FRUIT TYPES, BROOD-SIZE, GERMINATION AND SEEDLING MORPHOLOGY OF HOPBUSH [(DODONAEA VISCOSA (L.) JACQ.), FAMILY SAPINDACEAE]

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ARSTRACT

Dodonaea viscosa (L.) Jacq. was studied for its fruit types, brood size, germination and seedling morphology. The fruit of D. viscosa, a capsule, was associated with a varying number of reniform wings (2-4). Out of 818 fruits studied, the three-winged fruits predominated in number (88.02%). Two-winged fruits were less frequent (11.0%) and four-winged fruits were rare (0.98% only). The fruit showed two distinct regions. The central darker region is the capsule extending into pale -cream membranous wings. The main veins of the capsular region (brown) branched profusely to form a reticulum. The finer veinlets were generally single, linear, and straight. The veins from the capsular region entered the wings, they branched and running almost parallel to each other they traversed to the wing margin where they united with a prominent vein running along the margin of the wings. The number of locules in a fruit was generally equal to the number of wings associated with the fruit. The brood size varied from zero to five seeds per fruit and averaged to 1.64 ± 0.18 seeds. It was maximum in four-winged fruits (mean: 2.63 ± 0.498; ranging from 1 to 5) followed by three-winged large-sized fruits (2.08 ± 0.073 seeds per fruits; ranging from 1 to 4 seeds) and minimum in two-winged seeds - 1.35 ± 0.098 seeds per fruit in large fruits and 1.42 ± 0.076 seeds per fruit in case of smaller fruits. Seeds were hard subglobose, dark chocolate brown to black in colour around 3-4 mm in diameter. They were plugged at the micropyle and characterized with a pleurogram. Seeds glutinous (viscid shiny and sticky surface), lustrous and devoid of non-glandular hairs but dotted with resin glands of various sizes. The cells of testa surface were periclinally concave. The anticlinal walls were thick, smooth and curvy. The relief of the surface was reticulately patterned in variously shaped depressions in succession with raised anticlinal walls. The emergence of seedlings from soil was episodic. Within 20-day incubation the emergence reached to 50% of the sown seeds. The seedlings were Phanerocotylar-Epigeal and foliaceous type. Cotyledons were linear and opposite and embryo was coiled. Leaves alternate and dorsiventral. Cotyledons and leaves both were amphistomatic with higher stomatal density on the lower surface. Diverse stomatal types were observed - anomocytic, cyclocytic, tetracytic, anisocytic, staurocytic, 11/2 cyclic tetracytic and bicyclic anomocytic. Ventral surface of seedling leaf had 155.88 ± 5.08 (88.46 – 216.24) stomata per mm². Stomata were elliptical with prominent ledges. Subsidiaries were distinct and smaller on cotyledon and leaf but non-distinct of capsular wall. There were two types of trichomes. The non-glandular trichomes were only present on the margins of very young leaves and the glandular trichomes (capitate and stalked resin glands) were distributed not only on capsular wall and wings of the fruit but also on hypocotyl, both surfaces of cotyledons and leaves and seeds. The glands were more numerous on younger organs. The largest gland seen on ventral surface of leaf had diameter of the head c 84.45 µm (diameters: 78.3 and 90.6 µm at right angle) and the stalk c 20.0 µm in length. The results are discussed in the light of available

Key words: Dodonaea viscosa (L.) Jacq., fruit types, brood size, seedling morphology and ornamentation.

INTRODUCTION

Dodonaea viscosa (L.) Jacq. [Syn. Ptelea viscosa L. (vern. Hop Bush) is an evergreen woody, perennial and extremely hardy shrub. Dodonaeas are called Hop bush as they were used to make bear by early European Australians. The genus is named after Rembert Dodens (1517-1585; also called Rembertus Dodonaeus) and specific epithet from viscosus = sticky leaves (McDowell, 2007). It is widespread and distributed in Australia, S. Africa, N. America, China, India, Ceylon and Pakistan, (Abdulla, 1973) and UAE. It is a constituent species of scrub vegetation of low hilly areas of Pakistan and grown in many cities of Pakistan. The widely distributed *Dodonaea viscosa* evolved in Australia from its most recent common ancestor in the Late Pliocene to Early Pleistocene and subsequently split into two distinct, geographically based, intraspecific lineages (Harrington and Gadek, 2009).

Plants generally dioecious - rarely monoecious. It flowers in January to March. Inflorescence terminal panicle. Flowers greenish yellow and pedicellate. Sepals imbricate or valvate. Petals are absent. Stamens 6-8. Ovary triquetrous. Stigma trifid. The bark is in form of long thin strips. It possesses biologically active saponins (Wagner et al., 1987). D. viscosa is an important medicinal plant (Ghazanfar, 1994). It is rich in sapogenins and flavonoids (Ahmad et al., 1987; Nisar et al., 2015). It contains gallic acid, ellagic acid, rutin, apigenin and kaempferol (Nisar et al., 2015; Hamdi, 2017) and may be effective in developing baiting system against termites (Nisar et al., 2015). It may be used as antibacterial, analgesic, antiviral, anti-inflammatory, anti-ulcer, antioxidant, etc. (Rani et al., 2009, Riaz et al., 2012; Mohankumar and Asha, 2008, Anilreddy, 2009). In India, is used in rheumatism, gout, hemorrhoids, fractures and snake bite (Kirthikar and Basu, 1995; Nadkarni and Nadkarni, 1982). The bitter fruits are substitute for hops and yeast in making bear (Upholf, 1959; Kunkel, 1984; Perman, 1962; Facciola 1990). It is a drought and frost tolerant plant (McDowell, 2007) and salt tolerant too (Yousefi et al., 2017). It is a weed growth inhibitor (Ali, 2012) in Vigna mungo and Zea mays crop fields. Leaf and bark residues exhibit promoting effect on growth, yield and yield components, and several physiological parameters of growth of Vigna and Zea. Inoculation with growth promoting rhizobacteria is reported to improve its germination and growth resistance to high salinity (Yousefi et al., 2017). Its gregariousness and quick growth make it an excellent hedge plant. Its hedges are common in Dubai (UAE) raised through drip irrigation (Fig. 1). In Oud Metha Park, Dubai, it was found copiously laden with mature fruit crop in the months of April and May, In this paper it has been studied for its fruit types, brood-size and seedling morphology as D. viscosa is reported to be not only widely distributed but extremely polymorphic species especially in leaf and capsule morphology.

Climatic features of Dubai: UAE is located in Middle East, situated on Arabia Peninsula between Oman and Saudi Arabia bordering the Gulf of Oman and the Persian Gulf. It covers an area of 83,600 Sq. km. Its largest city is Dubai. Its landscape is sandy − extreme hot. Days are sunny all the year around. Humidity is discomfortingly high in coastal region. According to Köppen-Geiger classification (Köppen and Geiger, 1954; Peel *et al.*, 2007), its climate is of Bwh type (Tropical desert climate) and bioclimate as given by Holdridge (1947) falls into the category of Tropical Desert Bush formation. Climate of Dubai is described in Table 1. The record high temperature is 52 °C and record low 9°C. Average high temperature is 33.4 and average low 23.5°C. Rainfall is low − around 103.7 mm which is largely in winter months (December, January − March). Annual seawater temperature averages to 28.3 °C −varying 13.69%. UV index is high (11+) in summer. Evapotranspiration for Wadi Ham catchment is presented by Al Mulla (2005) to be ≥ 6 mm per day for March through September and maximum in May and June − around 8.5 mm per day.

