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BUZZ - POLLINATION IN *TRICHODESMA INDICUM* (L.) R. BR. (BORAGINACEAE).

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Abstract

Trichodesma indicum (L.) R. Br. (Boraginaceae) flowers throughout the year. Flower was protandrous and remained in bloom for ± 3 days. Buzz-pollination was carried out sternotribically by two species of *Anthophora* (Apidae). Pollen - ovule ratio and breeding experiments (hand-pollination treatments) revealed it as a facultative xenogamous species. It is a non-apomictic species as except emasculation treatment for apomixis, all the hand pollination treatments resulted in normal fruit and seed set.

Introduction

The genus *Trichodesma* L., is represented by c. 10 species, distributed in tropics and subtropics (Willis, 1973). According to Dukas & Dafni (1990) two species of the genus viz. *Trichodesma africana* (L.) R.Br. and *Trichodesma boissieri* Post., share a peculiar mode of pollination known as "Buzz-Pollination". In buzz-pollinated flowers bees usually collect pollen by vibrating the anthers rather than brushing or grooming them (Buchmann, 1983). The buzz-pollination has also been reported in *Borago* and *Onosma* of the family Boraginaceae (Buchmann, 1983; Corbet *et al.*, 1988).

In Pakistan, only four species of the genus *Trichodesma* are present (Nasir, 1989). Since no work has been done on the floral biology of any of the local species, therefore the account of pollination biology of *Trichodesma indicum* (L.) R.Br., the most common species of Pakistan is presented.

Materials and Methods

Morphology and Phenology: Young floral buds (N=50) were tagged in the Karachi University campus area to determine the morphology and phenology.

Insects: Foraging behaviour of flower visiting insects was recorded. The insects were collected by hand net, chloroformed and examined microscopically for the pollen load. Insects carrying pollen were evaluated as pollinators.

Pollen-Ovule Ratio: The pollen-ovule ratio of mature floral buds (N=30) was calculated following the method of Cruden (1977).

Breeding System: Hand pollination treatments for control, direct autogamy, geitonogamy, xenogamy and emasculation for apomixis were performed in mature floral buds (N=50 for each treatment), just prior to anthesis following the method of Radford *et al.*, (1974). From each treatment fruit length, breadth and weight, seed set, seed length, breadth and weight were determined. The data was statistically analyzed using one-way ANOVA and Duncan's Multiple Range Test (DMRT) following Gomez

& Gomez (1984).

Observations and Results

Morphology and Phenology: *T.indicum* an erect annual herb often occurs in clumps. Stems profusely branched and densely hairy; hairs dimorphic; shorter ones thin appressed, longer ones stiff with tuberculate bases. Basal leaves dry at anthesis, cauline leaves usually opposite, oblong-lanceolate or rarely ovate, entire dilated or amplexicaule at base, 2.5-11.25 x 0.75-3.5 cm. Inflorescence terminal, lax, few-flowered. Flowers regular, actinomorphic hanging upside down. Pedicels slender pubescent, 0.16-10.7 mm long. Calyx 5-partate, lobes oblong, 7-12 x 13-15 mm, base sagittate to auriculate, gradually narrowing towards the acute apex, slightly enlarged in fruits. Corolla pinkish blue with brown blotches at the throat, infundibuliform, 10-15 mm long, slightly surpassing the calyx, lobes semi orbicular, spreading, abruptly narrowed to filiform apices. Stamens 5, anthers introrse, densely villose, filaments very short, elongated connectives formed a cone around the style, with twisted appendages at the apex forming a central pore. Nutlets ovoid, glabrous 3-3.9 x 1.9-2.5 mm (Fig. 1).

Flowering occurs throughout the year but profusely after monsoon rain. An initiated bud took about 8-9 days to become a flower. Most of the flowers open in the evening at 17.00-18.00 h and few in the morning at 6.00-7.00 h. The individual flower remains in bloom for $\pm$ 3 days. After flower anthesis, following three phenological phases/stages may be recognised.

1) ***Staminate Phase:*** Freshly open flower has straight, pink petals, greenish cone with tightly twisted appendages and small apical pore. Anthers and pollen grains white, dehiscence of anthers starts in mature bud stage, just prior to flower opening. Anthers dehisce longitudinally which starts from the apex and continues towards the base. White pollen grains start to accumulate at the apex of cone in the twisted region. Immature (non-capitate) stigma remains within and just below the twisted portion of the cone with 6-7 mm long style. Nectar is secreted by the nectaries, situated at the base of the ovary and accumulates in the small corolla tube. This phase lasts for about 24h.

