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Study of floral biology of *Bidens pilosa* L.

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Abstract

The present paper indicates that *B. pilosa* displays vegetative, flowering and fruiting phases throughout the year. The plant produces heterogamous capitula with all ray florets opening on the first day and disc florets opening on the next four consecutive days. The ray florets are sterile while disc florets are fertile, dichogamous, protandrous, herkogamous, self-compatible, self-pollinating (vector-mediated) and facultative xenogamous. The disc florets display secondary pollen presentation. The plant is accordingly entomophilous but principally psychophilous. Disc florets produce non-dormant, long and short cypselas from the same capitulum. Seed dispersal is polychorous involving anemochory, anthropochory, zoochory and ombrohydrochory.

Keywords: *Bidens pilosa*, heterogamous head

Introduction

Gillett (1975) ^[1] reported that the genus *Bidens* is composed of approximately 230 species with worldwide distribution in tropical and temperate regions. Ballard (1986) ^[2] and Jaimes and Ramirez (1999) ^[3] noted that a few species of *Bidens* have been studied for their reproductive ecology in North and Central America.

Sheriff (1937) ^[4] classified *B. pilosa* into six varieties var. *pilosa*, var. *minor*, var. *radiata*, var. *bimucronata*, var. *calicicola*, and var. *alausensis* based on the morphological differences. Peng *et al.* (1998) ^[5] listed three varieties of *B. pilosa*, var. *minor*, var. *pilosa* and var. *radiata* in the Flora of Taiwan. Wu *et al.* (2004; 2010) ^[6-7] reported that the most obvious differences in morphology among these three varieties are their flowers.

The var. *minor* and var. *pilosa* are more widespread and more dominant than var. *radiata* in the native distribution range of *B. pilosa* species complex. The studies mentioned above indicate that the detailed studies on the pollination ecology of *Bidens pilosa* have not been done despite its widespread distribution in agricultural and non-agricultural areas. Keeping this state of information on this widespread species in view, the present study was contemplated to investigate the following objectives in *B. pilosa*: flowering phenology, flower morphology. This information is important to carry out further studies to develop strategies for its control in agricultural as well as non-agricultural areas if it is a menace.

Materials and Methods

The study area: Populations of *Bidens pilosa* growing in Sidhi district lies in between 81°-18' to 82°-42' longitudes and 23°-48' to 24°-42' latitudes north in the north-east corner of Rewa division of M.P. It is surrounded by the Mirzapur district of U.P. On the north-east side and by Korea district of Chattisgarh on the south side. The general physiography of this district is very hilly and undulating. The hottest month is usually 'May' but occasionally it may also be the 'June' if the onset of monsoon is delayed. Similarly the coldest month is January, but it may also be December. Rainy season is more or less equable. The post rains period is warm to hot, winters are mild but summers are severe.

Floral biology: Twenty mature but un-dehisced anthers from disc florets were collected from five randomly chosen plants and placed in a Petri dish. The pollen output per anther/disc floret and pollen-ovule ratio was calculated using the protocol given by Cruden (1977) ^[8]. Individual volumes of nectar were recorded for twenty-five disc florets and then the average volume of nectar per disc floret was determined and expressed in μ l. The capitula used for this purpose were bagged at mature bud stage, opened after anthesis and

squeezed nectar from each disc floret into micropipette to measure the volume of nectar. Based on nectar volume in individual disc florets, the total volume of nectar secreted in a capitulum was estimated. Similarly, the nectar sugar concentration at capitulum level was determined using a Hand Sugar Refractometer (Erma, Japan). Nectar analysis for sugar types was done as per the Paper Chromatography method described in Dafni *et al.* (2005) [9]. The sugar content/flower is expressed as the product of nectar volume and sugar concentration per unit volume, mg/ μ l. This is done by first noting the conversion value for the recorded sugar concentration on the refractometer scale and then by multiplying it with the volume of nectar/flower. Table 5.6 given in Dafni *et al.* (2005) [9] was followed for recording the conversion value to mg of sugars present in one μ l of nectar. Dinitrosalicylic acid method was followed for the first two sugar types while Resorcinol method for the last sugar type. The caloric reward of nectar/flower/day was measured as per the formula given in Heinrich (1975) [10]. He assumed that 1 mg of sugar yields 16.74 joules or 4 calories of energy and accordingly he used the formula for calculating the caloric reward of the nectar.

$$\frac{\text{Nectar volume } (\mu\text{l}) \times \text{Concentration of nectar } (\%)}{100} \times 16.74$$

Paper chromatography method described in Dafni *et al.* (2005) [9] was followed for identifying the amino acid types in the nectar of disc florets.

