

Morphological and biochemical variations in *Elaeagnus umbellata* Thunb. from mountains of Pakistan

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Elaeagnus umbellata Thunb. (Elaeagnaceae) is a native multipurpose plant from Himalayan regions of Pakistan. Its flowers and fruit are rich in vitamins, flavonoides, essential oil, lycopene and other bioactive compounds. Five populations of *Elaeagnus umbellata* from variable microenvironments of District Poonch were compared in terms of morphology and biochemistry using conventional and biochemical techniques. In spite of significant variability in morphological characteristics and vitamin C, seed oil and leaf chlorophyll content, *Elaeagnus umbellata* has great potential as a raw material for pharmaceutical industries, soil stabilization, reclamation, and for economic activities to the disadvantaged communities of the mountainous regions of Pakistan.

Key words: *Elaeagnus umbellata*, morphology, vitamin C, oil content, chlorophyll, Himalaya, district Poonch, Pakistan

Introduction

The topographic nature of Azad Jammu and Kashmir does not allow considerable production of cereal crops. The land is mostly terraced and fragmented on steep hills. These steep hills are mostly covered by conifer forests and not only enriched with fuel wood and timber but contains large diversity of medicinal plants, wild life, soil, micro flora and fauna of great value. A survey conducted in 1978–79 indicated that the medicinal plants of Azad Jammu and Kashmir were not only enough to provide raw materials for medicinal needs of the country but also have greater export potential to export the produce (AHMED et al. 1998). The conservation of these medicinal plants and their commercial cultivation will help to produce an ample quantity of valuable raw material for local and international pharmaceutical industry as well as a source of desirable genes for future generations.

Elaeagnus umbellata Thunb. a member of Elaeagnaceae family also called cardinal olive, autumn olive or autumn elaeagnus (DIRR 1998) is one of the valuable shrub of this area with inherent ability to grow under natural conditions of Azad Kashmir. It is a common me-

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dicinal shrub found in wild at height of 1300 to 1800 m above sea level in Azad Kashmir (SABIR et al. 2003). *Elaeagnus umbellata* is a large spreading, spiny-branched shrub often obtaining, 3.5 to 5.5 m in height and 3.5 to 5.5 m in width. The foliage is light green on top and a silvery green on the bottom (DIRR 1998). Leaves are alternate and petiolated in small lateral clusters on twigs (ECKARDT and SATHER 1987). Leaves are elliptic to ovate-oblong, 4–8 cm long, 1–2.5 cm wide, upper surface sparsely white lepidote, lower surface densely white lepidote, apex acute to sometime obtuse; petioles are 0.5–1.0 cm long, densely white lepidote. The drupes (fruits) are silvery with brown scales when immature, ripening to a speckled red in September to October (STERNBERG 1982). Fruit is fleshy, subglobose to broadly ellipsoid, 6–8 mm long. Fruits are 1.25 to 1.5 cm in size and start as a spotted light green in mid-summer turning red in the autumn (DIRR 1998). A mature plant can produce 0.9 to 3.4 kg of fruit per year, with the number of seeds ranging from 20,000 to 54,000 (ECKARDT and SATHER 1987).

Elaeagnus umbellata can fix atmospheric nitrogen (KIM et al. 1993, GARDNER 1958, GRAHAM 1964). It does very well on infertile soils because of its root nodules house nitrogen-fixing actinomycetes (STERNBERG 1982) and yields 236 kg of mineral nitrogen per ha in one year in upper 20 cm of soil (PASCHKE et al. 1989).

Companion planting of *Elaeagnus umbellata* with black walnut controls mycosphaerella leaf spots and walnut anthracnose which are important defoliating diseases of black walnut (KESSLER 1990). *Elaeagnus umbellata* is valued for the ability to prevent erosion and to attract wildlife (ZERGER 1980).