2) ***Pistillate phase:*** The petals start turning blue particularly at the middle vein, curved backward so that the brown blotches at the throat become more visible and exposed. Cone starts to become greenish-black, twisting of the appendages become loose and apical pore become wide open. Anthers become brown and dehisce completely early in this phase. Capitate stigma matures and comes out of the cone through the pore with 11-12 mm long style. Nectar is still available in the corolla tube. This phase lasts from 24-48 h after anthesis.

3) ***Post-pollination phase:*** After 48 h of anthesis, the corolla alongwith the cone fades and drops while calyx and gynoecium remain on the flower. Initiation of the fruit (i.e. swelling) is evident within 3-4 days after dropping of the corolla.

Insects: Mainly Hymenoptera and Lepidoptera were observed on the flowers of *T. indicum*. Nectar and pollen seem to be the main reward. Hymenoptera were the main pollinators particularly two species of *Anthophora* (Anthophoridae; Apoidae) found to be the only pollinators of this taxon. *Anthophora* sp.(A) is larger in size than sp.(B), however, both species share similar foraging behaviour. Insect first hoover around

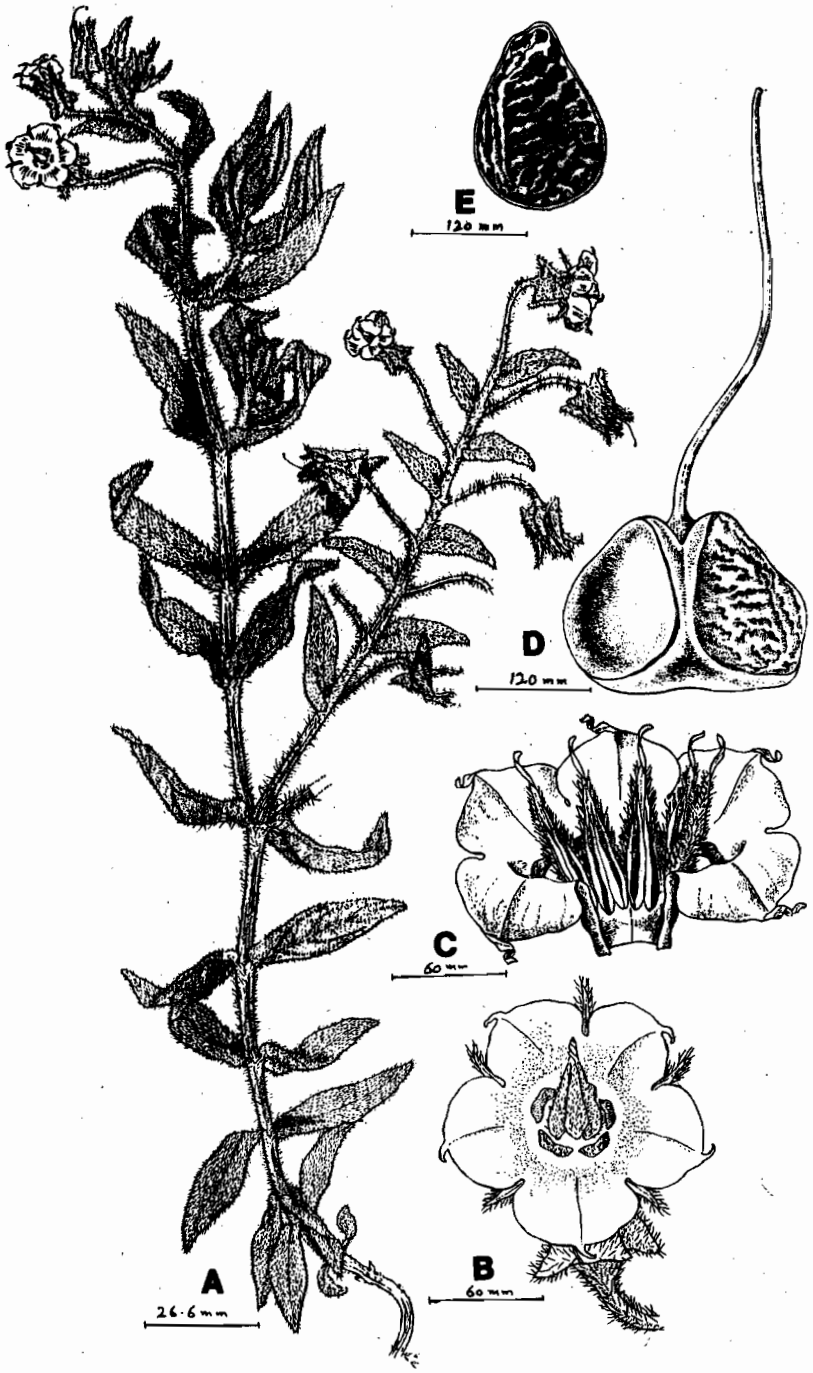

Fig. 1. *Trichodesma indicum*: A, habit ; B, flower with twisted cone ; C, corolla cut open to show the dehisced anthers ; D, mature carpel; E, nutlet.

several flowers of a plant or plants and then land on the selected one. Insect directly land on the anther cone of the inversely hanging flower and vibrates it. As the flower is protandrous and pollen grains are dispersed in the cone head so due to vibrations pollen grains come out from the cone through the pore and get deposited on under side (abaxial) of the insect body. Pollen grains get deposited mainly on insect's legs and abdomen while few on head, thorax, wings and proboscis. Apart from pollen collection, insect also try to sip the nectar from the base of the ovary in the short corolla tube by inserting its proboscis in the grooves present between the anthers at the base of the cone. The brown coloured blotches seem to direct the insect towards the grooves and thus act as the nectar guide. In pistillate phase, stigma comes out of the cone pore (i.e. exposed) and pollination occurs when bee touches the stigma during nectar collection. Nectar is available in both staminate and pistillate phase.

