3. RESULTS & DISCUSSION

Agronomic studies

Experiments were carried out at all the three selected sites to standardize the protocol of cultivation of *A.annua* in different agroclimatic conditions. The effect was assessed through various parameters. Results are presented site wise. Different stages of cultivation of *A.annua* can be observed in fig 3.1.The data on three field experiments conducted on three locations during Rabi seasons of 2010-11 and 2011-12 are presented in the table below (table 3.1 to 3.37). The view of different experiment plots can be seen in figure 3.1 to 3.4.

Experiment 1: Effect of different fertilizer treatments on plant height on *A.annua*.

i. Plant height

Site 1. Ratlam, Madhya Pradesh.

Height (cm)								
	30DAT		60DAT		90DAT		120DAT	
Treatment	I Year	II Year	I Year	II Year	I Year	II Year	I Year	II Year
T1	23.3	30.3	69.33	72.3	119.93	116.5	140.87	153.2
T2	30.06	32.03	90.33	95.3	161.6	169.3	206.3	216.3
T3	27.93	36.93	84.93	89.9	161.06	172.4	218.3	245.6
T4	26.4	30.2	88.53	96.3	161.93	178.3	209.6	234.4
S.Em±	1.4	0.4	1.1	1.7	1.2	1.5	1.4	1.7
C.D. (5%)	NS	1.4	3.9	5.8	4.2	5.3	4.8	6.0

Table 3.1: Effect of different treatments on plant height.
T1= control (no fertilizer use), T2= NPK: 100:60:40 Kg/ha, T3= NPKS: 100:60:40:40 Kg/ha, T4= NPKS: 80:40:40:40 Kg/ha

Site 2. Vadodara, Gujarat.

	Height (cm)							
	30DAT		60DAT		90DAT		120DAT	
Treatment	I Year	II Year	I Year	II Year	I Year	II Year	I Year	II Year
T1	25.3	29.3	65.3	61.2	110.7	102.5	155.2	147.9
T2	31.5	36.4	75.4	80.4	189.7	178.5	252.3	265.2
T3	35.2	40.6	81.4	90.2	195.1	186.2	269.7	278.1
T4	31.7	32.8	80.6	84.3	172.6	180.3	255.1	274.1
S.Em±	0.8	0.8	0.7	1.1	1.1	1.6	2.2	1.4

C.D.(5%)	2.9	2.7	2.5	2.3	3.7	5.5	7.5	5.0

Table 3.2: Effect of different treatments on plant height.

Site 3.Dehradun, Uttarakhand.

Height (cm)								
	30DAT		**60DAT**		**90DAT**		**120DAT**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	30.3	33.6	78.8	76.9	124.6	126.0	160.2	165.3
T2	34.8	38.8	88.8	85.8	178.8	175.9	225.7	235.7
T3	36.8	35.9	85.6	84.9	184.9	187.9	259.8	266.9
T4	33.8	34.5	85.9	86.4	173.4	180.8	247.8	252.8
S.Em±	1.2	1.0	1.8	1.7	2.6	4.2	4.4	5.8
C.D. (5%)	4.2	3.6	6.2	6.0	9.0	14.5	15.2	19.9

Table 3.3: Effect of different treatments on plant height.