Table 1	Climatic	characteristics	of Dubai *
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	Temperature (°C)							
Months	Record	Average	Average	D1 I	Rain	Rainy	Seawater	UV
	High	High	Low	Record Low	(mm)	Days	Temp. (°C)	index
J	31.8	26.1	17.8	9.0	18.8	4.4	23.4	6
F	37.5	27.4	18.6	10.0	25.0	3.0	21.9	8
M	41.3	31.6	20.9	11.0	32.1	5.8	28.2	10
A	43.5	34.2	23.2	15.4	7.2	2.6	25.5	11 +
M	47.0	38.6	26.1	18.1	0.4	0	29.8	11 +
J	50.9	40.7	28.5	21.2	0.1	0	31.6	11 +
J	52.0	41.2	31.2	23.4	0.1	0	32.7	11 +
A	51.5	41.8	31.5	25.1	0	0	33.5	11 +
S	48.12	39.8	28.7	23.5	0	0.2	33.1	11
0	42.4	35.6	24.3	17.0	1.1	0.5	31.3	8
N	38.0	32.2	21.0	14.8	2.7	1.0	28.6	6
D	33.2	28.3	20.3	12.2	16.2	2.8	25.4	5
Year	52.0	33.4	23.5	9.0	103.7	20.3	28.3	9.1



*, Data source: (http://en.wikipedia.org/wiki/Climate_of_Dubai#Climate_data) -date of retrieval - June 29, 2018. (Data being mainly the courtesy of Dubai Meteorological office, Seatemperature.org, Climatebase.ru, Weather Atlas; Gulf News, etc.

Fig.1. *D. viscosa* hedge-Oud Metha Park. DXB.







Fig. 2. A) Small twig showing spoon-shaped leaves and Three-winged fruits of *D. viscosa*. B and C are abnormal fruits of *Dodonaea viscosa* - B) Two-winged fruits and C) four-winged fruit. The Locular region is dark brown in colour and wings are lighter in colour.

MATERIALS AND METHODS

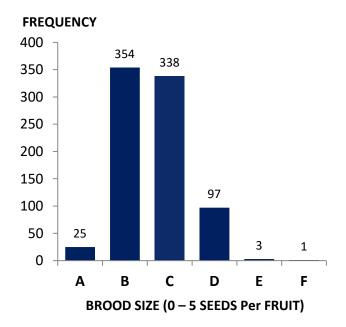
A sizeable number (c 900) of fruits of *D. viscosa* were collected. The plant's identification was confirmed following Abdulla (1973). The fruits were initially graded with respect to their size and wingedness (2, 3 or 4 wings). The fruit were dissected for their morphology and the number of seeds recovered from each fruit was recorded to adjudge the brood size following Uma Shaanker *et al.* (1988).

One hundred healthy untreated (unscarified) seeds of *D. viscosa* were sown in sandy garden loam soil in pots. They were daily irrigated with normal tap water in suitable amount. The emergence of seedlings was recorded daily up 20 days when the emergence count was stopped but pots were continued irrigating as usual to watch the fate of the residual seeds. The seedlings of *D. viscosa* were studied for their morphological characters including stomatal types.

Table 2. Location and dispersion pattern of brood size in various types of it	pattern of brood size in various types of fru	of brood	pattern 4	dispersion	and	 Location 	Table
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Parameters	Fruit types							
1 arameters	A	В	С	D	Е	F		
N	500	110	110	40	50	08		
Mean	1.59	2.08	1.52	1.35	1.42	2.63		
SE	0.033	0.073	0.060	0.098	0.0761	0.498		
CV (%)	47.29	36.92	41.51	46.07	32.89	53.54		
G1	0.169	0.107	0.376	0.944	0.741	-		
Sg1	0.109	0.230	0.230	0.374	0.3347	-		
G2	0.412	- 0.722	- 0.277	0.844	-0.627	-		
Sg2	0.218	0.457	0.457	0.733	0.662	-		
Minimum	Zero	1	Zero	Zero	1	1		
Maximum	3	4	3	3	3	5		

Acronyms: A, Three-winged normal size fruits; B, Three-winged large size fruits; C, Three-winged small size fruits; D, Two-winged large size fruits; E. Two-winged small size fruits, F, Four-winged fruits.



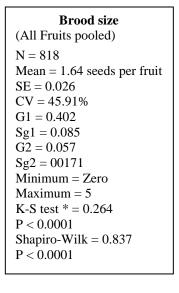


Fig. 3. Frequency distribution of Fruit types of *Dodonaea viscosa*. N = 818. Acronyms: A, No seed in fruit; B, One seed; C, two seeds; D, three seeds; E, four seeds and F, five seeds per fruit. *, Kolmogorov-Smirnoff test corrected with Lilliefors significance correction.

Seedlings type was described according to Vogel (1980) and Garwood (1996). Hickey (1979) and LWG (1999) were followed for description of cotyledon and leaf. To study stomatal types, the impressions of cotyledons and leaves were made with clear nail polish (Wang et al., 2006) and studied under compound optical microscope. Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. For scanning electron microscopy (SEM) air-

dried plant material was mounted on brass stub and coated with 250 °A thick layer of gold with JFC -1500 gold coater. SE micrographs were taken with JEOL JSM -6380A electron microscope. Images were saved on computer. The data were analyzed statistically (Zar, 2010).

RESULTS AND DISCUSSION

Fruit types and Brood size

The flowers of *D. viscosa* may be unisexual or bisexual. The fertilized fruits can take quite long to mature. Over this period the capsules may change their colour from a green or cream. The winged capsules are produced on female or bisexual flowers (McDowell, 2007). The fruit of *D. viscosa* is a capsule type with varying number of wings (Fig. 2). When dry they are generally yellowish cream or light brown in clour. Being in bunches, they appear quite decorative. Some 818 fruits of *D. viscosa* were studied (Table 2) and divided into six types: (I) Three-winged and normal-sized fruits (c. 1.8 cm across wings; N = 500), (II) Three-winged large-sized fruits (1.9 – 2.3cm across wings; N = 110), (III) Three-winged small-sized fruits (< 1.8 cm across wings; N = 110), (IV) Two-winged large-sized fruits (1.9 to 2.2cm across wings; N = 40), (V) Two-winged small-sized fruits (1.4 -1.8cm across wings; N = 50) and (VI) Four-winged fruits (1.9 – 2.5 cm across wings; N = 8). Obviously, three-winged fruits (type I, II and III) predominated in number (88.02%). Two-winged fruits were less frequent (11.0%) and four-winged fruits were rare (0.98% only). The ripe fruits of *D. viscosa* were reported to be on an average 18.2 ± 1.0 mm in diameter including wings (Burrows, 1995). We found larger winged fruits 14-23 mm in diameter and generally light brown in colour. Real capsule zone was 12-14 mm long and 15-19 mm broad. Capsular valves are membranous light, brown, green or maroon in colour and winged at the base. Fruit septicidal. The number of wings, as we found varied from 2-to 4. West (1980) has reported that Australian *Dodonaea* may bear 2 to 6 wings in a capsule.

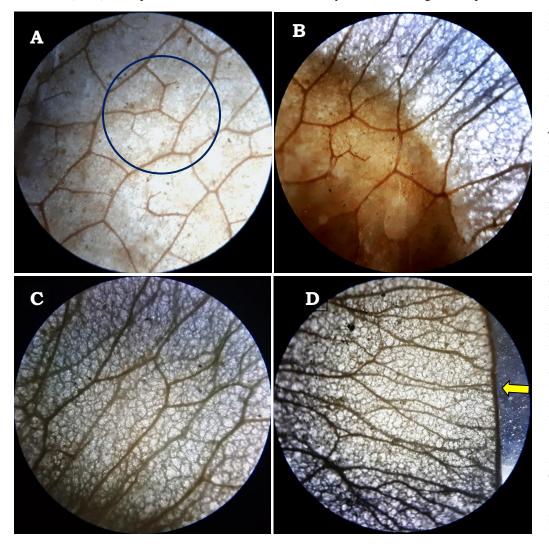


Fig.4. Venation as seen on the surface of the fruit. A, Capsular wall; B, the junction of capsular region with the wing (Capsular region is brown in colour and wing region is pale cream in colour); C, Wing region and D, Wing showing the edge of the wing margin bear vessel (shown with an arrow in yellow). The veinlets are generally linear.

