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Original Article

Standardization of Agrotechniques and Biochemical Assessment of *Crataegus oxyacantha* in Western Himalaya

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ABSTRACT

C. oxyacantha is a high valued medicinal plant of Rosacea family. It is used to cure cardiac disorder in ayurvedic medicines. A field experiment was laid out in 2004 in CSIR-IHBT farm, by using different quantity of FYM and various spacing. Low growth in plant height was observed in first five years with higher dose of FYM, but in 2015 the significant height growth was recorded. From 2008 to 2015 all types of FYM applications produced statistically significant yield of seed production except in 2012 and 2014. The 22.50t/ha was the most statistically significant dose of FYM in relation to seed yield. The spacing did not produce any significant results for seed production. A vegetative propagation trial of *C. oxyacantha* was also laid out using semi hard stem cuttings and some selected hormones (IAA, IBA, GA_3 and Abscisic acid) with different concentrations. Statistically significant shoot sprouting (78.35%) was recorded when IBA of 1000 mg/L was used followed by 67.74% in case of 1500 mg/L of the same hormone. While lowest shoot sprouting (27.85%) was observed using 2000 mg/L of Abscisic acid. A statistically significant 5.67 cm and 5.33 cm shoot lengths were observed using 2000 mg/L of IAA and 1000 mg/L of IBA, respectively. In the case of shoot tillers 3.33 was recorded in 1500 mg/l. of IAA. Two new compounds and 9 known compounds were isolated from fruit extract.

Key words: *Crataegus oxyacantha,* Hormones, Bioactive compounds, FYM, Spacing.

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INTRODUCTION

Crataegus oxyacantha (hawthorn) belongs to the family Rosacea. It is a middle deciduous spiny tree, distributed in Himalaya from 2500 to 4000 m amsl or near the tree lines of western Himalaya. In India, it is an endangered species. It is a native of Europe and North America. It's all parts such as bark, flower and fruits have medicinal importance, especially in cardiac disorders and leaves are used in herbal tea. Its cardiac and arrhythmia curing properties were studied.[1] Some workers have also reported its role in controlling various diseases like myocardial problem,[2] heart failure,[3] antioxidant, anti-inflammatory and antimicrobial infections.[4,5] It is also used in some other critical disease such as control of central nervous system (CNS), anxiety and depression.[6] A Homeopathic medicine has also been prepared from its flowers and fruits by Homeopathic pharmacopeia of India.[7] As food for heart has described.[8] Some of its phenolic constituents like hyperoxide, vitexin, quercetin and rutin, epicatechin, catechin and phenolic acids have pharmacological properties.[7]

MATERIAL AND METHODS

Field experiments were conducted from 2004 to 2016 in High Altitude Biology division farm of the CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur (1325 m. amsl. 32^{0}06'05"N, 76^0 34'10" E). The weather data was recorded by CSKH-PKV Palampur Table 1. The soil of the experimental field is silty clay loam in texture acidic in reaction (pH 6.4) high in organic carbon (2.4%) low level of N (198ha-1) medium in available P (23kg) and available K (538 kgha-1).

In agro-climatic conditions of Palampur, *C. oxyacantha* trees defoliated from the month of October and new shoot emerged in 1st week of April. Flowering also started within a week. Seed formation started immediately after 15-20 days of flowering. The seed matured in the month of July. When seed became red in color, its plucking was started. All the seed were plucked up to the end of August month. No disease has been reported in this plantation so far from July 2004. The seed formation though started in 2006, but it was properly started in 2007. Since then various growth parameters were recorded and presented in various tables. Statistically analyses have also been carried out every year.

Agrotechniques

Field experiment

An experiment was laid out for standardization the agrotechniques of *C. oxyacantha*. The stem cuttings

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were used for vegetative propagation. The diameters of stem cuttings were 6-8 mm and 8-10 cm which were less then pencil thickness.

