-3 days. Un-pollinated female flowers fall after 3-4 days. In pollinated female flowers, the corolla falls off after 4 days and calyx after 8-10 days. But, the style and stigma in withered form remain in place and crowns the fruit even after maturation.

Figure 1 *Mukia maderaspatana:* a. Anthesis initiation, b. Male flower, c. Stamens in the center of the flower, d. Insertion of stamens at the base of calyx tube, e. Pollen grain, f. Female flower, g. & h. Gynoecium within the flower, i. Pistil, j. 3-lobed stigma, k. Ovules.

The flowers were foraged by diurnal insects consisting of bees and butterflies. The bees foraged during 0700-1600 h maximum activity during 1100-1300 h (Figure 5) while the butterflies foraged during 0800-1500 h with maximum activity during 11-1200 h

(Figure 6). The bees included *Apis dorsata* (Figure 2a), *A. cerana, A. florea* (honey bees), *Trigona iridipennis* (stingless bee), *Anthophora cingulata* (Figure 2 e,f) (hairy-footed flower bee), *Xylocopa pubescens* (Figure 2b,c) (carpenter bee) and *Thyreus histrio* (Figure 2d) (cuckoo bee) (Table 1). Of these, the last two bee species foraged for nectar only while all other bees for both nectar and pollen. The butterflies included the Papilionid *Papilio polytes* (Figure 3a,b), Pierids *Catopsilia pyranthe* (Figure 3c), *Eurema hecabe* (Figure 3d), Nymphalids *Danaus genutia* (Figure 3e), *D. chrysippus* (Figure 3f), *Ariadne ariadne* (Figure 3g), Lycaenid *Euchrysops cnejus* (Figure 3h) and Hesperiid *Borbo cinnara* (Figure 3i) (Table 1). All butterflies foraged for nectar only. The bees made 84% and butterflies 16% of total foraging visits paid to both male and female flowers (Figure 7).

Figure 2 *Mukia maderaspatana:* a. *Apis dorsata* collecting nectar, b. & c. *Xylocopa pubescens* collecting nectar, d. *Thyreus histrio* collecting nectar, e. & f. *Anthophora cingulata* collecting nectar.

Figure 3 *Mukia maderaspatana:* a. *& b.* Papilionid butterfly, *Papilio demoleus*, c. & d. Pierid butterflies – c. *Catopsilia pyranthe*, d. *Eurema hecabe,* e-g. Nymphalid butterflies – e. *Danaus genutia*, f. *Danaus chrysippus*, g. *Ariadne ariadne*, h. Lycaenid butterfly, *Euchrysops cnejus*, i. Hesperiid butterfly, *Borbo cinnara.*

Figure 4 *Mukia maderaspatana:* a. & b. Growing fruits, c-f. Ripe fruits.