The wings of the fruit are reniform in shape and at sometime unequal. In one of the four-winged fruits, two wings were smaller and two relatively larger. In two-winged fruits, wings were generally symmetrical and equal in size. The wings should help in fruit dispersal by wind.

The brood size varied from zero to five seeds per fruit and averaged to 1.64 ± 0.18 seeds. It was maximum in four-winged fruits (mean: 2.63 ± 0.498 ; ranging from 1 to 5) followed by three-winged large-sized fruits (2.08 ± 0.073 seeds per fruits; ranging from 1 to 4 seeds) and minimum in two-winged seeds - 1.35 ± 0.098 seeds per fruit in large fruits and 1.42 ± 0.076 seeds per fruit in case of smaller fruits which didn't differ significantly with each other (t = 0.56, NS). The overall brood size (pooled sample) of *D. viscosa* varied around 45.9%. It was positively skewed and asymmetrically distributed as indicated by KS-test and Shapiro-Wilk tests (inset, Fig. 3). It is apparent from Fig. 3 that amongst the fruits inspected, there were 3.06% fruits with no seeds inside and 84.6% of the fruits had one or two seeds. Fruits with three seeds inside were only 11.86%. Fruits containing four and five seeds were 0.37 and 0.12% merely. There was only one fruit containing 5 seeds. It follows from the results that majority of fruits contain one or two seeds.

The dissection of fruits indicated that the number of locules in a fruit was generally equal to the number of wings associated with the fruit. Each locule produced two ovules (Karkare-Khushalani and Mulay, 1964) but some of them are invariably aborted. The reason of the seed abortion in this species is not known and needs further investigation.

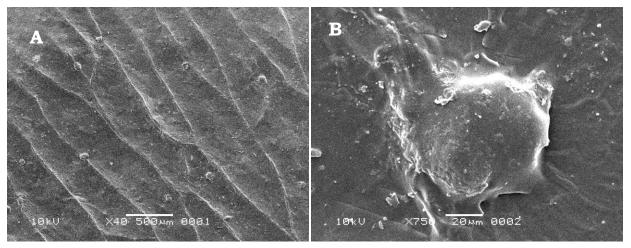


Fig.5. Surface of fruit wing of *D. viscosa* (A) at magnification of 40X showing glands on or near the vascular supplies B, A gland on the surface of fruit (Magnification: 750 X). Head diameter c 70um.

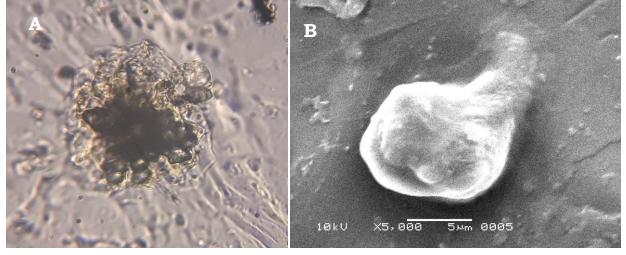


Fig. 6. OM view of a multicellular trichome (gland) on the surface of fruit wing. A, A large gland as seen on nail polish imprint at magnification of $45 \times 10 \times 10 \times 10 \times 10 \times 10 \times 10^{-2}$ polish imprint at magnification of $5000 \times 10^{-2} \times 10^{-2}$ K. The stalk of the gland is clearly visible.

Fruit surface (Venation, glands and stomata)

The fruit surface shows two distinct regions. The central darker region is the capsule extending into pale -cream membranous wings. The main veins of the capsular region (brown) branched profusely to form a reticulum. The finer veinlets are generally single, linear, and straight. The veins from the capsular region enter the wings, they branch and running almost parallel to each other they traverse to the wing margin where they unite with a prominent vein running along the margin of the wings (Fig.4). The veinlets are linear and straight (Fig. 4A). The Capsular wall and its extension in form of wings both on their surface bear glands. Such glands are multicellular and globular and attach to the wing surface through a short stalk (Fig. 5 and 6). Possibly, they are resin producing glands to protect fruit from invading insects. West (1980) reported that capsule has floating ability and their viscous nature prevent water to enter inside. The capsule wall bears stomata which are anomocytic (Fig. 7 and 8). Stomata admeasured $19.3 \times 11.3 \, \mu m$. The outer ledge shaped like dome Outer stomatal ledge aperture measured $11.9 \, \mu m$ in length.. No stomata were, however, seen on wing surface.

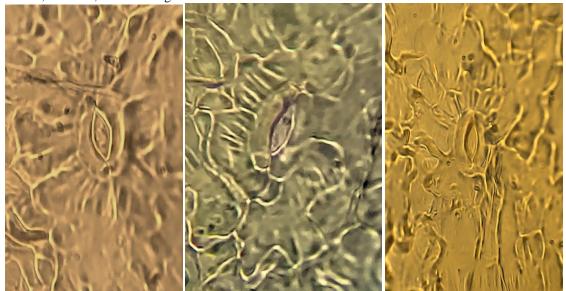


Fig. 7. Nail polish imprint of stomata (anomocytic type) observed on the capsular surface of the dry fruit of *D. viscosa*. Magnification 45 x 10 X with 4X zoom).

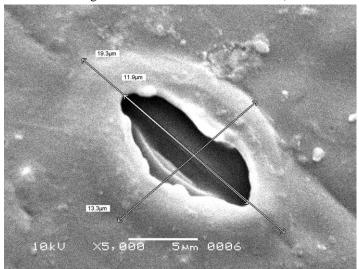


Fig. 8. SEM view of stoma (Size: 19.3 x 13.3μm including ledges) on the capsular wall of *D. viscosa*. Magnification: 5000 X.

Outer stomatal ledge aperture admeasured to 11.9μm in length.

Table 3. Size measurements of c 20-day old (3-4 leaved) seedlings of D. viscosa (N = 5).

Parameters	Hypocotyl	Cotyledon	Leaf	Epicotyl
Length (cm)	6.63 ± 0.046	2.65 ± 0.08	1.41 ± 0.13	1.23 ± 0.10
Width (cm)	0.10 ± 0.0	0.53 ± 0.021	0.59 ± 0.068	-



Fig. 9. (A) seeds of *Dodonaea viscosa*. The seeds are ovoid in shape, dark chocolate-brown in colour and shining on the surface.

- (B) Stereomicroscopic view of seed surface covered with resinous material secreted by capitate, multicellular glands.
- (C) The micropylar region of the seed. The micropyle is plugged.



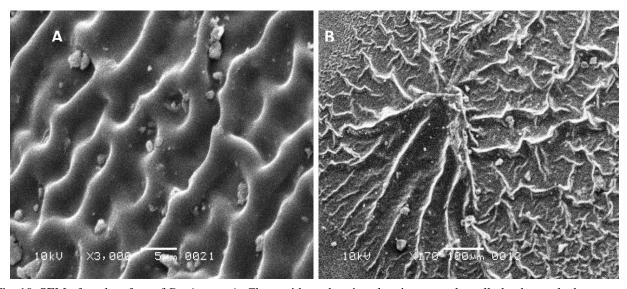


Fig. 10. SEM of seed surface of *D. viscosa*. A, Clear epidermal region showing several small glands attached on to the surface; B, Epidermal region covered with resinous material. The seed in image B is the same given in Fig. 9B.

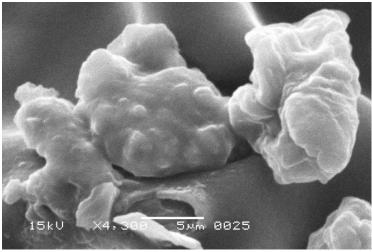


Fig. 11. SEM view of glands on the surface of a dry seed.