The lower portions of stem cuttings (2 inch) were dipped in different solutions up to 3h and the control was dipped in plane water. In the experiment some selected hormones, IAA, IBA, GA_3 and Abscisic acid was used with the concentrations of 1000, 1500 and 2000 mg/l, 100 no. stem cuttings were used for each concentration, it was replicate thrice time i.e. 300 no. stem cuttings for each concentration in polytunnel. The sprouting time was taken about 60 to 80 days. The mature seed was collected in September 2015; the seeds were dried in natural open field for three months and at -5^{0}C in cold chamber. After 3 months, the seed were pickup outside the cold chamber. In the case of seed, the dormancy period is two years so, it is very difficult and time consuming. So, the vegetative propagation is preferred by shoot cuttings for raising sapling of *C. oxyacantha*. In two-year time most of the seeds were damaged or infected by some and other causes in the soil and in a store.

Nursery techniques

The land was prepared well by manual digging, levelling, breaking soil clods, weeding and finally mixing it with well rotten FYM and sands. The semi hard brown stem cuttings were collected from our own field. These cuttings were 8-10 cm long and 6-8 mm in diameter with 5-6 nodes. The stem cuttings were collected in the last week of January 2016. The experiment laid out on 02.02.2016. The stem cutting was dipped in solution of selected hormones IAA, IBA, GA_3 and Abscisic acid for 3h. The stem cuttings planted at 10.0 cm x 10.0 cm spacing. All the used hormones promoting callus. Normally stem cuttings take 70-80 days for rooting. The rooted cuttings were hardened for 2 months before transplanting in the field. The water was given as or when required. The reading of sprouting of sprouts stem cuttings were recorded in last week of March and 1st week of April 2016.

Soil type

C. oxyacantha is a middle tree and deciduous in nature. It is very well grown in sandy, clay-loam soil and sub humid, temperate climate. It's like low or medium clay-loam soil.

Land preparation and manure application

First of all, wild bushes with rooted stock and secondary roots were removed. Tractor tillage 4-5 times with disc than cultivator and the soil was leveled by leveler. The soil texture should be fine without any clods. FYM 15t/ha was thoroughly mixed, some quantity of sand was also mixed in soil to make it porous in nature. For plantation 45.0 x 45.0 x 45.0 cm dimension pits were dugout. In the experiment, FYM application 15t/ha, 22.5t/ha,30t/ha and 37.5t/ha was applied, each plot was replicated 4 times. The FYM applications were given before planting and were repeated every year in the month of December. Because it is a deciduous middle tree, there was no any growth observed during October to March. The growth period was April to October. In the month of December FYM was very well mixed by manually by proper digging with phawra. In dormant period, the loss of roots very less and recovered subsequently. The five spacing S1-1.0x1.0 m, S2-2.0x1.0m, S3-2.0x2.0 m, S4-3.0x1.0 m and S5-3.0x2.0 m was applied for this experiment with 3 no. replications. The planting has been done in the month of July 2004. And some filling was covered in August 2004. Because it is hilly moist region, wild bushes and unwanted vegetation including weeds were frequently come up.

In all the treatments, plants were planted at the given spacing. At the time of planting, the soil sample of top soil and sub soil (15 cm depth) were taken and analyzed for physic-chemical properties. The soil was silty clay loam in texture. Normally acidic in nature, high organic carbon percentage, available NPK high and average.

Isolation of bioactive compounds from *Crataegus oxyacantha* fruit extract.

Collection of plant material

The fruits of *Crataegus oxyacantha* were collected from IHBT Institute's farm in the month of July. The fruits were shade dried for 15 days and then in hot air oven at 30-40°C. The fruits were pulverized electronic grinder machine in to coarse powder. The voucher specimens (PLP 007) collected was identified, processed and deposited in the Herbarium of CSIR-IHBT, Palampur, India.

Extraction and fractionation of plant material

The powdered material of fruits (13 kg) was percolated four times with 70% aqueous ethanol at room temperature and the extract was concentrated for further studies on rotary evaporator at 40°C. The fruit extract was found to be 1391.2 g. For fractionation, crude extract (600 g for fruit) was defatted with *n*-hexane and remaining extract was partitioned with ethyl acetate and *n*-butanol. The respective ethyl acetate and *n*-butanol extracts obtained were 128.4 (FE) and 180.9 g (FB).