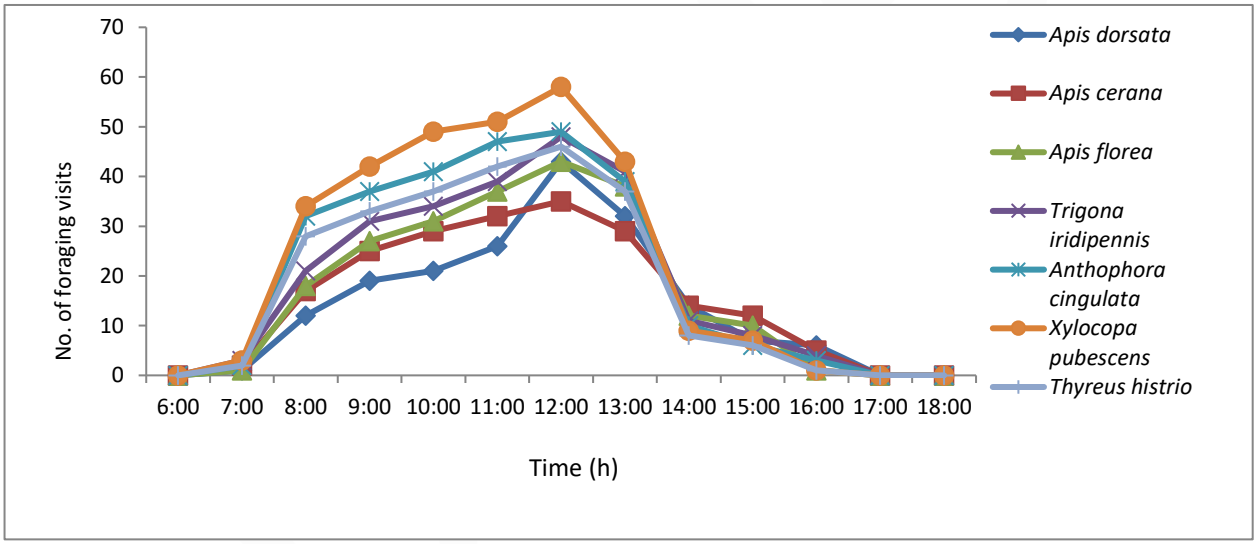

Figure 5 Hourly foraging activity of bees on *Mukia maderaspatana*

Table 1 List of insect pollinators on *Mukia maderaspatana*

Order/Family	Insect species	Forage sought
Hymenoptera		
Apidae	*Apis dorsata* F.	Pollen + Nectar
	Apis cerana F.	Pollen + Nectar
	Apis florea F.	Pollen + Nectar
	Trigona iridipennis Smith	Pollen + Nectar
	Anthophora cingulata F.	Pollen + Nectar
	Xylocopa pubescens Spinola	Nectar
	Thyreus histrio F.	Nectar
Lepidoptera		
Papilionidae	*Papilio polytes* L.	Nectar
Pieridae	*Catopsilia pyranthe* L.	Nectar
	Eurema hecabe L.	Nectar
Nymphalidae	*Danaus genutia* Cramer	Nectar

	Danaus chrysippus L.	Nectar
	Ariadne ariadne L.	Nectar
Lycaenidae	*Euchrysops cnejus* F.	Nectar
Hesperiidae	*Borbo cinnara* Wallace	Nectar