Fig 3.1: Different stages of cultivation of *A.annua*

Fig: 3.2: Experimental plot view of *A.annua*

Fig: 3.3: Experimental plot view of *A.annua*

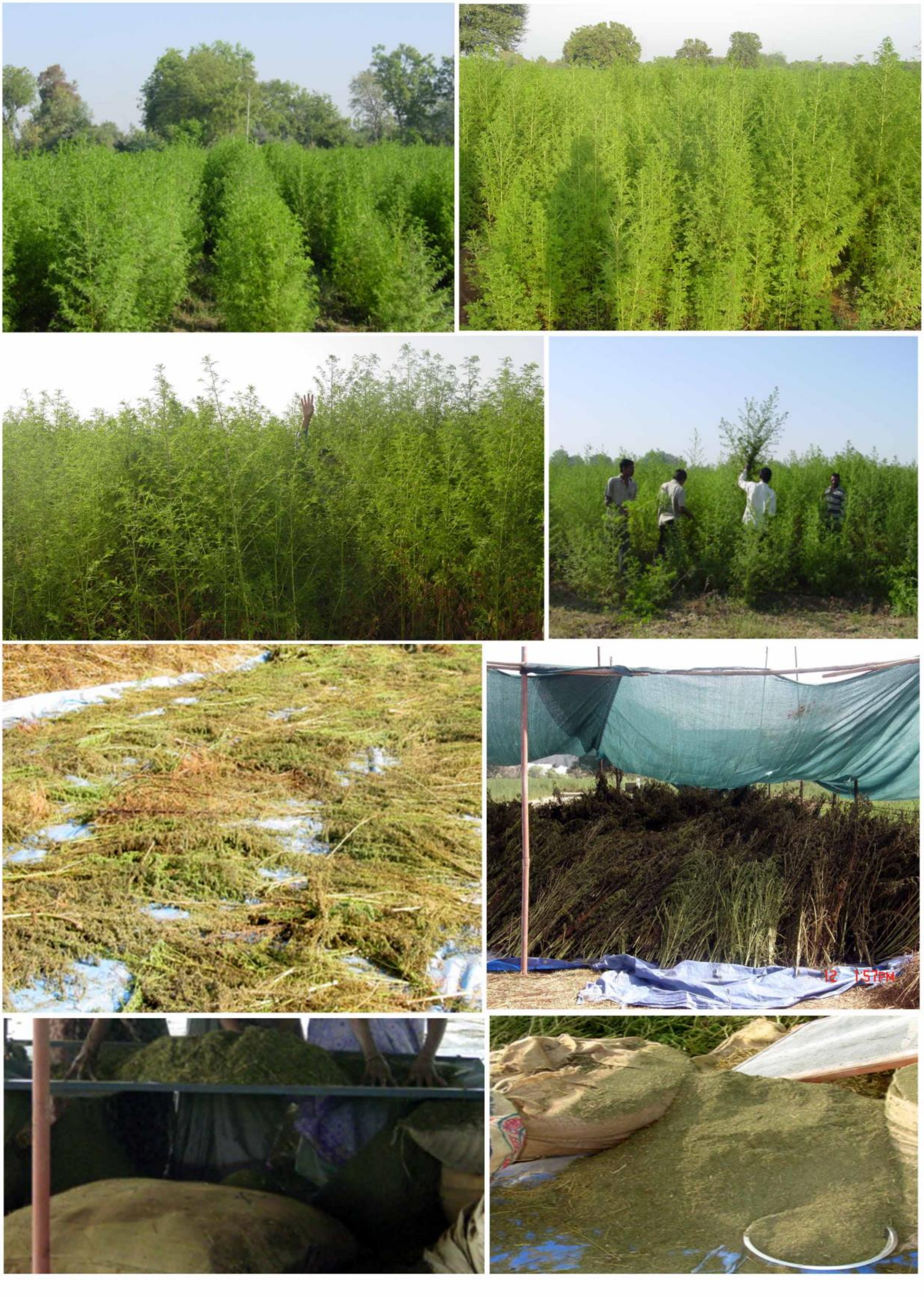


Fig 3.4: Harvesting and drying of *A.annua*

The data on plant height as influenced by four treatments (T1, T2, T3, and T4) at different plant growth stages at Ratlam, Vadodara and Dehradun are presented in Table 3.1, 3.2 and 3.3. Treatment significantly affected plant's final height with standard error of 1.7 having critical different 6 at 120 DAT. Over the period, in all three sites, the highest height was achieved in the plants treated with T3 while the plant height were low in T1 (control) in both years. Also treatment T1 and T2 had significant effect in increasing the plant height in all three sites.

The results confirm that nutrients need to be added proportional if they are to increase plant growth in nutrient limited systems. Boussadia *et al*. (2010) suggests that application of N in excess to the plants does not increase yield or vegetative growth but negatively affect their derived products.

Plant height fluctuated from 140.87 cm to 245.6 cm at Ratlam, 147.9 to 278.1 cm at Vadodara and 160.2 to 278.1 at Dehradun. Baytop (1984), Woerdenbag *et al* (1994), Ferreira and Janik (1996) reported that plant height of the wormwood ranged from 50.0 to 200.0 cm. In the present study, the plants were longer than the earlier studies. There are a number of factors affecting plant height such as different agricultural practices, growing conditions, climatic and soil properties and plant ecotypes. Mert (1999) reported that plant heights of different wormwoods ecotypes grown in Turkey ranged between 177.3 and 217.2 cm. Another study from Turkey reported plant height fluctuation in *A.annua* ranging from 268.0 to 291.3 cm (Ozguven *et al*., 2008)