Insect activity and pollination: The flower visitors were collected and identified with the representative specimens available with the environmental biology Department of A.P.S. University Rewa (M.P.).

Simultaneously, the insects were observed for their foraging behavior such as mode of approach, landing, probing behaviour, the type of forage they collected, contact with essential organs to result in pollination, inter-plant foraging activity. The insects were captured from the flowers during 1000-1200 h on five different days for pollen analysis in the laboratory. For each insect species, 10 specimens were captured and the proboscides were separated for examination to record whether they carry pollen grains or not. They were washed first in ethyl alcohol and the contents stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present.

Fruiting ecology, seed dispersal and germination: Field observations on seed germination and seedling formation were made to record the approximate number of generations produced during rainy season.

Results

Phenology: The plant is an annual branching herb with gracile habit. It grows in open hilly areas and waste lands but it is not very common. It often forms colonies covering the soil in open areas. The stems are quadrangular with grooved and hairy straggling branches and striate branchlets. The leaves are petiolate, trifoliolate and arranged oppositely; terminal leaflets are obovate to lanceolate, crenate-serrate, acuminate, base rounded to acute and sparsely hairy. It propagates only by seed mode. The seeds germinate and produce new plants during rainy season

which commences in June. The flowering occurs throughout the year if the soil is sufficiently wet but peak flowering occurs during September to November. The plant withers and dies in areas where the soil is dry, especially during summer season.

Flower morphology: A plant produces 28.68 ± 17.42 heterogamous capitula either singly or in groups as a corymb on long peduncles in leaf axils or terminally. Each capitulum consists of a long outer and shorter inner linear to lanceolate involucral bracts with fine hairy margins followed by odorless ray and disc florets. In each capitulum, ray florets are usually five but rarely six or seven while disc florets are 34.68 ± 5.80 ; the ray and disc floret ratio is 1:6. The calyx is reduced to pappus of barbed and strong bristles in both types of florets. The corolla is tubate and tipped with five actinomorphic lobes in both floret types but in ray florets, one lobe is ligulate. The ligulate ray floret is white with scarlet red base which serves as nectar guide. The ray floret is 8.1 ± 1.1 mm long and 4.9 ± 0.6 mm wide. Disc florets are yellow, 8.5 ± 0.5 mm long and 1.8 ± 0.3 mm wide. The ray florets lack stamens but have poorly developed pistil with ovary lacking ovule and residual two-armed style. The disc florets have five stamens with yellow filaments (1.2 mm long and 1 mm wide) and dark brown anthers (1.8 mm long and 1 mm wide).

Floral biology: The ray and disc florets open during early morning 0700-0900 h on clear sunny days (Table 1). The florets open completely on sunny days while they are partially open on rainy days. In a capitulum, all of the ray florets open on first day simultaneously; the disc florets open concentrically inwards from the next day for four successive days with 37% opening on day 1, 29% on day 2, 19% on day 3 and 15% on day 4. Individual disc florets take about 3 hours to open from mature bud phase. The disc florets are protandrous with anther dehiscence taking place during mature bud stage by longitudinal slits. The narrow anthers are united to form a hollow space into which pollen is liberated. At mature bud stage the style with its aligned stylar arms lies below the anthers, its forked arms are converged at this stage. During and immediately after anthesis, the style grows, elongates and passes through the anther tube brushing the pollen with its sweeping hairs. At this stage, the inner stigmatic surfaces are un-receptive and not exposed, the functional situation of which prevents the occurrence of autogamy. Such a form of pollen presentation is referred to as "secondary pollen presentation mechanism" which ensures the pollen availability to insects visiting the capitula on daily basis. The style with its aligned branches gradually diverge in the early hours of 2nd day; then the inner stigmatic surfaces attain receptivity and remain so until the end of that day. The stylar arms curve downwards completely exposing the receptive stigmatic surfaces. The pollen grains are bright yellow, circular, tricolporate, 21.1 ± 0.34 μ m in size and echinate. The pollen grains are 338.21 ± 64.18 per anther, 1691.06 ± 320.94 per floret and 58,644 per capitulum. The pollen-ovule ratio is 1691:1. The ray florets are nectarless while disc florets are nectariferous. Ray florets do not produce nectar. Disc floret produces 1.2 μ l of nectar which rises up as it accumulates in the floret due to narrow corolla tube by the end of 2nd day. Nectar is not secreted during the remaining period of floret life indicating that nectar is secreted only during staminate phase. A