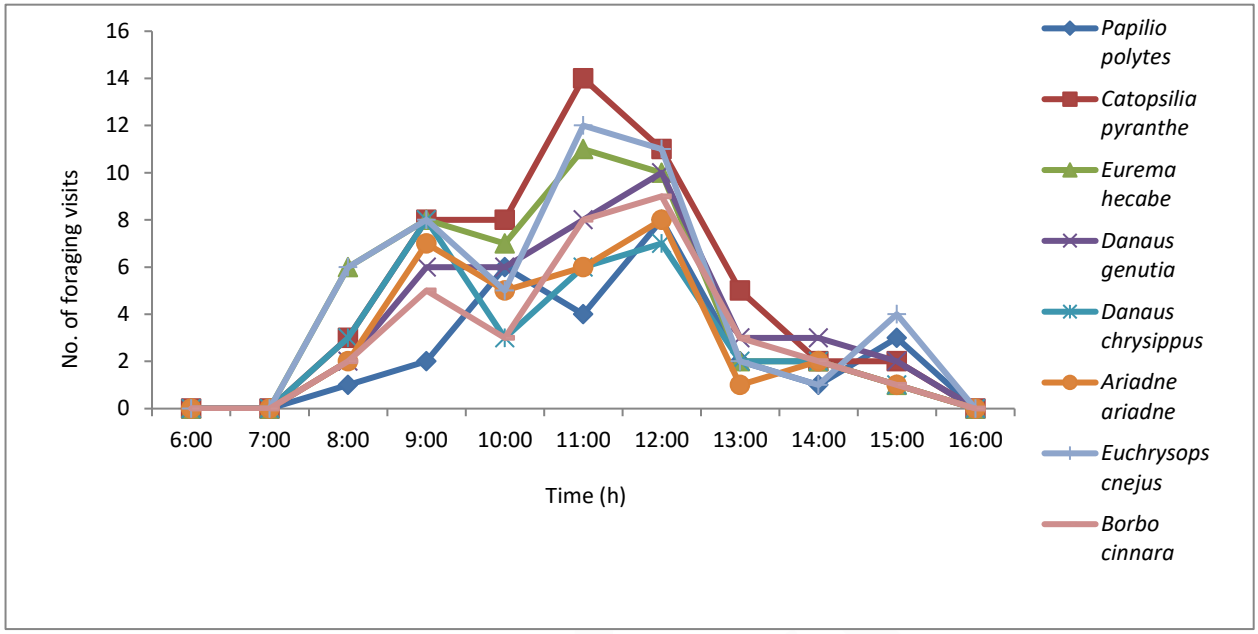

Figure 6 Hourly foraging activity of butterflies on *Mukia maderaspatana*

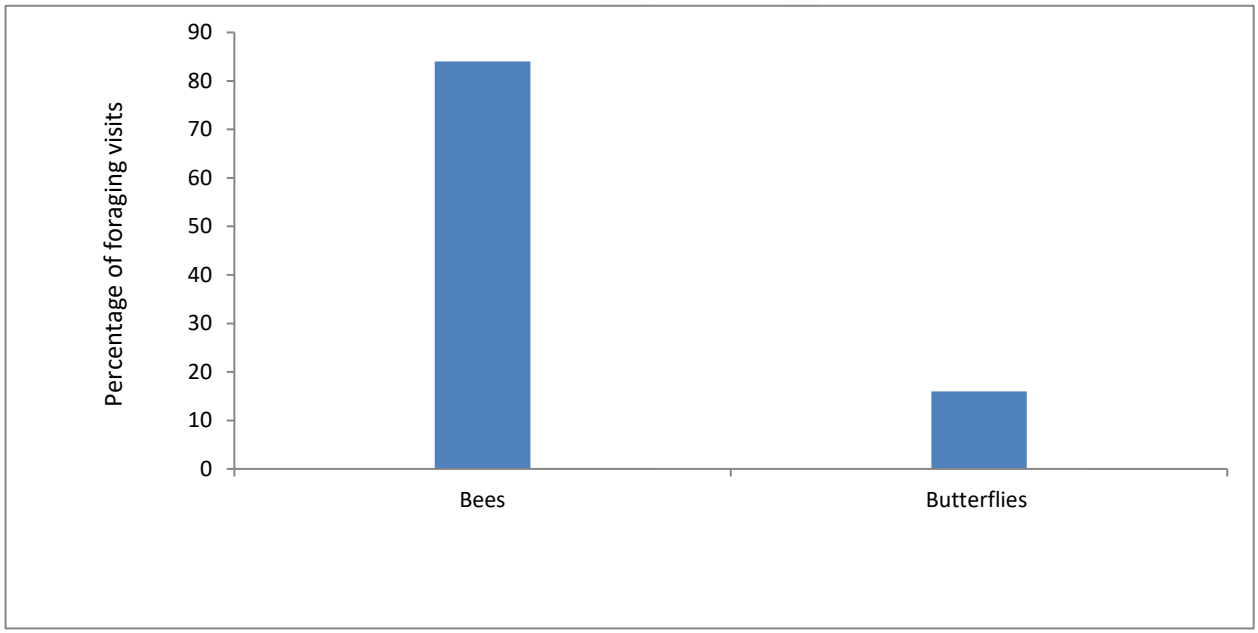

Figure 7 Percentage of foraging visits of bees and butterflies on *Mukia maderaspatana*

The flowers of both sexes are too small to provide landing place for either bees or butterflies. But, the corolla is appropriate for landing by *T. iridipennis* and to some extent by *A. florea* bees only. All other bees and butterflies landed on the stem and/or nearby leaf to probe the male and female flowers for forage collection; among these bees, *X. pubescens* is very large-bodied and the flowers with minute nectar quantity are quite inappropriate to the energy requirement of this bee. The nectar-foraging bees and butterflies probed the corolla base through the space between petals and stamens. Butterflies also probed the corolla base with their proboscis through the stamens in male flowers and through stigmatic lobes in female flowers. In male flowers, the bees while collecting nectar and/or pollen contacted the dehisced anthers on their ventral side and carried pollen ventrally whereas butterflies while collecting