Results obtained from the study in Ratlam showed plant height at 30 days after transplanting was highest in T2 (I year) and in T3 (II year) and lowest in T1 (I & II year). At 60 days after transplant highest plant height was found to be in T2 (I year), T4 (II year). At 90 DAT, T2, T3 & T4 plants had same height in I year but in second year, it was highest in T4. At 120 DAT, plant height was maximum in T3 in both the year. Height was lowest in T1 which is control. Similar results were obtained in Vadodara and Dehradun, where the range varied from 147.9-278.1 and 160.2-266.9 respectively. Height is crucial component of a plant species's ecological strategy. Plant height is also an important part of a coordinated suite of life history traits including seed mass, time to reproduction, longevity and the number of seeds a plant can produce per year (Moles & Leishman 2008). The height of plant is an important growth character directly linked with the productive potential of plant. An optimum plant height is claimed to be positively correlated with productivity of plant. Saeed (2001) found height of maize plants was maximum in those plants that received 150 kg//ha followed by 100 kg/ha while the shortest plants were those plants without treatment. Present study has maximum proportion of nitrogen and sulphur in treatment 3 which is responsible for increasing the height of the plant. Adediran and Banjoko (2003) observed that maize fail to produce good grain in plots without adequate nutrients. Inorganic fertilizer exerts strong influence on plant growth, development and yield (Stefano *et al* 2004). The availability of sufficient growth nutrients from inorganic fertilizers lead to improved cell activities, enhanced cell multiplication and enlargement and luxuriant growth (Fashina *et al* 2002). Luxuriant growth resulting from fertilizer application leads to larger dry matter production (Obi *et al* 2005) owing better utilization of solar radiation and more nutrient (Saeed *et al* 2001). There was substantial depletion of nutrients with the yields where no NPK fertilizer was applied. The significant increase in plant height reflects the effect of fertilizer nutrients N, P and K

Site 1.

ii. Biomass and Artemisinin content

	Fresh Herb yield T/ha		Dry stem yield T/ha		Dry leaves yield T/ha		% AMS	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	9.4	9.8	3.4	3.60	0.88	0.95	0.64	0.60
T2	27.61	27.9	8.76	9.60	3.14	3.6	0.77	0.71
T3	26.02	28.6	8.91	9.86	3.48	3.9	0.83	0.90
T4	23.82	24.2	7.03	7.59	2.35	2.8	0.76	0.79
S.Em±	1.1	0.3	0.2	0.3	0.1	0.2	0.1	0.1
C.D. (5%)	3.9	1.1	0.7	1.0	0.5	0.6	0.3	0.2

Table 3.4: Effect of different treatment on *A. annua* leaves yield & Artemisinin %

Fresh Herb yield, dry stem and dry yield of *A. annua* (T/ha):-

T1 which is control had fresh yield of 9.8 T/ha while T2 treatment has higher yield of 27.61& 27.9 T/ha for two years. Dry stem yield and dry leaves yield was maximum in T3 (8.91 & 9.86 T/ha) and T2 (8.76 & 9.6 T/ha) as compared with T1 treatment. Artemisinin content was found maximum in T3 treatment followed by T4 and T2.

iii. Yield

	Dry leaf/stem ratio		Leaves harvest index		Artemisinin yield Kg/ha	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	0.25	0.26	20.69	20.56	5.68	5.70
T2	0.35	0.38	26.39	26.39	24.18	25.56
T3	0.39	0.40	28.07	28.09	28.89	35.1
T4	0.35	0.37	25.10	25.05	17.91	22.12
S.Em±	0.0	0.0	1.1	0.6	0.5	0.6
C.D.(5%)	0.2	0.1	4.0	2.1	1.8	1.5

Table 3.5: Effect of fertility levels, and sulphur on quality parameters & Artemisinin yield performance.

Dry leaf to stem ratio and leaf harvest index was highest in T3 and T2 as compared with control. Artemisinin yield was exceptionally high in T3. Standard error was 0.5 and 0.6 with a critical difference of 1.8 and 1.5, which was significant.

Site 2

ii. Biomass and Artemisinin content

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	8.5	8.1	3.1	2.8	0.88	0.81	0.65	0.62
T2	28.3	28.6	9.1	9.5	3.26	3.5	0.75	0.74
T3	29.1	29.4	9.95	10.1	3.75	4.1	0.93	0.89
T4	25.6	26	8.15	8.3	2.93	3.65	0.82	0.81
S.Em±	0.6	0.6	0.2	0.3	0.1	0.1	0.0	0.1
C.D. (5%)	2.0	2.0	0.8		0.4	0.3	0.1	0.2

Table 3.6: Effect of fertility levels, and sulphur on *A. annua* leaves yield & Artemisinin % performance

Fresh herb yield, dry stem yield and dry leaves yield were maximum in plants treated with T3. Other than T3, T2 also showed significant result in increasing the yield. Artemisinin content was also found more in plant treated with T3 followed by T4 and T2 treatments when compared to T1 (control) which was fairly low.

iii. Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	0.28	0.29	22.11	22.44	5.70	5.02
T2	0.36	0.37	26.38	26.92	25.56	25.9
T3	0.38	0.41	27.37	28.87	35.1	36.49
T4	0.36	0.40	26.44	30.54	22.12	29.57
S.Em±	0.0	0.0	0.8	1.5	0.5	0.2
C.D. (5%)	0.1	0.1	2.6	5.0	1.6	0.8

Table 3.7: Effect of different treatments on dry leaf/stem ratio, leaves harvest index and Artemisinin yield performance

Dry leaf/stem ratio was seen more in plants treated with T3 with a minor difference from T2 and T4. The standard error was zero. Leaves harvest index was also maximum in T4. Artemisinin yield was highest in T3 treated plants. The standard error was 0.5 with a critical difference of 1.6.