General experimental procedure

Melting points were determined on a Bronsted Electrothermal 9100. Mass spectra were recorded on QTOF-Micro of Waters Micro mass. NMR experiments were performed on Bruker Avance-300 spectrometer. For open column chromatography (OCC) silica gel (60-120 mesh), for dry column chromatography (DCC) silica gel-H and for flash column chromatography (FCC) silica gel (230-400 mesh) was used. Thin layer chromatography (TLC) was performed on silica gel G (on glass plates) and precoated silica gel 60 F_{254} plates. The detection of chemical constituent's present in the extract/fractions was carried out by iodine vapours, UV-detection and spraying reagents. The solvent systems used to resolve the chemical constituents and spraying reagents used for the detection are summarized below:

Isolation of secondary metabolites

The ethyl acetate fraction of fruit extract (FE) was subjected to DCC using CH_2Cl_2: MeOH: H_2O (100:13.5:16.5) as eluting solvent and seven fractions (Fr.) from FEa-FEg were collected on the basis of TLC pattern. The Fr. FEa was re-chromatographed using OCC with CH_2Cl_2: MeOH: H_2O (100:13.5:16.5) and again seven Fr. (FEa1-FEa7) were obtained. FEa1 yielded colourless needles of *β*-sitosterol **1** (40 mg), whereas, *β*-sitosterol-3-*O*-*β*-D-glucoside **2** (31 mg) was obtained from FEa6. Similarly, FEb was subjected to OCC in CH_2Cl_2 with increasing polarity of MeOH and 35 fractions of 25 ml each were collected. Fr. 4-10 yielded oleanolic acid **3** (15 mg) whereas ursolic acid **4** (67 mg) was purified from Fr. 19-32 using reverse phase silica (MeOH-H_2O). A white precipitate of bis-(2-ethylhexyl) phthalate **5** (19 mg) was obtained from FEc. The Fr. FEe was re-chromatographed using FCC in isocratic mode with CH_2Cl_2: MeOH: H_2O (100:18:20) and five fractions (FEe1-FEe5) based on TLC were obtained. FEe1 was re-chromatographed over silica using OCC with CH_2Cl_2: MeOH: H_2O (100:13.5:16.5) to yield quercetin **6** (10 mg) and (2*R*,3*R*)-taxifolin **7** (14 mg) as yellow and brownish yellow powder, respectively.

The *n*-butanol fraction (FB) of fruit extract was subjected to DCC using EtOAc: $CHCl_3$: MeOH: H_2O (15:8:4:1) in isocratic manner and nine fractions (FBa-FBi) were collected on the basis of TLC. Fr. FBc was re-chromatographed over FCC using EtOAc: $CHCl_3$:MeOH: H_2O (15:8:1:0.5 to 15:8:2:0.5) and five fractions were obtained. The Fr. 3 yielded vitexin-2″-*O*-rhamnoside **8** (32 mg) whereas, Fr. 5 afforded

Table 1: Weather parameters during 2004 to 2016 recorded at CSKHPKV, Palampur (H.P.)

Years	Temperature	Temperature	Relative Humidity (%)		Bright Sun	Rainfall
	Maximum (°C)	Minimum (°C)	RH (Max)	RH (Min)	shine hrs	mm
2004	24.90	13.40	65.20	55.00	384.50	2206.40
2005	20.08	10.90	59.23	54.15	345.80	2860.80
2006	20.18	11.83	55.12	46.25	343.80	2730.20
2007	19.86	11.19	57.61	45.54	359.30	1848.60
2008	19.65	10.88	56.90	47.20	328.40	2304.80
2009	20.47	10.61	59.49	45.84	348.00	1768.40
2010	25.62	13.52	73.57	56.23	345.44	2569.60
2011	23.90	13.17	81.31	67.24	331.41	2500.60
2012	24.01	12.81	70.96	56.18	348.23	2421.99
2013	23.55	13.05	75.55	61.18	328.41	3142.00
2014	23.30	12.41	72.74	59.15	327.62	1886.10
2015	23.65	12.53	77.72	64.41	318.30	2630.80
2016	24.61	12.55	75.26	60.22	345.19	2230.80

Table 2: Effect of growth hormones on shoot sprouting of *Crataegus oxyacantha*.