capitulum produces an average volume of 41.6 µl of nectar during its lifespan; the nectar sugar concentration is 25 ± 1.43% with 0.38 mg of sugar containing 1.52 calories of energy at floret level and 11.36 mg of sugar containing 45.44 calories of energy at capitulum level. The sugar types present in the nectar include sucrose, glucose and fructose; they are present in that order of dominance. The nectar contains four essential amino acids (tryptophan, histidine, lysine and arginine) and six non-essential amino acids (cysteine, serine, aspartic acid, glycine, cystine and glutamic acid); they are present in that order of dominance. The ray and disc florets wither away on 3rd day and fall off on 4th day. In fertilized disc florets, the tubate corolla and stamens gradually fall off after the initiation of fruit formation.

Table 1: Anthesis of disc florets as a function of time in *Bidens pilosa*.

Time (h)	No. of disc florets anthesed								Total
	Day 1	%	Day 2	%	Day 3	%	Day 4	%	
06:00	-	-	-	-	-	-	-	-	-
07:00	4	12	3	9	3	8	2	6	35
08:00	6	17	5	14	3	8	2	6	45
09:00	3	8	2	6	1	3	1	3	20

First, all ray florets open at once on the first day of anthesis of capitulum. The next day onwards disc florets open for four consecutive days. Total no. of disc florets per capitulum 35.

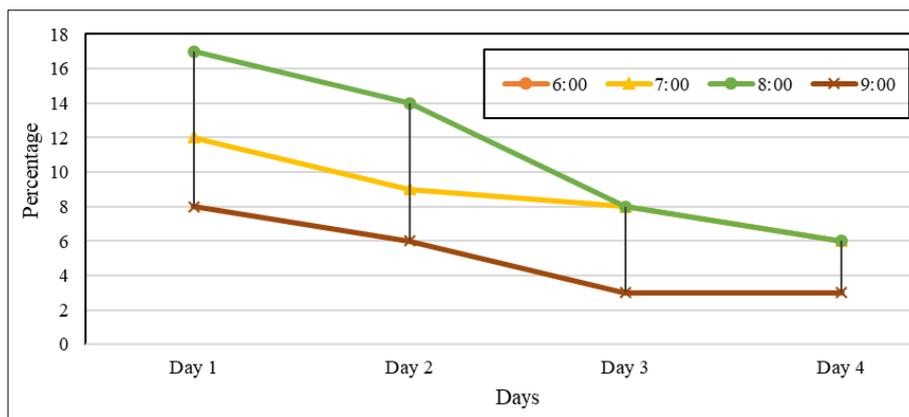


Fig 1: Anthesis of disc florets as a function of time in *Bidens pilosa*

Discussion

Bidens pilosa is a therophyte herb and occupies roadsides, disturbed sites and cultivated fields in tropical and subtropical climates (Peng *et al.*, 1998; Grombone-Guaratini *et al.*, 2004) [5, 11].

The plant disappears in moisture and nutrient-deficient soils, which is usually evidenced during dry season. Therefore, it can be said that soil moisture and nutrient content plays an important role in providing stimulus for the growth and reproduction of this plant.

Peng *et al.* (1998) [5] reported *B. pilosa* has three varieties in Taiwan. They are var. *radiata*, var. *minor* and var. *pilosa*. The capitula in the first two varieties have both ray and disc florets while those in the last variety has only disc florets. Huang and Kao (2015) [12] reported that *B. pilosa* var. *radiata* is a tetraploid and self-incompatible while var. *minor* and var. *pilosa* are hexaploids and self-compatible. Treier *et al.* (2009) [13] reported that there is a link between polyploidy and invasiveness. All three varieties of *B. pilosa* are polyploids; var. *minor* and var. *pilosa* with hexploidy are non-invasive while var. *radiata* with tetraploidy is invasive in Taiwan. Further, these authors stated that non-invasive varieties of *B. pilosa* are more widespread and more dominant than invasive var. *radiata* in the native distribution of *B. pilosa* species complex. Keeping this in view, they stated that polyploidy alone cannot explain the dominance of non-invasive varieties and suggested that there are some other traits such as the ability of vegetative reproduction and breeding system that contribute to dominance or invasiveness of *B. pilosa* varieties. In the present study, the distribution and floral morphological characters of *B. pilosa* probably indicate that this weed represents var. *pilosa* which is dominant in cultivated and