Site 3

ii. Biomass and Artemisinin content

	Fresh Herb yield T/ha		Dry stem yield T/ha		Dry leaves yield T/ha		% AMS	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	9.8	9.6	3.4	3.6	0.9	0.88	0.61	0.63
T2	28.1	27.5	10.1	10.0	3.8	3.7	0.75	0.79
T3	27.0	26.8	10.4	10.3	4.01	3.8	0.9	0.92
T4	24.7	24.3	9.1	9.4	3.5	3.6	0.81	0.87
S.Em±	0.7	0.7	0.1	0.2	0.1	0.1	0.00	0.00
C.D.(5%)	2.4	2.4	0.4	0.6	0.3	0.5	0.00	0.1

Table 3.8: Effect of fertility levels, and sulphur on *A. annua* growth

Fresh herb yield was highest in T2 followed by T3 while dry stem yield was more in T3 followed by T2 and T4. Standard error was 0.7 and critical difference was 2.4 which were significant. Dry leaves yield was maximum in T3 as compared with other treatments and control.

iii.Yield

	Dry leaf/stem ratio		Leaves harvest index		Artemisinin yield Kg/ha	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	0.26	0.24	20.93	19.64	5.49	5.54
T2	0.38	0.37	27.34	27.01	28.50	29.23
T3	0.39	0.37	27.83	26.95	36.09	34.96
T4	0.38	0.38	27.78	27.69	28.35	31.32
S.Em±	0.2	0.3	0.5	0.8	1.2	0.6
C.D.(5%)	0.5	0.1	0.1		2.3	0.6

Table 3.9: Effect of different treatments on dry leaf/stem ratio, leaves harvest index, Artemisinin yield.

Dry leaf/stem ratio was maximum in all the treatment as compared to T1. Leaves harvest index also had similar result where all the treatments T2, T3, T4 affected the plant in identical way. Artemisinin yield was highest in T3 as compared to other treatments. Standard error was 1.2 with critical difference of 2.3 at 5%. Figure 3.5 showing artemisinin bands on HPTLC plate of three different regions.HPTLC chromatogram of standard artemisinin (R_f: 0.09), Calibration curve of standard artemisinin at 540 nm, HPTLC chromatogram of *A. annua* L leaf sample and 3D view of HPTLC chromatogram can be seen in figure 3.6,3.7 and 3.8 respectively.