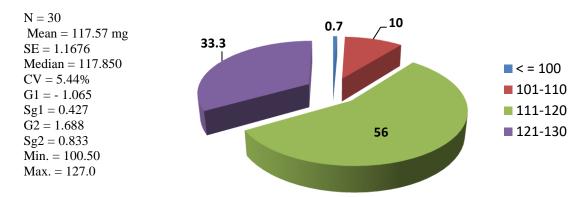


Fig. 12. *Per cent* frequency distribution of 10-seeds weight (mg) of *D. viscosa* excluding very small shriveled seeds which constituted a large part of seed population on numerical basis.

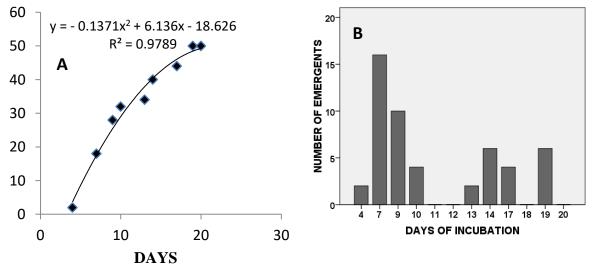


Fig. 13. Emergence of seedlings in relation to incubation time (days). A) Final germination and B) Number of new emergents.



Fig. 14. Emergence of seedling of *D. viscosa*. Cotyledons come out of soil even still enclosed within testa (A). Cotyledons remain coiled even within seed and on emergence for some time. Hypocotyl is curved on emergence (B) but it straightens in 2-3 days when cotyledons are fully expanded at right angle; C and D, Coiled cotyledons – emerged from the testa. (see Fig. 15 also).

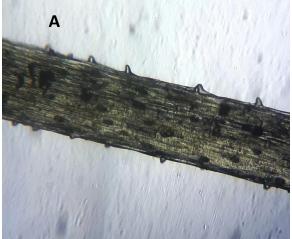


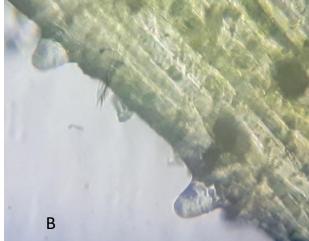
Fig. 16. A, 12-day old seedling bearing leaves (A) in a plane cross to the cotyledons. B, Dorsal surface of young leaf (5 mm² in size) showing a number of glands and the viscous exudation around them (Magnification: 10 x15 X).





Fig.17. A) The basal part of the hypocotyl turned to be purple in colour in some seedlings and B) the venation of young leaf (enlarged view) of the seedling leaf - brachidodromous type.





Seeds

Seeds are subglobose, dark chocolate brown to black in colour (Fig. 9A) and around 3-4 mm in diameter. The seeds are hard coated and plugged at the micropyle (Fig. 9C). The seed coat is said to be impervious to water. Burrows (1995) reported seeds to measure 3.1 ± 0.09 mm in length and 2.3 ± 0.14 mm in width. Endosperm is consumed in mature seeds (Karkare-Khushalani and Mulay, 1964). Pleurogram present (Fig. 9B). Seeds are glutinous (viscid shiny and sticky surface). They are lustrous and devoid of non-glandular hairs but dotted with resin glands of various sizes (Fig. 11). Testa surface in low magnification appears as 'ripples in the water' in relief (cf. Davies, 2008) but in higher magnification the epidermal cells are periclinally concave. The anticlinal walls are thick, smooth and curvy. The relief of the surface reticulately patterned in variously shaped depression in succession with raised anticlinal walls (Fig. 10). The concave periclinal surface walls of seed coat have also been shown in Brassicaceae (Kasem *et al.*, 2011).

In our studies, excluding small shriveled seeds, the weight of 10-seeds averaged to 117.5 ± 1.1676 mg and varied only 5.44% (Fig. 12) i.e. c 11.8 mg per seed. Burrows (1995) reported seeds of *D. viscosa* weighing 0.0095g i.e. 9.5mg per seed from New Zealand. The weight of 1000-seeds of Hop bush was reported to be 10.27g from Malagha village, Khouzesthan province, Iran (i.e. single seed weight around 10.27mg per seed (Yousefi *et al.*, 2017). The average single seed weight of *D. viscosa* from Shakar Puria, Islamabad (Pakistan) was reported to be 0.0084g (Qadir and Lodhi, 1971). The differences in seed weight observed among the localities may be due to geographically induced variations or differences in the selection of seeds.

The seeds of *D. viscosa* are desiccation tolerant and live quite longer in laboratory (Ludwig *et al.*, 1996) and survive for longer period of time if stored dry (Rani *et al.*, 2009).

Germination

Germination of *D. viscosa* was undertaken to obtain seedlings from unscarified seeds by sowing in soil. The emergence of seedlings was episodic. Two seedlings were observed to emerge after four days of incubation. The second crop of seedlings emerged on 7th when 16 new emergents were recorded. Ten more seedlings emerged on 9th day and continued to emerge in varying number on subsequent days. Within 20-day incubation the emergence reached to 50% of the sown seeds (Fig. 13 A and B). There was, however, a long tail of germination as the residual seeds in the pot continued to germinate sporadically in 1 or 2s in the pot even after months. It appears that seeds in hand had varying degree of seed dormancy i.e. some seeds germinated quickly and some quite late.

There are reports that seeds of *D. viscosa* from China (Baskin *et al.*, 2004), Pakistan (Qadir and Lodhi, 1971; Hussain *et al.*, 1991) and Botswana (Tietema *et al.*, 1992) germinated substantially *in vitro* (Petri dishes) without any treatment. Nasr *et al.* (2013) have also reported around 50% germination of freshly collected seeds of *D. viscosa* from Iran without any treatment. Physical dormancy in *D. viscosa* seeds has, however, been reported by some workers due to water impervious seed coat (Burrows, 1995; Baskin *et al.*, 2004; Phartayal *et al.*, 2005; Cook *et al.*, 2008; Rani *et al.*, 2009; Benitez-Rodriguez *et al.*, 2013; Jaganathan and Liu, 2014). Since on scarification, seeds germinate in higher numbers (> 90%), this dormancy is rated due to impervious testa and not due to the immature embryo. Jaganathan and Liu (2014) have speculated that seeds used in Nasr *et al.* (2013) experiment might have been collected before full maturity. In our experiment with untreated seeds we also obtained 50% germination (but in soil) within an incubation period of 20 days with seeds that were dry-stored for around two months. We intended to obtain seedling for morphological studies and have not designed experiment to investigate dormancy. It appears that detailed investigation should be undertaken with respect to the seed dormancy in this species.

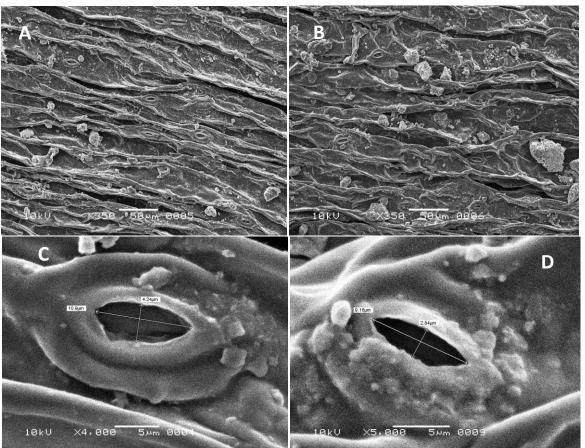


Fig. 19. SEM of a dry cotyledon. A, dorsal surface; B, Ventral surface C, stoma on dorsal surface (Outer stomatal ledge aperture c. 10.9 x 4.24 μm; 4000X) and D, stoma on ventral surface (Outer stomatal ledge aperture c. 9.16 x 2.84 um; 5000X). Glands are profusely present on the cotyledonary surface besides granules of epicuticular waxy crystalloids and dry resinous material.