forest sites with moist soils but it is not invasive. However, further studies on vegetative and reproductive aspects are needed for confirmation.

Dichogamous protandry and self-incompatibility have been reported to be functional in Asteraceae (Allen *et al.*, 2011) [14]. Dichogamous protandry is a mechanism that reduces the impact of pollen-pistil interference on pollen import and export (Barrett, 2002) [15] and reduces rates of self-fertilization and enhances out-crossing (Harder *et al.*, 2000) [16]. Self-incompatibility is another evolutionary strategy to avoid self-fertilization and inbreeding (Nettancourt, 2001) [17]. In Asteraceae, it usually occurs at the stigmatic surface either by failure of germination of self-pollen grains or by the arrest of pollen tube growth (Allen *et al.*, 2011) [14]. The present study shows that *B. pilosa* exhibits dichogamous protandry and self-compatibility. The ray florets representing female sex are sterile due to non-production of ovule by ovary. The disc florets are bisexual and fertile with staminate phase on day 1 and pistillate phase on day 2 characterize dichogamous protandry characterizing temporal dioecy. The florets also have a physical barrier between the site of pollen deposition and stigmatic surfaces characterizing herkogamy which favors xenogamy, and pollination occurs during pistillate phase. The vector-mediated selfing occurs due to availability of self-pollen as a consequence of centripetal opening of disc florets in the capitulum on consecutive days. Such a mating system has been reported in *Bidens segetum* (Grombone-Guaratini *et al.*, 2004) [11]. In this study, high fruit set rate within the capitulum and in open-pollinations at population level in *B. pilosa* indicate the function of self-compatibility and self-pollination in this plant. This finding agrees with the report of Huang and Kao (2014) [18] that hermaphrodite disc florets

of *B. pilosa* var. *pilosa* are self-compatible, self-pollinating and set fruit in bagged capitula in Taiwan, and also with the report of Grombone-Guaratini *et al.* (2004)^[4] that *B. pilosa* is self-compatible and self-pollinating. The pollen/ovule ratio recorded for *B. pilosa* (1,691:1) in this study falls in the range of pollen/ovule ratio (244.7-2,588) for facultative xenogamy provided by Cruden (1977)^[8] and hence this plant is facultative xenogamous with low self-incompatibility. In this breeding system, xenogamy enables the plant to increase genetic heterogeneity which favors its establishment in heterogeneous and variable environments (Hsu, 2006)^[19] while geitonogamy facilitates to increase its population in the currently growing sites or other sites with similar environment. The allied species, *B. alba* and *B. subalternans* are also self-compatible and self-pollinating (Grombone-Guaratini *et al.*, 2004)^[4]. But, a congener, *B. segetum* is self-incompatible as it does not set fruit in spontaneous and hand-self pollination modes (Valentin-Silva *et al.*, 2016)^[20]. Therefore, it can be stated that *Bidens* genus has both self-compatible and self-incompatible species with which they became widespread while some of them became invasive.

Mani and Saravanan (1999)^[21] stated that butterflies are important pollinators of Asteraceae and represent nearly 75% of all visitors. Because their irregular, unpredictable and random foraging behavior increases out-crossing rate which in turn increases genetic variation. These authors also stated that Syrphidae flies are also important pollinators. Sun and Ganders (1990)^[22] reported that butterflies are important foragers of *Bidens* species. Grombone-Guaratini *et al.* (2004)^[4] reported that *Bidens pilosa* var. *pilosa* is principally pollinated by bees, especially solitary bees which tend to visit the capitula randomly promoting out-crossing while butterflies are of minor importance as they do not carry pollen on their bodies. The present study shows that butterflies are principal pollinators while all other insects are supplementary pollinators of *B. pilosa*.

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