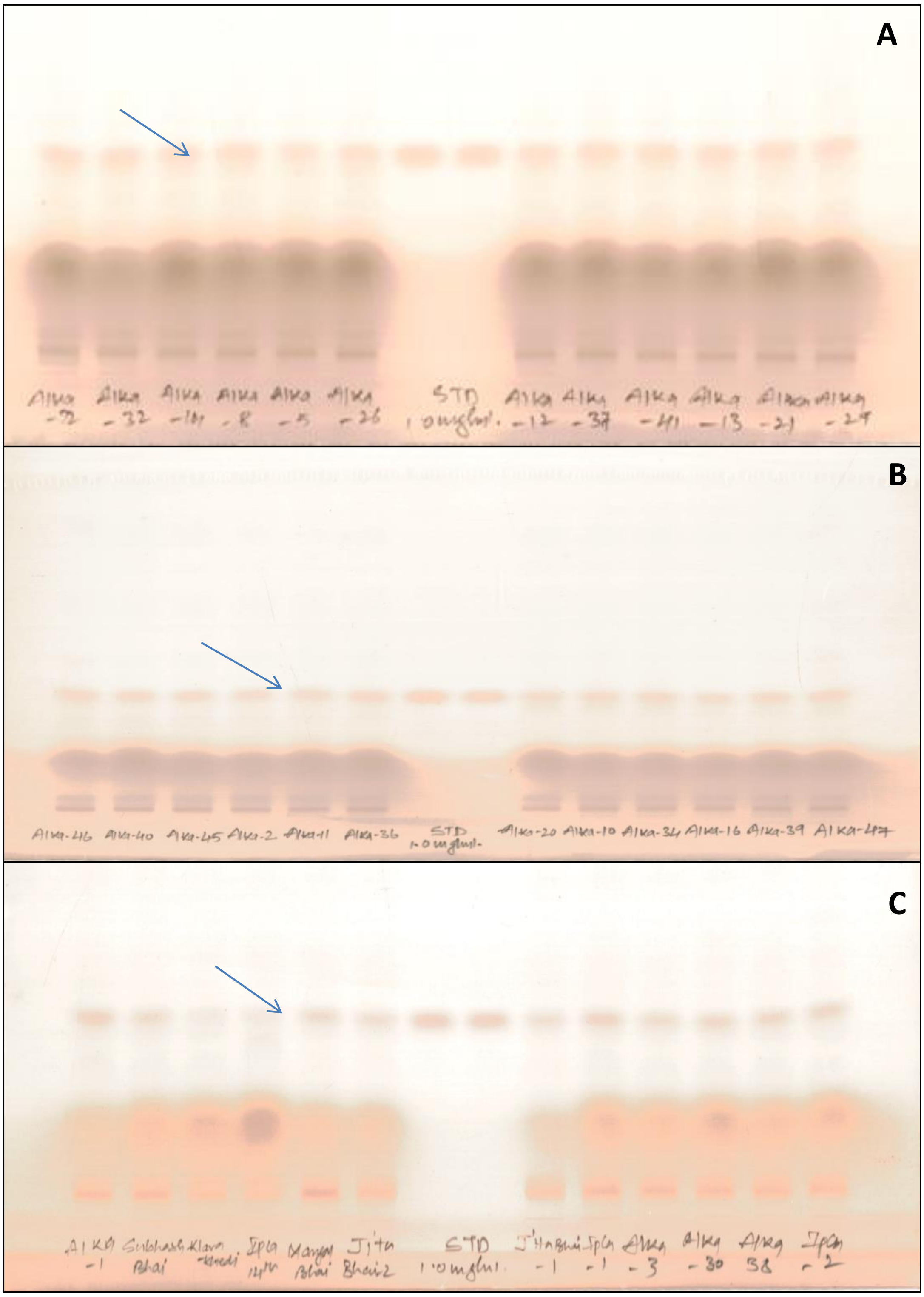


Fig 3.5: HPTLC plate, arrow showing artemisinin bands

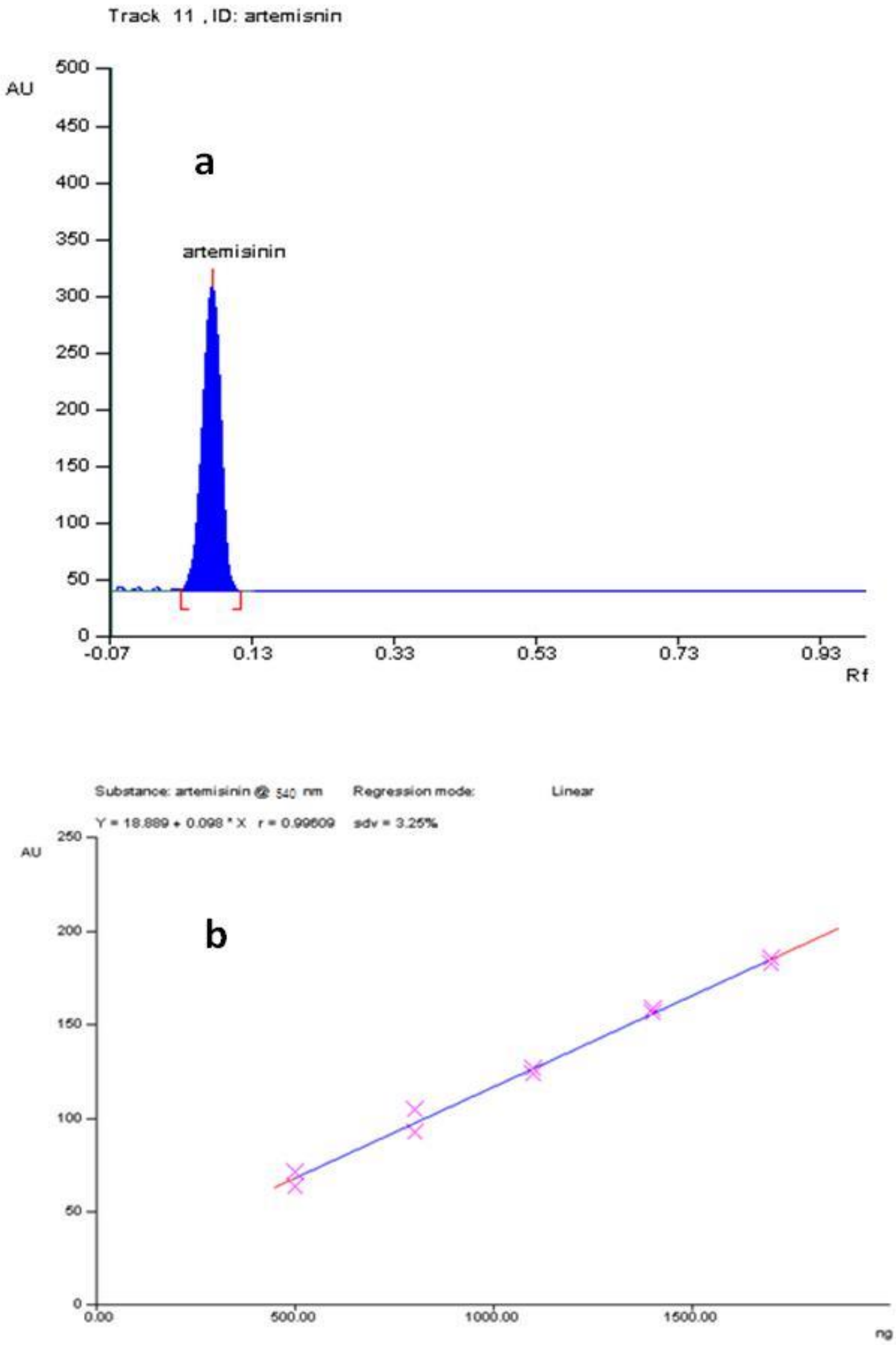


Fig.3.6: (a) HPTLC chromatogram of standard artemisinin (R_f: 0.09)