nectar contacted the dehisced anthers with their proboscis, forehead and occasionally the foremost part of abdomen and carried pollen on all these parts. The body washings of bees and butterflies indicated that all carry pollen on their bodies but pollen carrying ability varied with each species. The mean pollen recovered from the body washings varied from 129.2 to 208.2 in case of bees and from 18.2 to 28.4 in case of butterflies (Table 2). In female flowers, the bees and butterflies while probing contacted the stigmatic lobes first and then proceeded to corolla base for nectar collection; in this probing behavior, they invariably transferred pollen from male flowers to the stigmata of female flowers. Both bees and butterflies visited male and female flowers without any discrimination. The foraging activities of bees and butterflies effected geitonogamic and xenogamic pollinations only as individual flowers had either male or female organs but not both. Therefore, the plant is vector-dependent for pollination.

Table 2 List of insect pollinators on *Mukia maderaspatana*

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Bees				
Apis dorsata	10	92-307	208.2	55.45
Apis cerana	10	66-214	140.5	42.9
Apis florea	10	43-214	129.2	46.26
Trigona iridipennis	10	53-231	131.3	47.32
Anthophora cingulata	10	87-236	161.5	46.61
Xylocopa pubescens	10	78-252	167.1	56.83
Thyreus histrio	10	68-164	115.9	24.8
Butterflies				
Papilio polytes	10	15-40	27.8	7.40
Catopsilia pyranthe	10	11-29	20.8	5.24
Eurema hecabe	10	10-38	25.4	7.4
Danaus genutia	10	12-34	23.3	6.37
Danaus chrysippus	10	10-42	28.4	8.27
Ariadne ariadne	10	11-28	18.2	5.9
Euchrysops cnejus	10	8-25	19.1	4.25
Borbo cinnara	10	12-31	22.1	6.20

Fruits mature within a month and produced from solitary and fascicled flowers. They are initially green and red when mature (Figure 4a-f). Fruit is a globose berry clothed with a few coarse hairs and 12-15 mm diameter containing grey colored scrobiculate seeds enclosed in mucilaginous sap. The seeds are 4 mm long, 2 mm broad, and moderately compressed. Fruits are indehiscent but sometimes open exposing the seeds which then float to the ground.

4. DISCUSSION

Gokhale et al. (2011) reported that *Mukia maderaspatana* is a salt tolerant species that is widespread in sand dune, sandy beaches and mangrove forest lands indicating that it has all inherent adaptations to grow as halophyte or glycophyte under varied ecological conditions. In this study, *M. maderaspatana* is found to grow in damp areas in inland and coastal wetland areas. In mangrove lands, it is restricted to the landward areas while in inland areas; it flourishes well in damp areas near canals, agricultural fields and riverine banks. Its occurrence in only landward side of mangrove forest and inland damp areas indicates that *M. maderaspatana* is largely a land plant but also has adaptations to grow as a glycophyte in low saline soils. Further, it also grows well in damp sand dunes. This species is characteristically an annual herbaceous climber but can grow as a perennial if soil ecological conditions are favorable. The flowers being bright yellow in color are quite prominent and attractive against light to dark green foliage to the pollinator insects.

Bomfim et al. (2016) reported that Cucurbitaceae members display different types of sex expression such as monoecy, andromonoecy, gynoecy and hermaphroditism. In this study, *M. maderaspatana* has been found to exhibit monoecy with male and female flowers borne in different fascicles on the same plant. This monoecious sexual system completely avoids the occurrence of autonomous or vector-mediated autogamy but does not prevent the occurrence of geitonogamy which is an evolved form of

selfing. However, the occurrence of geitonogamy could be reduced by the plant by limiting the production of female flowers on days of maximum production of male flowers or production of male and female flowers on alternate days at plant level. Detailed field studies on this aspect may throw more light whether the plant has any such strategy to reduce geitonogamy and promote xenogamy.

Renner and Schaefer (2010) stated that Cucurbitaceae flowers usually open early in the morning and anther dehiscence occurs before anthesis. Delaplane and Mayer (2000) reported that Cucurbitaceae flowers show stigma receptivity during anthesis but it is more receptive after anthesis. In this study also, *M. maderaspatana* flowers open in the morning hours and anthers dehisce shortly before anthesis indicating that flowers are adapted for diurnal pollination and exhibit weak protandry due to attainment of stigma receptivity during the process of anthesis. In fact, protandry whether it is weak or strong is not a matter of concern since the flowers are unisexual and pollen flow should occur between male and female flowers.