PGR mg/l	Sprout Cutting no.	Sprouting %	Tiller No.	Plant Height cm
IAA-1000	40.00	47.62	2.67	2.67
IAA-1500	35.33	56.08	3.33	4.00
IAA-2000	39.00	51.53	2.67	5.67
IBA-1000	60.33	78.35	1.67	5.33
IBA-1500	42.00	67.74	2.00	3.33
IBA-2000	46.67	52.03	2.00	3.33
GA_3-1000	37.33	50.45	2.33	5.00
GA_3-1500	39.00	55.71	2.00	4.00
GA_3-2000	45.00	53.57	3.00	3.33
Abscsic Acid-2000	26.67	27.85	1.67	2.67
SEm±	2.85	3.72	0.42	0.50
CD at 5%	8.48	11.05	NS	1.48

(2*R*,3*R*)-taxifolin-3-*O*-*β*-D-xylopyranoside **9** (16 mg). The fraction Fbe was chromatographed over reverse phase silica (MeOH-H_2O) which provided isoquercitrin **10** (21 mg) and it was purified with sephadex-LH-20. The yellow precipitates of rutin **11** (11 mg) were obtained from FBf.

RESULTS

The results of seed germination were poor (3.7%) and statistically non-significant on higher application of hormones. In case of semi hard stem cutting, which were used for vegetative propagation by using some selected hormone treatments, the sprouting time was 60-80 days Figure 1A. The statistically significant results (78.35%) was recorded in case of 1000 mg/l IBA followed by 67.74% growth using 1500 mg/l IBA. In the case of shoot tiller parameters, it was maximum in 1500 mg/l IAA and minimum in Abscisic acid 1.67 number. But these results were statistically non-significant. In terms of plant height IAA produced statistically significant 5.67 cm, and parallel in IBA 1000 mg/l, followed by GA_3 100 mg/l Table 2.

The plant height was recorded during 2005 to 2009 and lastly in 2015. All the results were statistically analyzed and found non-significant Table 3. The FYM application could not influence the plant height in all the years. However, the seed were produced in 2006, but the yield was very low. In the year 2007 also, the seed yield was recorded statistically non-significant. But from 2008 to 2011, it was statistically significant Table 4. Again in 2012 and 2014, the seed yield was non-significant. In 2015, all FYM applications produced higher and statistically significant yield as compared to other years. In 2016 the statistically significant seed yield was recorded. It was observed that the seed yield was highly affected by local weather conditions. At the time of flowering and seed setting, the high wind, high rainfall and big size hailstorms highly affected the seed yield leading to statistically non-significant result. In all observations, the plant spacing did not affect the plant growth as well as seed yield Table 6,7,8, but the dose of FYM produced high and statistically significant results Table 4,5. For commercial utilization, these harvested seeds were dried under shade conditions Figure 1B, C,D,E. To avoid moisture, the seed were dried in aluminum trays (iron mesh) and every alternate day the seed were transferred and changed to its previous position. The dried seeds are weighed and stored in jute bags, which are now ready for sale.

Two new compounds from *Crataegus* sp. and 9 known compounds were isolated from the ethyl acetate and n-butanol fractions of fruit extract of *Crataegus oxyacantha*. The new compounds are Bis-(2-ethylhexyl) phthalate and (2*R*,3*R*)-Taxifolin-3-*O*-*β*-D-xylopyranoside; whereas, the

Table 3: *Crataegus oxyacantha* FYM trial observation for plant height.