(b) Calibration curve of standard artemisinin at 540 nm.

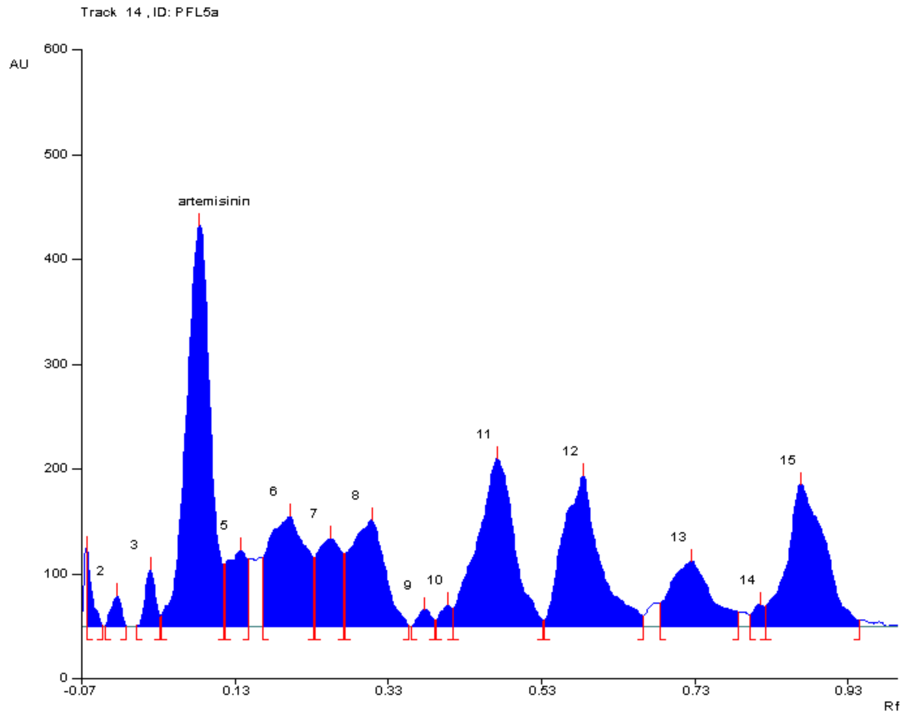


Fig. 3.7: HPTLC chromatogram of *A. annua* L leaf sample.