The *Elaeagnus umbellata* berry is an excellent source of vitamins and minerals, especially vitamin A, C, E, flavonoids and other bioactive compounds. It is also a good source of essential fatty acids (MATTHEWS 1994). 100 g of *Elaeagnus umbellata* fruit contains 69.4 g of moisture, 14.5 g of total soluble solids, 1.15 g of organic acids, 8.34 g of total sugar, 8.13 g of reducing sugars, 0.23 g of non-reducing sugars and 12.04 mg of vitamin C. The total mineral content of the fruit as represented by its ash is 1.045% (DIRR 1998). It also contains lycopene, β -carotene, lutein, phytofuluene and phytoene. The lycopene content per 100g ranged from 10.09 to 53.96 mg in fresh fruit from the naturalized plants and from 17.87 to 47.33 mg in the cultivars with red-pigmented fruit. Cultivar with yellow fruit has only 0.82 mg/100 g fresh weight of fruit. In contrast, fresh tomato fruit which is the major dietary source of lycopene, has lycopene content of 0.88 to 4.20 mg per 100 g. This newly identified source of lycopene may provide an alternative to tomato as a dietary source of lycopene and related carotenoids (KOHLMEIER et al. 1997, FORDHAM 2001). Lycopene is widely believed to protect against myocardial infarction (KOHLMEIER et al. 1997) and various forms of cancer (CLINTON 1998), including prostate cancer (GIOVANNUCCI et al. 1995). Thus, *Elaeagnus umbellata* shows potential as a deterrent to heart disease, cervix and gastrointestinal tract cancer (MATTHEWS 1994).

The floral volatiles of *Elaeagnus umbellata* contain palmitic, C₁₄ to C₂₀ fatty acid, methyl esters, eugenol, 4-methyl phenol, phenylacetate aldehyde and (E)-2-nonenol. The seeds are used as a stimulant in the treatment of coughs. The seed oil is used in the treatment of pulmonary affections (CHOPRA et al. 1986). Fruits can be used in raw or cooked form (HEDRICK 1972). Fruit is juicy, pleasantly acidic and can also be made into jams or other preserves (FACCIOLA 1990, REICH 1991: 113–120).

Materials and methods

Morphological analysis

Five morphologically variable populations from District Poonch were selected for morphological comparisons. Fruits were collected from different populations and stored in the refrigerator at 4 °C for biochemical analysis. The morphology of *Elaeagnus umbellata* was compared for five different natural populations from District Poonch, Azad Jamu & Kashmir. There were three replications and five representative plants from each replication for comparison. The plants had been randomly selected and tagged indicating the population and replication. In addition five branches were randomly tagged for other characters indicating average size of main branches on the main stem, average number of thorns on the main branches, average number of fruit bunches per branch, average number of fruit berries on fruiting bunches, average number of berries on main branches, average number of leaves on the main branches and the leaf area. Finally the average data were analyzed statistically by following the method of STEEL and TORRIE (1980).

Biochemical analysis

Ascorbic acid content of berries

Ascorbic acid was determined using phenol indophenol dye method (AOAC 1984). 10 g of the fresh berries/fruits were blended with metaphosphoric-acetic acid extracting solution. 5 ml of the filtrate extract were then titrated with standard indophenol to pink end point. Experiment was repeated three times.

Lipid content of berries

Oil contents from the berries/fruits of different plants were used for the analysis of lipid content according to the standard methods (AACC 1983). Samples were dried in an oven at 105 °C for 6–12 hours. 10 g of dried sample was used for extraction of oil in Soxhlet apparatus (30–40 °C) for 6 hours using diethyl ether as solvent. The solvent was removed under vacuum and the residual oil was dried over anhydrous Na₂SO₄. Experiment was repeated three times. Analytical grade chemicals were used for extraction of oil.

Chlorophyll Content

Chlorophyll was extracted from 1 cm² area of fresh leaves of *Elaeagnus umbellata*. An extraction of chlorophyll was done in 80% acetone and absorbance was measured at 663 and 645 nm with UV spectrometer for chlorophyll a and b respectively. Chlorophyll contents were calculated according to the method of ARNON (1949).

Statistical analyses

All comparisons between the different parameters of the morphological characters were done using ANOVA. The results of biochemical analysis were expressed as a mean of three determinations ±SD.

Results

Morphological characters

Mean Values of some morphological characters among the populations of *Elaeagnus umbellata* are compared in table 1. Plant height was reported from 247.66 cm to 317.26 cm. The plant canopy ranged from 656.16 cm to 727.5 cm. Stem girth ranged from 3.93 cm to 5.26 cm, the size of thorns on stem from 1.12 cm to 1.91 cm, the number of branches per plant from 47.33 to 54.47.33, number of thorns on branches from 1.66 and 2.33, size of thorns on the main branches from 0.72 cm to 1.74 cm, and the number of leaves per branch from 98.0 to 194.66. Leaf area ranged from 6.46 cm² to 9.25 cm², number of leaves per branch from 102 to 107, number of seeds in 1 kg fruits from 5488.33 to 5966.66, 1000 berries pulp weight ranged from 21.56 gm to 27.56 gm, and the weight of 1000 seeds was found to be in the range of 170 to 183 grams.