FYM	Plant height (cm) 2005	Plant height (cm) 2006	Plant height (cm) 2007	Plant height (cm) 2008	Plant height (cm) 2009	Plant height (cm) 2015
15t/ha	173.58	225.00	230.99	297.92	346.69	450.00
22.5t/ha	159.67	210.45	242.54	291.25	357.19	425.42
30t/ha	175.71	227.79	238.06	268.33	330.94	407.50
37.5t/ha	156.79	218.21	230.19	283.00	322.19	425.83
SEm±	5.07	7.09	8.95	11.74	24.61	9.19
CD at 5%	NS	NS	NS	NS	NS	NS

Table 4: *Crataegus oxyacantha* FYM trial observation for seed weight (kg/plot).

FYM	Seed weight (kg)2007	Seed weight (kg)2008	Seed weight (kg)2009	Seed weight (kg)2010	Seed weight (kg)2011
15t/ha	0.45	0.98	0.41	4.66	12.20
22.5t/ha	0.97	1.10	0.48	4.80	16.69
30t/ha	0.67	0.84	0.43	6.18	16.89
37.5t/ha	0.51	1.42	0.34	7.81	14.36
SEm±	0.30	0.24	0.06	0.84	1.58
CD at 5%	NS	0.77	0.19	2.69	5.07

Table 5: *Crataegus oxyacantha* FYM trial observation total seed weight kg/plot.

FYM	Seed weight (kg)2012	Seed weight (kg)2013	Seed weight (kg)2014	Seed weight (kg)2015	Seed weight (kg)2016
15t/ha	18.08	12.44	3.13	46.00	8.00
22.5t/ha	24.35	17.69	4.44	54.50	8.13
30t/ha	27.00	20.31	4.81	48.00	7.75
37.5t/ha	22.13	11.75	3.19	48.25	5.50
SEm±	3.25	2.53	0.77	4.35	1.75
CD at 5%	NS	8.09	NS	13.93	5.60

Table 6: *Crataegus oxyacantha* spacing trial observation for plant height.

Spacing	Plant height (cm) 2005	Plant height (cm) 2006	Plant height (cm) 2007	Plant height (cm) 2008	Plant height (cm) 2009	Plant height (cm) 2015
S1=1x1m	169.99	207.90	229.42	275.00	359.56	444.86
S2=2x1m	172.25	205.78	223.00	299.03	335.42	453.19
S3=2x2m	176.56	212.89	229.55	280.00	324.58	424.58
S4=3x1m	181.38	212.42	234.72	297.29	336.83	425.14
S5=3x2m	176.17	223.33	236.00	262.50	278.33	469.31
SEm±	9.04	11.51	13.13	10.90	17.88	18.14
CD at 5%	NS	NS	NS	NS	NS	NS

Table 7: *Crataegus oxyacantha* spacing trial observation for seed weight (kg/plot).

Spacing	Seed weight (kg)2007	Seed weight (kg)2008	Seed weight (kg)2009	Seed weight (kg)2010	Seed weight (kg)2011
S1=1x1m	0.82	1.05	0.55	2.45	13.07
S2=2x1m	1.03	1.62	0.39	5.88	11.82
S3=2x2m	0.79	1.30	0.93	7.73	13.02
S4=3x1m	0.81	1.23	0.89	4.23	11.30
S5=3x2m	0.97	1.53	0.34	7.12	11.33
SEm±	0.49	0.36	0.10	1.27	2.01
CD at 5%	NS	NS	NS	NS	NS

Table 8: *Crataegus oxyacantha* spacing trial observation total seed weight (kg/plot).

Spacing	Seed weight (kg)2012	Seed weight (kg)2013	Seed weight (kg)2014	Seed weight (kg)2015	Seed weight (kg)2016
S1=1x1m	19.52	19.17	5.83	47.00	5.33
S2=2x1m	14.13	20.17	7.17	50.67	6.33
S3=2x2m	20.17	21.33	7.25	50.00	9.33
S4=3x1m	14.55	15.17	3.92	40.67	4.67
S5=3x2m	23.37	27.68	10.67	54.67	8.33
SEm±	5.48	4.64	2.29	13.65	1.98
CD at 5%	NS	NS	NS	NS	NS

Figure 1: Vegetative propagation by stem cuttings, R&D field experiment and mature staging of fruit, different stage of seed drying under shade of *C. oxyacantha.*