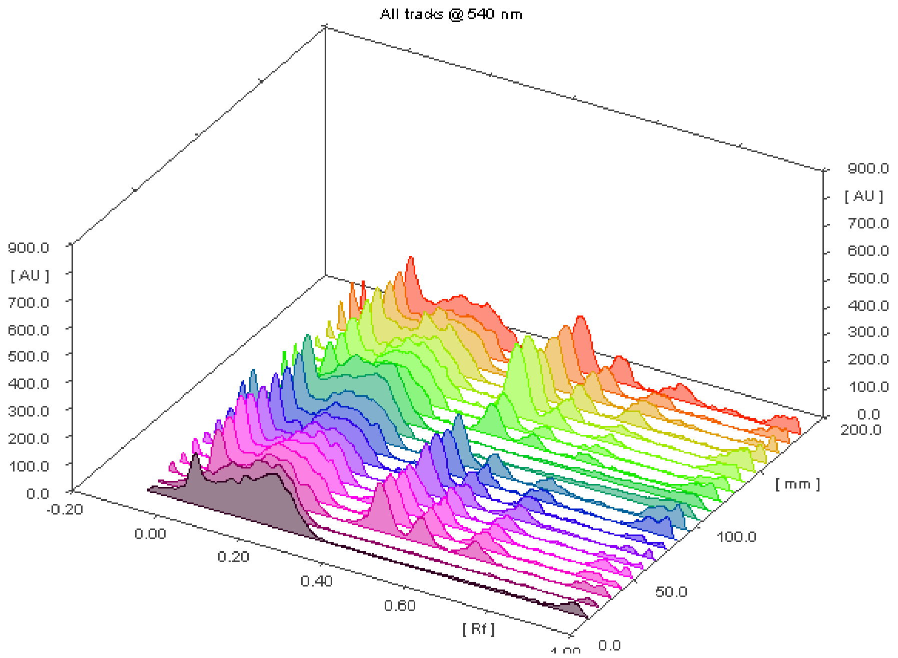


Fig.3.8: 3D view of HPTLC chromatogram of *A. annua* L. leaf extract.

Babatola *et al*., (2002) reported that increasing level of fertilizer application was observed to increased growth and yield of crops. The untreated plants were almost stunted in growth as they had to rely on native soil fertility. Same study also showed that NPK fertilizer application increased the stem girth.

Fresh herb yield was less in control of both the year while it was highest in T2 in I year and T3 II year in Ratlam. At Vadodara maximum was 29.1 T/ha in T3 while in Dehradun 27.5 T/ha was recorded at T2. Ogbomo and Egharevba (2009) reported that fruit yield of tomato can be increased through higher planting density and NPK fertilizer application as the yield per hectare. Sowing date and nitrogen levels had significant effect on grain yield. Carlone and Russell (1987) reported that grain yield was increased by 78.0% as nitrogen rate was increased from 0 to 80 kg/ha.The application of fertilizers is one of the primary methods for improving the availability of soil nutrients to plants. Fertilizing can changes rates of plant growth, maturity time, size of plant parts, phytochemical content of plants (Mevi *et al*., 2003). Stefano *et al* (2001) reported that fertilizer application resulted in luxuriant growth with excessive leaves. Higher number of leaves of fertilizer treated plants contributes to a better canopy and suppression of weeds.

Dry stem yield was recorded highest in T3 in both years as compared to T1 which had lowest yield in the present study. At Ratlam, Vadodara and Dehradun maximum yield was 9.86, 9.95, 10.4 T/ha respectively. Increasing nitrogen rates caused an increase dry herbage yield of wormwood. Ozguven *et al*., (2008) reported dry herbage yields ranged from 8997 and 10,920 kg/ha according to year and nitrogen rates. Mert (1999) reported dry herbage yields were between 15,472 and 26,099 kg/ha. Total dry matter was higher in plants that received 600kg NPK/ha than those that received 0 and 400 kg/ha the dry matter production at 600 kg/ha was higher in total dry matter that at

400, 200 and 0 kg/ha by 11.76%, 19.49% and 115% respectively. Olufolaji *et al*., (2002) in a comparative evaluation on soil and foliar applied fertilizer on growth and yield of *Celosia argentea* reported increased in total dry matter. This may be attributed to NPK being part of the essential nutrients required for the production of the meristematic and physiological activites such as leaves, roots, shoots, dry matter production, etc leading to an efficient translocation of water and nutrients, interception of solar radiation and carbon dioxide.

Dry leaves yield was found fluctuating between 2.8 to 9.86 T/ha with the maximum yield in T3 and lowest in T1. Vadodara and Dehradun had almost same yield with 4.1 and 4 T/ha while Ratlam had 3.9 T/ha. Higher amount of dry leaf implicates to more amount of artemisinin. % Artemisinin was also maximum at T3 in Vadodara (0.93%), as compared to other two sites.