Both *Anthophora* species start visiting the flowers from morning till dusk, however, two peak visiting periods were observed i.e., from 12.00-14.00 and 17.00-18.00 h. *Anthophora* sp. (A) spends 2-8 sec., per flower and a flower can be visited 1-6 times in an hour as observed in peak visiting period, whereas *Anthophora* sp.(B) spends 2-5 sec/h and visits a flower 1-3 times within an hour.

Other Hymenopteras like *Chrysis* sp., and *Colletes* sp., also visit flowers but do not carry pollen grains. Certain Lepidopteras like small butterflies also visit the flowers. They usually land on the petals and collect the nectar through the grooves by inserting their long proboscis without touching the tip of the cone. They do not carry pollen grains and are regarded as non-pollinators.

Pollen-Ovule Ratio: Average number of pollen grains per anther was 5126.8 $\pm$ 30.7 while average number of anthers per flower was 5 and average number of ovules per flower was 4. So the pollen-ovule ratio was 6408.5. According to Cruden (1977), with this value of P/O, the taxon is classified as xenogamous.

Breeding System: With the exception of apomixis, fruits were formed in all the treatments. Fruit set among the treatments was not analyzed statistically as many of them were damaged by grazing or cutting. However, the data of fruit length, breadth and weight, seed set, seed length, breadth and weight was analyzed from the remaining undamaged available fruits. Seed set remains the same i.e. 4/fruit in all the treatments.

Fruit length and breadth differed significantly in all the treatments (Table 1). DMRT showed significantly greater fruit length in xenogamy as compared to autogamy and geitonogamy; in control as compared to autogamy. However, non-significant differences have been observed in fruit length between xenogamy and control, between geitonogamy and control and between geitonogamy and autogamy. Fruit breadth was significantly greater in xenogamy as compared to autogamy, while non-significantly differed in rest of the treatments (Table 1).

Difference in fruit and seed weight was non-significant among all the treatments. However, DMRT showed significantly greater fruit weight in xenogamy than autogamy while no difference was observed among rest of the treatments (Table 1).

Seed length and breadth were significantly different among all the treatments. Seed length was significantly greater in control and xenogamy as compared to geitonogamy and autogamy while it was similar (non-significant) between control and xenogamy, between geitonogamy and autogamy. Seed breadth was significantly lower in autogamy

Table 1. Effect of different pollination treatments on *Trichodesma indicum*.