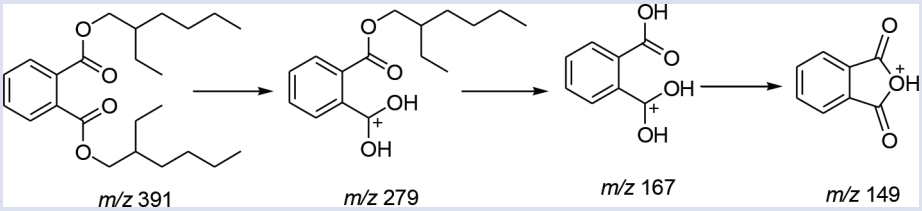

Figure 2: Mass fragmentation of 5.

Table 9: ^{1}H (300 MHz) and ^{13}C NMR (75.4 MHz) spectral data of 5 in $CDCl_3$.

Position	δ_H (*m*, *J* Hz)	Δc	Position	δ_H (*m*, *J* Hz)	δc
1	7.56 (*dd*, 3.0 and 6.0)	131.2	7	1.33-1.38 (*m*)	30.7
2	7.74 (*dd*, 3.0 and 6.0)	129.1	8	1.33-1.38 (*m*)	29.3
3	-	132.8	9	1.33-1.38 (*m*)	23.3
4	-	168.0	10	0.89-0.96 (*m*)	14.3
5	4.18-4.28 (*m*)	68.5	11	1.40-1.45 (*m*)	24.1
6	1.60-1.70(*m*)	39.1	12	0.89-0.96 (*m*)	11.3

Table 10: ^{1}H (300 MHz) and ^{13}C NMR (75.4 MHz) spectral data of 9 in CD_3OD.

Position	δ_H (*m*, *J* Hz)	Δc	Position	δ_H (*m*, *J* Hz)	δc
2	5.10 (*d*, 9.0)	84.3	2′	6.72 (*m*)	116.4
3	4.72 (*d*, 9.0)	78.1	3′	-	148.0
4	-	196.3	4′	-	147.1
5	-	164.8	5′	6.91 (*m*)	116.9
6	5.90 (*d*, 3.0)	97.0	6′	6.72 (*m*)	121.5
7	-	169.6	1″	3.82 (*d*, 4.0)	103.7
8	5.90 (*d*, 3.0)	98.0	2″	3.20 (*m*)	76.4
9	-	164.1	3″	3.33 (*m*)	74.0
10	-	103.2	4″	3.52 (*m*)	71.5
1′	-	129.6	5″	3.91 (*m*)	66.0

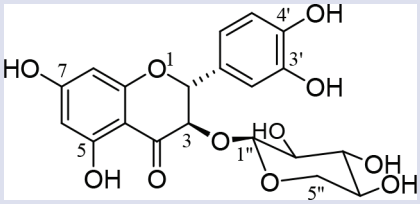

Figure 3: Structure of 9.

known isolated compounds are vitexin-2″-*O*-rhamnoside, isoquercitrin, (2*R*,3*R*)- taxifolin, oleanolic acid, ursolic acid, *β*-sitosterol, quercetin, rutin and *β*-sitosterol-3-*O*-*β*-D-glucoside.

Bis-(2-ethylhexyl) phthalate (5)

The compound **5** was obtained as reddish-brown precipitate from the ethyl acetate fraction of fruit of *C. oxyacantha*. The ESI-MS (+ve) spectrum showed a molecular ion peak at *m/z* 391 $[M+H]^+$ corresponding to the molecular formula $C_{24}H_{38}O_4$. The fragments at *m/z* 279, 167 and 149 with the consecutive loss of aliphatic chains were obtained according to the fragmentation pattern as shown in Figure 2.