Tab. 1. Mean values of some morphological traits in *Elaeagnus umbellata* V=Variety

Traits	V1	V2	V3	V4	V5
Plant height (cm)	317.2±16.6	300.33±8.67	278.0±10.1	281.53±6.12	247.66±13.7
Plant canopy (cm)	713.6±25.1	727.5±18.2	721.26±13.1	702.66±23.6	656.16±35.1
Stem girth (cm)	4.26±0.71	5.26±0.72	3.93±1.1	4.83±0.61	4.23±0.65
No. of thorns per stem	8.33±0.01	8.66±0.07	5.66±0.10	3.66±0.15	6.0±0.09
Size of thorns on stem (cm)	1.53±0.04	1.91±0.02	1.12±0.05	1.53±0.03	1.47±0.001
No. of branches per plant	47.33±10.1	54.0±9.5	52.0±7.1	54.0±5.6	54.0±9.0
Size of branches per plant (cm)	64.46±3.84	52.73±6.64	50.26±4.15	60.91±4.96	57.51±11.1
No. of leaves per branch	194.66±16.1	106.66±12.1	107.33±9.1	102.33±5.1	98.00±15.7
Leaf area (cm ²)	9.25±0.20	7.84±0.11	6.43±0.15	7.46±0.12	6.56±0.21
No. of berries per branch	39.67±0.51	28±1.1	100±6.5	74.67±3.1	58.33±2.1
No. of berries per bunch	2.66±0.01	3.0±0.02	4.0±0.1	3.66±0.03	3.0±0.04
1000 berries pulp weight (g)	28.60±5.1	24.00±4.1	26.70±10.2	7.40±2.5	21.56±1.5
1000 seed wt.(g)	173.60±17.1	169.64±15.2	176.73±14.6	183.03±13.02	181.83±11.1

Tab. 2. Mean square values for different morphological traits among different populations of *Elaeagnus umbellata*. NS = non significant at 0.01 level of probability, * -significant at 0.01 level of probability

SOV	df	Plant height	Plant canopy	No of thorns	Size of thorns	No of branches per plant	Size of branches	No of leaves per plant	Leaf area	No of fruit bunches	No of fruit berries per branch	1000 berries pulp weight	1000 berries seed weight	Stem girth
Blocks	4	1717.4 ^{NS}	16930.3	0.067	0.039	21.667	44.185	495.800	0.197	22.067	260.867	9.733	80.554	2.298
Populations	10	2048.2 ^{NS}	2424.0 ^{NS}	12.76*	0.236*	25.067 ^{NS}	101.054*	5019.93*	3.866*	108.93*	2441.433*	18.981*	94.132 ^{NS}	0.856 ^{NS}
Total	14	3765.6	19354.34	12.827	0.275	46.734	145.239	5515.73	4.03	130.997	2702.3	28.714	174.686	3.154

Biochemical analysis

The vitamin C content in *Elaeagnus umbellata* ranged from 14.1% to 14.3% in different samples of *Elaeagnus umbellata* (Tab. 2). The oil content in the seeds of *Elaeagnus umbellata* was estimated in the range of 5.91 to 6.1%. Maximum oil was reported in V1 (6.1%) while minimum oil in V2 (5.91%). The oil in pulp was also extracted and was found in the range of 7.60 to 8.06%. Again maximum oil was reported in V1 (8.06%) while minimum in V2 (7.60%). Thus, the pulp of *Elaeagnus umbellata* had greater quantity of fatty oil as compared to the seed. The amount of chlorophyll was also estimated in the leaves of *Elaeagnus umbellata* and found in the range of 5.2 to 6.5 mg cm⁻². Maximum chlorophyll was reported in V1 (6.5 mg cm⁻²) while minimum in V5 (5.2 mg cm⁻²).

Discussion

The significant differences ($p \leq 0.01$) among most of the traits were found, except for plant height, plant canopy, stems girth, number of branches and seed weight (Tab. 3). It implies that the populations of *Elaeagnus umbellata* from different areas of district Poonch vary among themselves. Plant height and width (canopy) 3.5 to 5.5 m described in earlier studies from North America (DIRR 1998) is little more than the present investigation. However, such variation may be due to population specificity and the adaptation of plants in different environmental or soil conditions (SZAFONI 1989). DIRR (1998) measured the length and width of the leaves as 4–8 cm long, 1–2.5 cm wide. His results are inline with our results with some variation among the populations (Table 1). Our results indicated less number of seeds/Kg compared to the results of earlier investigations (ECKARDT and SATHER 1987), where 1 Kg fruits had about 20,000 seeds. This variation may be due to the larger size of fruits having more pulp in our investigation. In North America *Elaeagnus umbellata* was introduced but in Azad Kashmir there is natural stand, therefore the variation may be due to its better performance at natural stand. The comparison of pulp and seed weight indicated that the fruits had lot of pulp hence 1 kg of fruit had less number of seeds as found

in the earlier investigations (ECKARDT and SATHER 1987). The populations compared were also variable in various characters indicating the potential for future improvement.