Baskin *et al.* (2004) have also opined that since *D. viscosa* is a polymorphic, worldwide species and physical dormancy in seeds of this species have been reported from Australia, Brazil, Hawaii, Mexico and New Zealand, the low level of dormancy reported for seed lots of *D. viscosa* in China, India and Pakistan is probably due to collection of seeds before they dried to the critical moisture content for development of water-impermeability of the seed coat.

Senna multijuga and Plathymenia reticulata are tropical tree species native to the Brazilian Atlantic Forest and the Brazilian Cerrado, respectively. Seed-coat dormancy variation was evaluated within and among natural populations of these two species (Lacerda et al., 2004). Scarified and non-scarified seeds from different plants within populations were germinated at 28° C. Mean germination percentages of non-scarified seeds tended to be higher for *P. reticulata* populations (40 and 62%) than for *S. multijuga* populations (9 and 35%). After scarification, germination percentages increased significantly in both species, with all populations showing mean values above 84%. The level of seed dormancy, evaluated through the experiment with non-scarified seeds, differed significantly within and among populations of both species (P < 0.05). The values of the coefficient of genotypic determination of dormancy in these species is of genetic were high for populations of both species (P = 0.85). Although this coefficient was an overestimation, since it included non-genetic maternal effects, its high values suggested that a considerable part of the phenotypic variation in seed origin (Lacerda et al., 2004). They concluded that variation in seed dormancy can be an important factor for increasing genetic diversity in populations of these species, making them able to respond to environmental changes. There appears a need to further investigate dormancy in *D. Viscosa* seeds from arid regions. Variation of seed dormancy in this polymorphic worldwide species may be hypothesized to increase genetic diversity of the species to cope with environmental heterogeneity of the dry and hot tropical world.

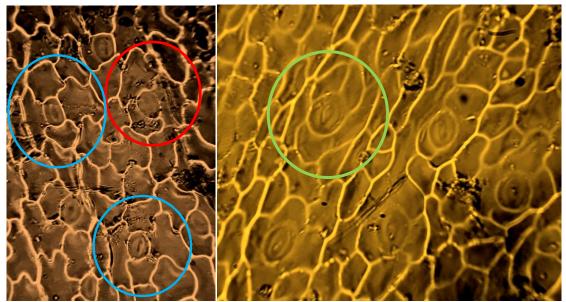


Fig. 20. Microscopic view of upper surface (exposed to sun) of cotyledon of three-day old seedling of *D. viscosa* showing tetracytic (Blue circle), anisocytic (Red circle) and anomocytic stomata (Green circle). The anticlinal walls of the epidermal cells are straight to curvy. Magnification: 45 x 10 X.

Jaganathan and Liu (2014) have suggested that as regards to the germination in natural populations of *D. viscosa*, high summer temperature rising above 60°C (in surface soil) of the tropics should likely be a factor breaking dormancy which they asserted as an adaptive mechanism to tolerate summer droughts that is common in dry tropics. This type of dormancy behaviour is similar to that reported in *Rhynchosia minima* by Shaukat and Burhan (2000). Exposure of seeds to high temperature appears to render seed coat permeable due to the appearance of micro-fractures in the seed coat on dry storage at higher temperature (Khan and Sahito, 2017) in case of hard-coated seeds of *Acacia stenophylla*. The desiccation of seeds after ripening may be a crucial factor in developing physical dormancy in *D. viscosa* seeds. However, it also appears that there should exist variation of seed dormancy in this species across its geographic range of distribution. *Dodonaea viscosa* is rated to be an extremely diverse and difficult taxonomic group. West (1980) has treated it as *Dodonaea viscosa* complex, considerably polymorphic and worldwide in distribution. Beside it, the seeds of *D. viscosa* are differentially glutinous (covered with resinous material partially or over substantial surface area) and have plugged micropyle. What sort of bearing these two factors may have with *D. viscosa* seed germination needs to be investigated?

Seedling Characteristics

The seedlings of *D. viscosa*, according to the Garwood (1996) classification were Phanerocotylar-Epigeal and foliaceous type. In majority of the seedlings, cotyledons came out of the soil while they were still enclosed in seed coat (Fig. 14, 15, and 17A). It was obviously due to the rapid elongation of hypocotyl. Hypocotyl was curved forming a loop in early stage which became erect with some time. Vogel (1980) classified *Dodonaea* seedling as Macaranga type. In this type germination commences with the emergence of radicle which elongates and pushes the envelopments by stretching hypocotyl. Hypocotyl continues elongation longitudinally but remains thin for quite longer time. Initially, it is curved in a loop above soil. It straightens and turns erect and lifts the cotyledons with or without coverings above the soil. Cotyledons stay with the seedling for quite long time. In this type seedling has no additional source of nutrition after the cotyledonary expansion and consequently dependent on the photosynthates produced by green parts of the seedlings. This is the most common type of germination – nearly 50% of the Malesian woody genera belong to this type (Vogel, 1980).

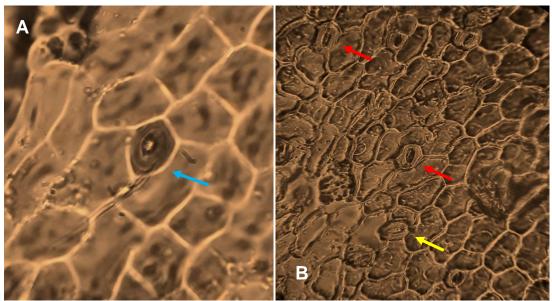


Fig. 21. Microscopic view of lower surface of cotyledon of three-day old seedling of *D. viscosa* showing staurocytic stoma (A, blue arrow) surrounded by four cells (two of the conjoint walls are polar and two are lateral). And a number of anomocytic (red arrows) and tetracytic stomata (B, yellow arrow)); Magnification: 45 x 10 X.

The growth of the epicotyl was quite delayed. The basal part of hypocotyl was often purple in colour and upper shiny green (Fig. 17A). Cotyledons were darker green than the hypocotyl. Cotyledons are opposite, thick, dorsiventral and long - somewhat tapering to form an acute apex. They are sometimes unequal. Cotyledonary midrib is prominent on ventral surface. Dorsally, midrib region forms a long groove (Fig. 16A). Like leaf, cotyledons are plano-convex in Transverse section Cotyledons are coiled in early seedling. The leaves are simple, alternate, hairless, cuneate at base, exstipulate, subsessile, oblanceolate to apathulate, linear obovate, apex obtuse and sometime mucronate, entire or slightly lobed. They bear resin glands profusely which are more numerous and active in young leaves (Fig.16 B). In early seedlings leaves were shiny green, sessile to subsessile and spoon-like in shape. In seedling the primary leaf was infrequently seen to be toothed at the apex as also reported by West (1980) in genus Dodonaea. Venkatesh et al. (2008) also reported the surface of leaves to be shining more or less viscid with a resinous exudation. Vein termination number was reported to be 10-14 per mm² by Venkatesh et al. (2008) and 9.8 -11.1-14.5 by Santhi et al. (2016). Venation of leaf is brachidodromous type (Fig. 17B). Guerin et al. (2012) have asserted that climate change is leaving visible mark on at least one subspecies of the plant in South Australia. The leaves of D. viscosa subsp. angustissima have narrowed by 2mm over the past 127 years - a 40% decrease in width as temperature in this region has increased by 1.2 °C. Leaves are said to be slightly cyanogenic (Lassak and Mc Carthy, 2011).