	ONE-WAY ANOVA					DUNCAN'S MULTIPLE RANGE TEST (DMRT)			
Parameters	SV	df	SS	Ms	F-value	Treatments	Rank	Mean	
Fruit length	Treatments	3	3.21094	1.07031	5.74251**	Control	2	5.375	ab
						Autogamy	4	4.875	c
	Error	28	5.21875	0.18638		Geitonogamy	3	5.1875	bc
	Total	31	8.42968			Xenogamy	1	5.750	a
Fruit breadth	Treatments	3	1.96093	0.65364	3.50698*	Control	2	4.125	ab
						Autogamy	4	3.6875	b
	Error	28	5.21875	0.18638		Geitonogamy	3	4.000	ab
	Total	31	7.17968			Xenogamy	1	4.375	a
Fruit weight	Treatments	3	1.56937	0.52312	2.27291ns	Control	2	2.7875	ab
						Autogamy	4	2.28125	b
	Error	28	6.44437	0.23015		Geitonogamy	3	2.5625	ab
	Total	31	8.01375			Xenogamy	1	2.84375	a
Seed length	Treatments	3	1.77343	0.59114	8.40740***	Control	1	4.75	a
						Autogamy	4	4.25	b
	Error	28	1.96875	0.07031		Geitonogamy	3	4.3125	b
	Total	31	3.74219			Xenogamy	2	4.75	a
Seed breadth	Treatments	3	0.33594	0.11198	4.36231*	Control	1	3.000	a
						Autogamy	4	2.750	b
	Error	28	0.71875	0.02567		Geitonogamy	3	2.937	a
	Total	31	1.05469			Xenogamy	2	3.000	a
Seed weight	Treatments	3	0.04785	0.01595	1.81286ns	Control	2	0.3937	a
						Autogamy	4	0.3187	a
	Error	28	0.24635	0.00879		Geitonogamy	3	0.3562	a
	Total	31	0.2942			Xenogamy	1	0.4212	a

ns= non-significant $P>0.05$; *= $P<0.02$; **= $P<0.005$; ***= $P<0.001$. Means sharing the same letter do not differ significantly $P>0.05$.

than the other treatments which exhibit similar seed breadth among themselves (Table 1).

Discussion

T. indicum flower throughout the year. Flower was protandrous and remained in bloom for ± 3 days, staminate phase lasted for about 24h while pistillate phase for about 24-60h after anthesis. In pistillate phase petals turn blue from pink, similarly colour changes in corolla of other members of Boraginaceae has also been reported i.e., yellow turn white in *Cryptantha breviflora*, *Cryptantha nana*, *Myosotis sauveolens*; yellow centre changes to bluish-purple in *Heliotropium curassavicum*; yellow centre changes to red, red-blue, red-purple in *Myosotis australis*, *Myosotis discolor* and *Moltkopsis ciliata* respectively (Gori, 1983).

T. indicum exhibits many characters of Buzz-pollinated plants as described by Buchmann (1983) such as clumped distribution and long flowering period of plants; individual flower inversely hanging, small-medium size, of short duration i.e., 2-3 days, showy with blue, pink, white, purple corolla; anthers introrse, in the form of anther cone with apical pore; pollen abundant, small, dry and white; long exserted slender style with small capitate stigma. *Anthophora* species as pollinators with sternotribic pollination. The only exception is the presence of nectar, which can be explained in the light of generalization made by Dukas & Dafni (1990). According to them buzz-pollination in Boraginaceae taxa may represent an intermediate step/stage and a possible evolutionary pathway between/from usual oligandrous nectariferous flowers and typical buzz-pollinated flowers.

P/O data indicates *T. indicum* as a xenogamous species, however, hand pollination experiments revealed a mix breeding system i.e., self-compatible, facultative xenogamous. Facultative autogamy-xenogamy has also been predicted for *Microsteris*, *Heliotropium* and *Lapulla* species (Boraginaceae) by Cruden (1977), on the basis of P/O alone (i.e. without hand pollination experiments). All the hand pollination treatments resulted in normal fruit and seed set with the exception of emasculation treatment for apomixis.

The results of the present study suggest that *T. indicum* is a facultative xenogamous plant which promotes cross pollination via sternotriby and protandry yet maintains the capacity to self pollinate. A breeding system which promotes cross pollination (thereby facilitating genetic variability) while maintaining the capability to self pollinate is obviously adaptive.

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