Earlier studies indicated 12.04 mg of vitamin C per 100 gram of fruit (GRAHAM 1998), which was little lower as compared to our investigation. This variation may also be due to the variation in populations or the climatic factors. The presence of essential oils in fruits and flowers has been indicated in the earlier literature (POTTER 1995) but the actual amount has not been mentioned. The present investigation thus, indicated larger amount of fatty oil in seed as well as in the pulp of the fruit. The therapeutic value of *Elaeagnus umbellata* against heart diseases and others may be due to the presence of high amount of oil in fruits. The Plant oil and the phytosterols are known to have anticoagulant properties which are highly suitable for lowering the blood cholesterol and angina (FORDHAM 2001). The variability among the populations in terms of total seed and pulp oil may be due to their inherent or genetic variation, which could be exploited by breeding better varieties of the plant (SABIR et al. 2003).

The genera of family Elaeagnaceae, *Elaeagnus* and *Hippophae*, can withstand variable temperature ranging from -43 to 55 °C (AHMAD and KAMAL 2002). These plants can also grow at variable height from sea level ranging from 1200–2100 m and can thrive at variable soil pH ranging from 5.5–9.5 respectively (RONGSEN 1992). Additional benefits of these plants species are the fixation of atmospheric nitrogen with the help of actinorhiza symbiosis in their roots (GARDNER 1958, GRAHM 1964). Further the plant produce berries in large amount, which are rich in active medicinal compounds including essential oils, vitamins and phytosterol (MATTHEWS 1994, RONGSEN 1992). The berries of *Elaeagnus umbellata* also contain large amount of lycopene which has anticancer properties (KOHLMIEIER et al. 1997, FORDHAM 2001). *Elaeagnus umbellata* is native to district Poonch Azad Kashmir and support wild life in the area too for feeding on fruits and leaves. Its wood is used for fuel and plant is thorny hence, used as fence around the fields. Because of its multidimensional importance the populations were compared to evaluate their genetic variability for breeding desirable plants with high yield of berries and active compounds as well as easy harvesting. The populations were taken from variable environmental conditions thus, indicated significant amount of variability in traits, number of thorns on stem, size of thorns on stem, size of branches per plant, number of leaves per branch, leaf area, number of fruit berries per bunch, number of fruit berries per branch and 1000 berries pulp weight, and also in biochemical's including vitamin C, pulp and seed oils (Tabs. 1, 3). Such variability is very important to breed better varieties of plant suitable to the local area.

Tab. 3. Comparison of concentrations of vitamin C, oil content and chlorophyll in different populations of *Elaeagnus umbellata*

Populations	Vitamin C (mg/100g)	Oil in Seed (g/100g)	Oil in pulp (g/100g)	Chlorophyll (mg/cm ²)
V1	14.1±0.10	6.1±0.10	8.06±0.12	6.5±0.05
V2	14.16±0.15	5.91±0.08	7.60±0.17	6.2±0.01
V3	14.3±0.10	6.06±0.11	7.63±0.15	5.9±0.10
V4	14.2±0.10	6.0±0.11	7.80±0.26	5.5±0.11
V5	14.3±0.11	6.01±0.07	7.87±0.25	5.2±0.20

Conclusions

The investigation provided source of basic information and establishment of *Elaeagnus umbellata* in the mountainous areas of Azad Kashmir where the rapid soil erosion has become an alarming threat. This shrub can effectively control soil erosion and its biochemical importance can make it suitable plant for economic activities to the poor masses of the region. The medicinal components of berries will provide very cheap raw material for national and international pharmaceutical industries benefiting the humanity at large. Due to high content of Vitamin C *Elaeagnus umbellata* can be used in making fruit juices and soft drinks. The high content of seed and pulp oil may have commercial importance and will help the local community for marketing their farm produce with additional benefits. Population of variety 1 was recommended best among the different populations for the commercial use.

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