D.~viscosa seedlings at 20^{th} day of age (Table 3) had hypocotyl around 6.63 ± 0.046 cm in length and 0.10 cm in width. Cotyledons were 2.65 ± 0.08 cm long and 0.53 ± 0.021 cm in width. Epicotyl was shorter than the hypocotyl (1.23 \pm 0.10 cm long) with 3-4 leaves. Hypocotylar surface was found to be studded with papillae (presumably precursor cells of glandular trichomes) (Fig. 18). They were unicellular, round-headed, shiny and protruding out of

the epidermis. Viscous more or less transparent material (presumably resin) was produced by the young leaves. This may be an adaptation against herbivory. Dried leaves were brittle and dry resinous material was clearly seen on the surface (Fig. 24).

The embryo of *D. viscosa* was observed to be bent or coiled type (Fig. 15). Such type of embryo is also reported from other species of family Sapindaceae by Cook *et al.* (2008) e.g., *D. hispidutus*, *D. aptera*, *D. hackettiana*, *D. petiolaris*, *D. ptarmicaefolia and D. stenozyga*. However, several species of Family Sapindaceae represented folded type of embryo e.g., *Alectryon conatus*, *Allophylus cobbe*, *Atalaya hemiglauca*, *Cunaniopsia anacarobioides*, *and C. parvifolia*.

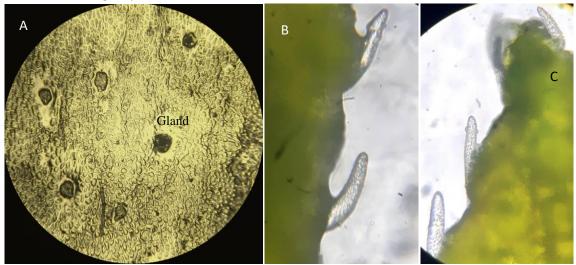


Fig. 22. A, General surface view of dorsal surface of young leaf of *D. viscosa* (Magnification: 10 x 10 X). B and C, The unicellular straight to curved trichomes on the margins of very young leaf (2 x 1 mm in size) of seedling.

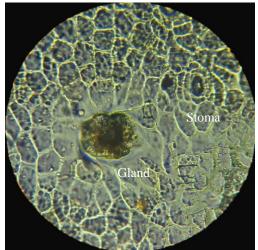


Fig. 23. Dorsal surface of young leaf of *D. viscosa* showing a peltate multicellular gland and a cyclocytic (special anomocytic) stoma (1½ cyclic) – note that the subsidiaries are distinct and smaller in size than the ground epidermal cells and in a ring. Epidermal cells with straight to somewhat curvy anticlinal walls.

Non-glandular Trichomes

The non-glandular trichomes were only seen in very young leaf (around 2 mm² in size) of the seedling. They were unicellular, straight to curved in shape and present only on the margins of leaves. The trichomes averaged to 63.15 ± 3.192 µm in length (N = 15) varying from 38.41 to 80.0 µm (Fig. 22 B and C). Simple unicellular trichomes are also reported from sapindaceous *Cardiospermum halicacabum* by Mohd. Norfaizal *et al.* (2017).

Epidermis:

The epidermal cells were somewhat irregular in shape, had straight to curvy anticlinal walls (Fig. 23 and 26). Onuminya and Adediran (2018) had reported epidermal cell shape to be irregular abaxially and polygonal adaxially

with anticlinal walls straight on both surfaces. Oil cavities are said to be the characteristic of foliar epidermis (Kirtikar and Basu, 1995). Our results are in agreement with Mohd. Norfaizal *et al.* (2018) who reported the epidermal anticlinal walls to be generally straight to curvy in Malaysian *D. viscosa*.

Stomata

Diverse stomatal types were observed in *D. viscosa*.

Capsular surface – Anomocytic (Fig. 7 and 8). Stomata: c $19.3 \times 13.3 \mu m$ in size. Outer stomatal ledge aperture: $11.9 \mu m$.

Capsular wings – No stomata seen on wings.

Cotyledonary Stomata – Cotyledons are amphistomatic (Fig. 19). The upper cotyledonary surface of 3-day old seedlings showed a variety of stomata (Fig. 20 and 21). Stomata were predominantly anomocytic and tetracytic (9.7%) types. Anisocytic stomata were infrequent (3.97%) and staurocytic rare (1.32%). The epidermal cells had straight to curvy anticlinal walls (Fig. 21). The stomata were raised above the epidermal ground surface. The lower cotyledonary surface of 3-day old seedlings showed staurocytic, anomocytic and tetracytic stomata. Some stomata had common subsidiaries between them.

Foliar Dorsal surface – The leaves are amphistomatic. The stomata on the dorsal surface of leaf were 1½ cyclic cyclocytic type (Fig. 23, 26A), 1½ cyclic tetracytic type (Fig. 26C and D) and bi-cyclic cyclocytic type (Fig. 26B). Stomata showed outer stomatal ledges or rim from the guard cells surface like an incomplete roofed dome. Stomatal pore deeply situated (Fig. 26) in a dome. Anticlinal walls of the epidermal cells were straight to curvy. Subsidiaries were distinct being quite smaller than the ground epidermal cells. Stomata were elliptical in shape and small.

Foliar Ventral surface – The leaves are amphistomatic. Stomata were generally cyclocytic (Fig. 27). Stomata were elliptical in shape and sometimes in clusters. Ledges were large forming dome like structure. Stomata small admeasuring c. 19.3 x 14.2 μ m in size and outer stomatal ledge aperture c. 13.0 μ m (Fig. 28). Subsidiaries smaller than epidermal ground cells. Hypostomatic leaves, however, do occur in Family Sapindaceae as *reported by Norfaizal et al.* (2017) in *Cardiospermum halicacabum*.

Not much literature is available on stomatal diversity in Sapindaceae (Metcalfe and Chalk, 1950, 1979; Adeyemi et al., 2011; Solis and Ferrucci, 2006; Venkatesh et al., 2008; Pole 2010; Santhi et al., 2016; Mohd. Norfaizal et al., 2017, 2018). Metcalfe and Chalk (1979) described anomocytic and paracytic stomata in Sapindaceae. Santhi et al. (2016) described cyclocytic (a special type of anomocytic stomata) in this species. Venkatesh et al. (2008) reported anomocytic type of stomata on leaves of D. viscosa as they were reported to be surrounded with indistinct subsidiaries. Hill et al. (2014) have not mentioned about the stomatal type in their paper on temperature influence on stomata in D. viscosa subsp. angustissima but figure 1 of their paper appeared to show cyclocytic (special anomocytic) type stomata on the leaves, which were round in appearance. Mohd. Norfaizal et al. (2017) reported anomocytic stomata in a sapindaceous plant, Cardiospermum halicacabum. Mohd. Norfaizal et al. (2018), however, on investigating 43 taxa of 19 genera of Malaysian Sapindaceae, reported four types of stomata in Sapindaceae - tetracytic, paracytic, staurocytic and anomocytic types on mature leaf surface. They reported staurocytic type of stomata in D. viscosa and paracytic type in Paranephelium macrophyllum and Xerospermum noronhianum. According to them stomatal types, anticlinal wall patterning and stomatal index had good taxonomic value in Sapindaceae. Adeyemi et al. (2011) stated there are two types of stomata observed in 50 taxa of African Sapindaceae - anomocytic and paracytic while another study described anomocytic stomata in South-American Cardiospermum (Solis and Farrucci, 2006). This study reports anisocytic stomata first time from cotyledonary surface of *D. viscosa*. We could not find paracytic stomata from *D. viscosa*.

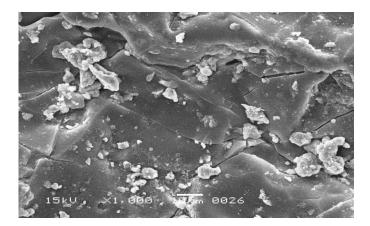


Fig. 24. SEM of dorsal surface of leaf showing its brittleness on drying and the scattered material, presumably the dried viscous material secreted by the glands present on the leaf surface.