Different authors have reported that Cucurbitaceae members are visited by a wide range of insect pollinators consisting of bees, wasps, ants, butterflies, flies and beetles (McGregor, 1976; Free, 1993; Delaplane and Mayer, 2000). *Mukia* is known as a genus of moths of Crambidae. Its flowers are tubular, sweetly scented and pollinated by sphingids and other nocturnal moths (Pitkin and Jenkins, 2004). In this study, *M. maderaspatana* with mildly scented flowers is never visited either by diurnal or by nocturnal moths. It is visited and pollinated by bees and butterflies only. In this species, male flowers offer both nectar and pollen while female flowers offer only nectar as floral reward(s) indicating that male flowers are more profitable than female flowers for bees collecting both nectar and pollen. Bees such as *Xylocopa* and *Thyreus* collecting only nectar use male and female flowers as nectar source and they obtain similar levels of energetic reward from both flower sexes. The same stands true for butterflies also since they collect only nectar. But, among bees, pollen foraging bees which also collect nectar are predominant pollinators as they are mostly involved in pollen transfer between male and female than nectar foraging insects. The pollen is sticky, granulose and appears to have a layer of oily pollen kitt which serves as an important nutrient for pollen collecting bees. This study is in conformity with the report by Renner and Schaefer (2010) that pollen foraging bees are the predominant pollinators of Cucurbitaceae members and also in conformity with the note by Vasil (1960) that pollen of Cucurbitaceae is usually covered with a thick layer of oily yellow to orange-colored pollenkitt that serves as a vital source of lipids (Dobson, 1988), carotenoids (Hesse, 1993; Lunau, 1995), flavonoids (Wiermann and Gubatz, 1992), proteins and carbohydrates (Parkinson and Pacini, 1995; Santos et al., 2003). Both bees and butterflies with their visits to male and female flowers effect geitonogamous and xenogamous pollinations. Therefore, *M. maderaspatana* is both melittophilous and psychophilous.

Renner and Schaefer (2010) reported that in Cucurbitaceae, fruit or seed dispersal is mostly by animals, more rarely by wind or gravity. In this study, *M. maderaspatana* produces indehiscent fruits with several seeds. Usually, they fall down when mature and ripe and subsequently seeds are released from mucilaginous sap of the fruit upon decomposition. During the study period, the animals such as birds or bats never visited the plant for ripe fruits indicating that these animals might be utilizing some other reliable fruit source available in the area or the fruits are not adapted for feeding and subsequent dispersal by birds or bats. Different authors reported that *M. maderaspatana* is valuable as vegetable and in folklore medicine for treating diabetes in humans and also for treating hygroma in cattle in various communities across the distribution range of this species (Reddy and Sudarsanam, 1987; Bettencourt and Konopka, 1990; Seidemann, 2005; Petrus et al., 2011; Srilatha and Ananda, 2014). Since *M. maderaspatana* grows extensively in damp places both inland and coastal areas, it can be promoted for utilization especially as a vegetable and listed in the list of vegetable crops. Further, this species can also be included as a potential candidate in restoration programs for coastal habitats due to its ability to form dense patches quickly covering the land and adding organic matter upon death leading to the fertilization and stabilization of the sandy soils.

5. CONCLUSION

Mukia maderaspatana is a herbaceous monoecious climber and widespread both in inland and coastal wetland habitats. It is a seasonal bloomer but flowers throughout the year if the soil is damp. The flowers are unisexual and essentially require pollen-vectors for pollination. The pollinators include bees and butterflies only. Fruits are indehiscent berries, disperse by gravity and subsequently release seeds upon decomposition. The plant is useful as a vegetable and in folklore medicine and in treating hygroma in cattle. Further, it is a potential candidate for inclusion in coastal restoration programs due to its ability to form thick patches quickly, add organic matter upon death and stabilization of the sandy soils.

Author's contributions

All authors contributed equally.

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Conflict of Interest:
The authors declare that there are no conflicts of interests.

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