The ^{1}H NMR spectrum showed two aromatic protons at δ_H 7.74 (*dd*, *J*=3.0 and 6.0) and 7.56 (*dd*, *J*=3.0 and 6.0) integrating for two protons each. In addition, multiples in the aliphatic region were also observed. The ^{13}C NMR spectrum showed signals for 12 carbons including two methyl's, five methylene's, three methane's and two quaternary carbons. The presence of only two aromatic protons with *ortho* and *meta* coupling double doublets suggested that the compound must have an *ortho*-disubstituted benzene ring bearing the same substituent in both positions. The aromatic proton at δ_H 7.74 (*dd*, *J*=3.0 and 6.0) showed HMQC correlation with the carbon at C-2 (δ_C 129.1), while HMBC correlations with that of C-1 (δ_C 131.2), C-3 (δ_C 132.8) and C-4 (δ_C 168.0). The methylene multiple at δ_H 4.18-4.28 (H-4, *m*) was attributed to C-5 (δ_C 68.5) based on its HMBC correlations with neighbouring carbons. Its correlation with C-4 (δ_C 168.0) indicated that it was linked to the carbonyl carbon through an oxygen atom *i.e.* has an ester linkage. The methyl multiple at δ_H 0.89-0.96 (H-10 and 12, *m*) integrating for twelve protons and correlations with methylene carbons at δ_C 23.3 and δ_C 24.1 were attributed to the positions C-10 (δ_C 14.3) and C-12 (δ_C 11.3). Hence, based on NMR (Table 9) and mass spectral data and its comparison with the literature values,[9] the structure of **5** was established as bis-(2-ethylhexyl) phthalate. This is the first report on the isolation of this compound from the *Crataegus* spp.

(2*R*,3*R*)-Taxifolin-3-O-*β*-D-xylopyranoside (9)

The compound **9** was isolated as brown powder from fruit extract of *C. oxyacantha*. The ESI-MS spectrum displayed the molecular ion peak at *m/z* 459 $[M+Na]^+$ and 437 $[M+H]^+$ corresponding to the molecular formula $C_{20}H_{20}O_{11}$. One peak at *m/z* 306 also appeared due to the loss of a pentose (132U). The NMR spectra of compound **9** were like that of taxifolin with the additional signals for a pentose Figure 3. The NMR values of the sugar were consistent with the xyloparyanoside.

The H-2 and 3 protons resonated at δ_H 5.10 (*d*, *J*=9.0) and 4.72 (*d*, *J*=9.0). According to data published in the literature (Hosoi *et al.*, 2006), a coupling constant of 9.3-11.3 Hz correspond to *trans* form of taxifolin 3-*O*-arabinoside, which means that there are two possible configurations, either the (+)-*trans* form (2*R*,3*R*) or the (-)-*trans* form (2*S*,3*S*). The configuration was determined to be 2*R*,3*R* by the optical rotation data *i.e.* $[\alpha]_D^{25}$ +20.9°, which was consistent with that of reported.[10] The glycosylation at C-3 (δ_C 78.1) was evident from the downfield shift of C-3 and the upfield shift of C-2 (δ_C 84.3) and C-4 (δ_C 196.3) due to *β*-effect. Hence, based on the spectral data (Table 10) and literature

values,[11] the structure of compound 9 was attributed to (2*R*,3*R*)-taxifolin-3-*O*-*β*-D-xylopyranoside. To the best of our knowledge this is the first report on the isolation of (2*R*,3*R*)-taxifolin-3-*O*-*β*-D-xylopyranoside from *Crataegus* spp.

DISCUSSION

C. oxyacantha has a very high medicinal value and in this article, we have tried to standardize the propagation its techniques by using semi hard stem cuttings. Later in the field trial, the effect of different doses of FYM and various spacing on growth and biomass yield of seeds was studied. In the propagation trial, through stem cuttings, the statistically significant results were obtained in IBA when it was used in the concentration of 1000 mg/l and 1500 mg/l Table 2. Due to pretreatment of stem cuttings by IBA, its endogenous level of hormones was increased due to availability of exposed surface area at the cut ends, which in turn promoted for the rooting.[12] Similar finding has been reported,[13,14] our results are fully in agreement to their findings. The hormones IAA, IBA, GA_3 and Abscisic acid activate enzymes and mobilize reserve food material, which result in initiation of cell division, cell elongation and formation of callus in cut ends of stem cuttings, leading to its rooting.[15,16] Our results are in agreement of findings,[14] who observed stimulation of plant height, leaves numbers, root length by these hormone treatments in another Himalayan medicinal plant *Taxus baccata*. Some other workers have also reported the same type of findings.[17,18]