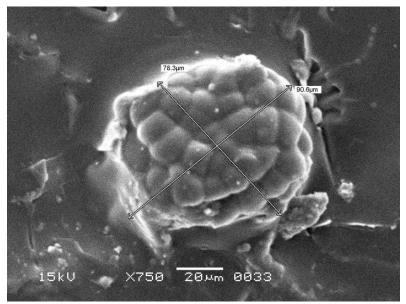
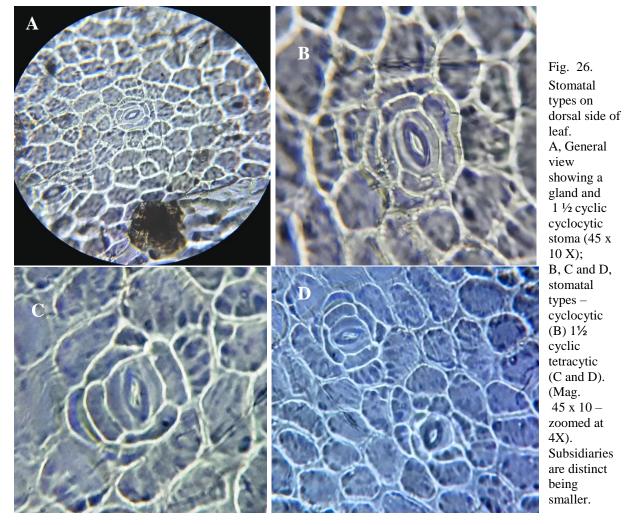


Fig.25. SEM of a multicellular capitate gland attached on the ventral leaf surface; Head diameter averaging to 84.45 μm . Stalk is short c. $20 \mu m$ in length.



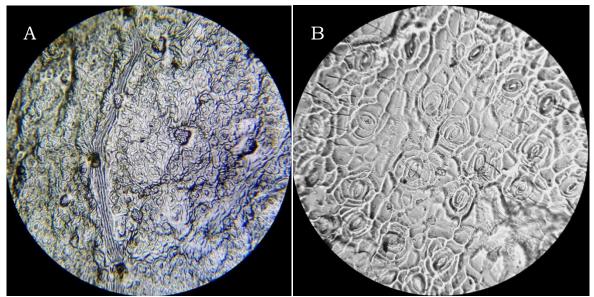


Fig. 27. Ventral surface of leaf. A, general view showing several glands and stomata (5 x 10X). B, Stomata generally cyclocytic type (45 x 10X).

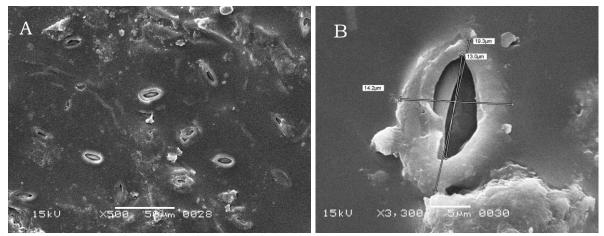


Fig. 28. SEM view of ventral surface of leaf showing stomata. A, Magnification: 500X; B, single stoma (magnification 3300X) admeasuring 19.3 x 14.2 μm and the outer stomatal ledge aperture: 13.0 μm. A gland may be seen near stoma.

A significant relationship between mean summer maximum temperature and stomatal density in *D. viscosa* subsp. angustissima along a latitude gradient in Southern Australia has been reported by Hill *et al.* (2014). The stomatal density ranged from 109 to 392 stomata / mm² across the latitude gradient $32^{\circ}S - 27^{\circ}S$ and stomatal size varied from 426.6 to $1104.2 \, \mu m^2$. There was negative relationship between stomatal size and latitude. No stomatal parameters related with elevation gradients. There was a significant positive relationship between mean maximum temperature of the hottest month and the stomatal density (Hill *et al.*, 2014).

Stomatal density on cotyledon

Stomatal density was higher on lower surface of the cotyledon (mean: 149.85 ± 4.089 stomata per mm² varying from 78.6 to 226.7 stomata per mm² as compared to the upper cotyledonary surface (mean: 74.50 ± 5.06 stomata per mm²) varying from zero to 176.9 stomata per mm². Stomatal density tended to distribute normally on upper surface (K-S test = 0.92, p < 0.20). On lower surface the distribution varied with the test type. K-s test indicated non-normal distribution and Shapiro-Wilk test indicated the tendency of stomatal distribution to be normal (Table 4).

Stomatal density on leaf

The leaves of *D. viscosa* were found to be amphistomatic but ventral surface tended to have much higher number of stomata per mm² (155.88 \pm 5.08, 88.46 – 216.24) as compared to the dorsal surface (50.53 \pm 2.29, zero to

98.27 per mm²). Both tests of normality testing indicated normal distribution of stomata on ventral surface. On dorsal surface the normality tests tended to behave differentially. K-S test indicated non-normal distribution but Shapiro-Wilk test indicated tendency of normal distribution of stomata (Table 4). Stomatal density was reported to be 27 (36 \pm 3) 45 on adaxial surface and 29 (46 \pm 2) 56 stomata per mm² on abaxial surface by Onuminya and Adediran (2018). On abaxial surface Santhi *et al.* (2016) reported much lower stomatal density (25.1 – 34.1- 40.3 stomata per mm²). Al-Aani *et al.* (2016) reported stomata to admeasure 21-25 μ m (mean = 23.60 μ m) on adaxial surface and 12 – 17 (mean 16.20 μ m) on abaxial surface - more or less comparable size range as recorded in this study.

redonary and roma stomatar density per min in seedings of D. viscosa.							
Parameters	Cotyledonary s	tomata per mm ²	Foliar stomata per mm ²				
Farameters	Upper surface	Lower surface	Dorsal surface	Ventral surface			
N	50	65	85	50			
Mean	74.5036	149.854	50.5325	155.8825			
SE	5.0634	4.08928	2.2863	5.07833			
Median	68.8028	157.2836	49.1449	152.3491			
CV (%)	48.06	22.00	41.71	23.04			
G1	0.189	0.164	- 0.076	0.015			
Sg1	0.337	0.297	0.261	0.317			
G2	0.221	-0.473	- 0.162	- 1.256			
Sg2	0.662	0.586	0.517	0.662			
Minimum	Zero	78.63	Zero	88.46			
Maximum	176.92	226.07	98.27	216.24			
K-S test* (p)	0.092 (0.200)	0.110 (0.05)	0.133 (0.0001)	0.131(0.031)			
Shapiro-Wilk	0.980 (0.550)	0.971(0.136)	0.976 (0.112)	0.940 (0.014)			
Distribution	S	S	S	AS			

Table 4. Cotyledonary and foliar stomatal density per mm² in seedlings of *D. viscosa*.

Resin Glands

Multicellular differentially-sized capitate trichomes (glands) were present over all organs of D. viscosa seedlings – hypocotyl (Fig. 18, gland precursor cells), cotyledons (Fig. 19, 29 C), both surfaces and margins of leaves (Fig. 16B, 22, 23, 25, 29 A and B), capsule wall (Fig. 5, 6), and seed (Fig. 10, 11A). Several of these glands were yellowish-brown in colour (Fig. 23, 26A). The density of glands on young leaf (0.86 cm²) of seedling averaged to 5.65 ± 0.853 per mm² varying from zero to 19.64 glands per mm² (Table 5). The density of glands varies with species to species of Dodonaea (West, 1980) and presumably with age of the organ. Young leaves are reported to have higher gland density as also reported by West (1980) from Australia and pointed out by Al- Asmari $et\ al.$ (2013) from Kingdom of Saudi Arabia. The presence of a gland appeared to suppress the occurrence of stomata on the leaf surface as is evident from the data presented in Table 5. Stomatal density was found to be significantly lower in those frames of microscopic vision where gland (s) occurred along with the stomata. In frames without the occurrence of gland (s), the stomatal density was higher. Also, some stomata were found to be surrounded by a number of resin glands around (Fig. 30).

Table 5. Glands and stomata density per mm² of a young leaf (c. 86 mm² in size) of *D. viscosa* seedling (Ventral surface).

Statistics	Glands*	Stomata accompanied with glands (A) in frames of vision	Stomata not accompanied with glands (B) in the frames of vision	Pooled sample stomata (C)
N	40	22	18	40
Mean	5.65	156.21	206.95	184.54
SE	0.8530	6.1650	6.281	5.434
CV (%)	81.12	81.51	12.87	18.62
Minimum	Zero	88.5	167.1	88.5
Maximum	19.64	226.10	245.7	245.7

*, Frequency of occurrence of glands = 55% of the frames of vision. The three categories of stomata (A, B and C) were significantly different from each other as given by paired t-tests – not shown here.

^{*,} Kolmogorov-Smirnoff test corrected for Lilliefors significance correction. Acronyms: CV (%), coefficient of variability; g1, skewness; g2, kurtosis; Sg1, SE of skewness; Sg2, SE of kurtosis; AS. Asymmetrical; S, symmetrical. Size of cotyledon studied: L x W = 2.0×0.5 cm and size of leaf studied: Young, 1.2cm².

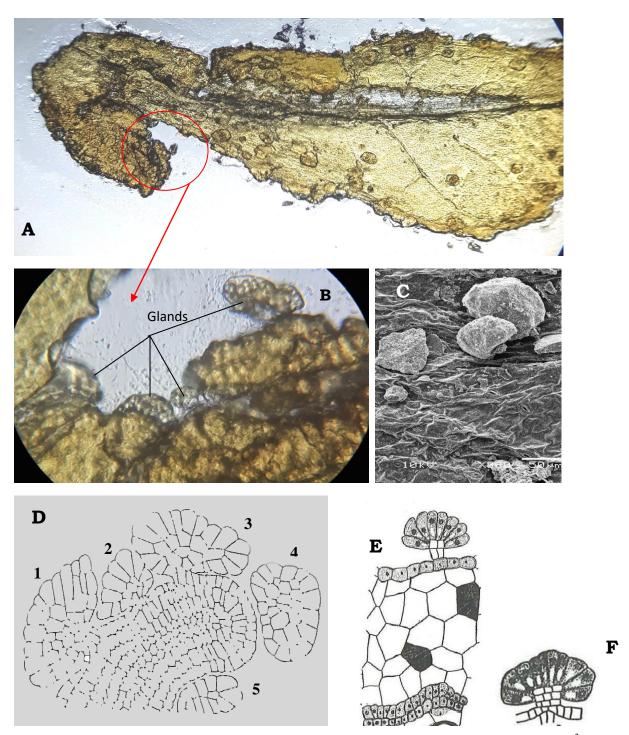


Fig. 29. A) Dorsal surface of leaf showing large number of young and mature glands of young leaf (5mm² in size) of *Dodonaea viscosa* as seen at magnification, 5 x 10X; B, Magnified view to show gland (45 x 10X); C, SEM view of dried solid glands scattered on the surface of dry young cotyledon; D, Mature glands crowded and overlapping on the surface of young leaf (X 120) as reported by Collins (1920). The images E and F are mature multicellular glands on seed epidermis (D) and ovary wall (E) adopted from Karkare-Khushalani and Mulay (1974). Images: A, B and C are from the present work.

These glands had multicellular head and multicellular stalk. Such glands have also been reported on ovary wall, seed surface and petiole of *D. viscosa* also by Karkare-Khushhlani and Mulay (1964) (Fig. 29 E and F). Collins (1920) was the first to describe these glands (Fig. 29 D). He described the developmental stages of resin-secreting

glands of some Australian plants including *D. viscosa*. The resinous viscous material secreted by these glands is considered to be an anti-herbivory tool. Langenheim (2003) has defined resin as primarily a lipid-soluble mixture of volatile terpenoids and /or phenolic secondary compounds that are 1) usually secreted in specialized structures located either locally or on the surface, 2) of potentially significance in ecological interactions. The surface resins are probably of greater significance as protection of vulnerable plant surfaces.

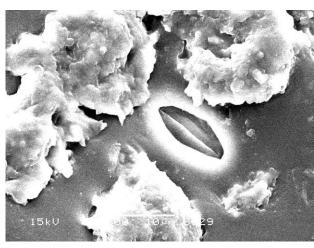


Fig. 30. SEM view of ventral surface of a dry leaf of 20 day-old seedling showing an stoma surrounded by several (at least more than five) capitate glands engulfed in their own secretion, most probably dried resin.

According to Collins (1920) glandular trichomes in *D. viscosa* resemble the *Melanodiscus* type. The mature glands were reported to be large peltate which may overlap one another and spread to cover considerable area of the epidermis. He reported that pedestal of the gland composed of 2-3 rows of cells which may become more numerous by later divisions. As regards to the ontogeny of glands, Collins (1920) described that first is the projection of papillose cell. It nucleus divides and the cell divides vertically not more than two times in the initial stages. Then, there are oblique divisions resulting in projecting mass almost spherical in contour. Now cells enlargement takes place. A series of irregular divisions in later stages of development may occur. Similar ontogenic details regarding development of glands in *D. viscosa* were later given by Karkare-Khushalani and Mulay (1964). They emphasized that gland precursor cell become papillate, protrudes out and the divides twice by vertical divisions to form multicelled stalk. The terminal cell of the stalk by further divisions forms the head of the gland.

The largest gland observed on leaf in our studies had quite large head admeasuring 90.6 and 78.3 µm in diameter at right angle. It comprised some 32 cells and stalk measured c 20 µm in length (Fig. 25). The glands were presumably more abundant and active in younger leaves of seedlings and secreted viscous resinous material forming a sheet or localized patches over leaf as a defensive tool against insect herbivory. The leaves are quite brittle when dry; the resinous material could be seen scattered on the young leaf surface under high magnification (Fig. 24). The occurrence of glandular hairs and resinous layer have been reported in several plants of families Mimosaceae, Euphorbiaceae, Sapindaceae, Boraginaceae, Dicrastylidaceae, Lamiaceae, Myoporaceae, Solanaceae and Goodeniaceae (Dell, 1977). Sometimes the resinous sheet may be broken or in isolated patched and in *Eremophia granitica* resin may amount as high as 29.5% of leaf dry weight (Dell, 1977). Glandular capitate trichomes have also been reported on abaxial and adaxial surfaces of leaf blade of *D. viscosa* from four resting formations in Paryne Estadual Acarai by Silva *et al.*, 2017) and in Ethiopian *D. viscosa* population by Kosel (2015). The size of the mature large gland as observed by us is almost comparable to the diameter of a mature resin-yielding glandular head reported to be 95-115µm by Dell and McComb (1978). According to them the resin content of such a glandular head may amount to 0.5µg and mature leaves may yield 22mg of resin per leaf.

D. viscosa leaves are highly nutritive, high in water content, high N and low C: N ratio. It is protected by chemical compounds secreted by glandular trichomes. The importance of glandular trichomes in herbivory defense was demonstrated in experiments with Aristolochia californica (Fordyce and Agarwal, 2001) and Liabum mandonii (Molina-Montenegro et al., 2006) in which predation and oviposition were found to be negatively correlated. Dell (1977) reported sheet of resin over leaf in several Western Australian plants including several species of Dodonaea (D. viscosa as well) to reduce transpiration loss of water. This sheet, according to Dell (1977) appears not to alter qualitatively the wavelengths of light available for photosynthesis although may reduce the amount of light entering the leaf. The glandular trichomes in D. viscosa appear to have multiple functions in its ecology and need further investigation.

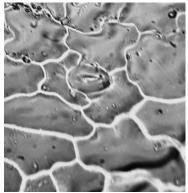
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PS: An anisocytic stoma present on the upper mature cotyledonary surface of *D. viscosa*.

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