The plant growth was recorded from 2005 to 2009 continuously and then in 2015. It was observed that in early five years, it was growing and showing increasing trends. It was 65 to 80% higher in 2005 as compared to 2006 Table 3. While in 2007 it was 128% more than of 2008. From 2008 to 2009, the growth was slightly slowed down to about 112%. Again, it was reduced from 2009 to 2015 from 125 to 135% Table 3. It means after a certain period, the growth became stable in this plant as the energy and reserve food materials got utilized in different physiological, metabolic and seed production processes. The reserved food gets accumulate in the seeds and the overall growth status goes down.

In the different FYM applications, since 2008 onwards statistically significant results were obtained. The FYM, application has increased the overall production of seed Table 4,5. In middle, some year as 2014, 2016. The seed production was reduced drastically due to high wind, untimely rain and heavy hail storm at the time of flowering and seed formation. Especially, in the month of April and May, every year, these are natural calamities that highly affected seed yield. Most of the flowers fallen in soil due to high wind, rain and hail storm. The same type of studies carried out in our Institute.[16,19] In most of the parameters various FYM application behave, separately plant species to species. In most of cases 22.5 t/ha dose produced increasing trend in comparison to lower and higher doses of FYM Table 5,6. It was recorded that NPK are major nutrients, which play an important role in growth and yield of the crop.[20,21] They further confirmed that a certain amount of nutrient (fertilizer) is required at a certain extent. Beyond a saturate stage, system did not allow it and no positive response was reported in terms of growth and yield.[22] In our experiment after five years 2009, 22.5t/ha produced higher plant growth, while in 1st year lower doses are effective but higher dose 37.5t/ha produced lower growth. Even in next five year means in 2015 lower dose 15t/ha was produced better growth performance of plants. However, in terms of plant height all the results were statistically non-significant Table 6. In terms of seed production again 22.5t/ha produced better yield in comparison to other doses in 2007 and 2008 years. After ten years in 2015, in the end the statistically significant seed yield was recorded in 22.5t/ha FYM application. In this year no any adverse of local weather or environmental conditions i.e. no high wind, no hail storm no heavy rains were recorded. But see in 2016 again the seed yield highly destructed by local environmental conditions caused low crop yield Table 7,8. It was reported that 50 kg N ha-1 was sufficient rather than higher doses.[23,24] It has also recorded that excessive N supply reduced the yield and biomass.[25]

Therefore, our purpose was to develop agrotechniques for its domestication for the global society. This will reduce our dependency on these natural resources mean as farmers can grow it in their field following our recommendations.

CONCLUSION

The purpose of this study was to standardize the agrotechniques of cultivation and nursery raising of *C. oxyacantha* leading to yield and biomass production of the crop. Besides, the standardization of agrotechniques, its secondary metabolites have also been studied. Two new compounds have also been reported from its fruit extract. Chemical evaluation of seeds have also been compared based on its quality and quantity. For these study, some R & D experiment were laid out. First of all, the nursery was raised from its semi hard stem cutting in conjunction with different concentrations of IAA, IBA, GA_3 and Abscisic acid hormones. Statistically significant sprouting was recorded in IBA-1000 mg/l 78.35% followed by IBA 1000 mg/l. For 10 years (2005 to 2015) duration, local climatic condition played an important role in seed yield as it got affected by high wind velocity, hail storms and high rainfall. Most of the flowers were fallen on ground surface. Spacing was not affected. Growth in plant height was not significant. Seed yield was statistically significant. FYM application showed its original activity and a saturations point. These parameters are statistically significant.

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SUMMARY

- Domestication of endangered and threatened species, *Crataegus oxycantha.*
- Fruiting at lower altitude, while the crop is of high altitude.
- Standardization of agrotechniques.
- Two new compounds was reported.

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