The yield of plants depends upon several production factors. Among these, proper balanced nutrition plays a significant role. Among the nutrient requirements of plants, nitrogen and potassium are two essential elements for plant growth and directly interact and affect the growth and development (Barzegarkhou, 2007.). Nitrogen is considered as one of the essential macronutrients required by plants for their growth, development and yield (Singh *et al*., 2003.). Nitrogen deficiency generally results in stunted growth and chlorotic leaves caused by poor assimilate formation that leads to premature flowering and shortening of the growth cycle. The presence of N in excess promotes development of the above ground organs with abundant dark green tissues of soft consistency and relatively poor root growth. Aminifard *et al*., (2010) with study responses of eggplant to different rates of nitrogen under field conditions were reported that fertilization with 100 kg nitrogen/ha resulted in the highest average fruit weight and fruit yield. Postini and Rostami (2000) while studying physiological and agronomical responses of tobacco to nitrogen fertilizer application were reported that, the nitrogen usage showed significant effect on amount of CGR, NAR, dry leaf yield, nitrogen percentage and income per hectare.

One of the other important macronutrients required by the plants for optimum growth and yield is potassium. Potassium plays a critical role in different physiological and biochemical process in plant. It plays a major role in protein synthesis, ion absorption and transport, photosynthesis and respiration (Barker and Pilbeam, 2007). Farrokh *et al*., (2011) reported effect of nitrogen application of dry leaf yield, wet stalk yield; total dry weight and total wet weight were significant.

Suphur is an important nutrient and one of the key macroelement essential for optimal plant growth. Sulphur is taken up from the soil solution by the plant in the sulphate form (SO_4^{2-}). It is a component of methionine, cysteine and cystine, three of the 21 amino acids which are the essential building blocks of proteins. Sulphur is also a component of key enzymes and vitamins in the plant and is necessary for the formation of chlorophyll. In legumes sulphur is necessary for the efficient fixation of nitrogen by the plant.

The use of inorganic fertilizers on crops increased yield as Adediran and Banjoko (2003) observed that there was substantial depletion of nutrients with the yields where no NPK fertilizer was applied. Inorganic fertilizers exert strong influence on plant growth, development and yield (Stefano *et al*., 2004).

Experiment 2:- Effect of plant population density on *A.annua* growth

i. Plant height

Density affects the height of the plant. *A.annua* plants were grown at different densities from T1 to T6 to assess the best result. At 30DAT the height was maximum in T6 while lowest was found in T3. At 60DAT, height increased at T5 compared to T6. At 90and 120DAT, it continue to increase at T5. The standard error was 0.48& 0.77 with a critical difference of 1.53 and 2.44.At site 2, maximum height at 30DAT was observed in T2 and T3 in first year which got decreased in second year, but T6 had constant increase in height. At 60, 90 and 120 DAT, height was found to be increased at T5.At site 3 also, initially the height was more in T6 at 30DAT but increased in T5 at later stages i.e. 60, 90 and 120DAT. The standard error was 3.67 & 2.26 with a critical difference of 11.5 and 7.12.

Site 1.

Height (cm)								
	30DAT		**60DAT**		**90DAT**		**120DAT**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	48.00	56.3	77.80	85.30	121.20	130.7	180.8	177.6
T2	50.6	58.9	77.60	86.90	127.6	135.1	210.5	215.9
T3	40.8	47.9	70.80	80.30	131.4	139.5	247.0	249.6
T4	49.6	47.6	93.20	98.60	161.0	172.5	278.5	289.2
T5	55.3	52.0	115.40	130.60	181.2	180.3	286.3	320.6
T6	59.6	57.3	106.20	115.70	172.0	186.4	278.6	298.7
S.Em±	0.51	0.41	0.70	0.46	0.84	0.31	0.48	0.77
C.D.(5%)	1.61	1.31	2.22	1.44	2.66	0.98	1.53	2.44

Table 3.10: Effect of plant population density on *A. annua* plant height.
[T1 (100X100 cm), T2 (75 X 75cm), T3 (60 X 60 cm), T4 (45 X 45 cm), T5(30 X 30 cm), T6(50 X 30 cm)]

Site 2.

Height (cm)								
	30DAT		60DAT		90DAT		120DAT	
Treatment	I Year	II Year	I Year	II Year	I Year	II Year	I Year	II Year
T1	52.3	51.3	89.60	81.30	148.6	140.2	200.6	215.3
T2	59.6	52.9	90.40	96.20	155.9	156.3	211.3	234.5
T3	58.6	55.3	97.60	105.30	164.3	169.4	245.0	265.2
T4	51.6	56.3	106.80	115.30	173.0	185.7	269.3	278.3
T5	55.3	54.2	150.70	165.30	230.6	221.3	306.5	315.3
T6	57.7	55.0	147.30	152.40	210.1	192.3	297.3	296.1
S.Em±	0.83	0.61	0.67	0.43	0.37	0.20	0.38	0.12
C.D. (5%)	2.62	0.87	2.11	0.61	1.17	0.29	1.20	0.17

Table 3.11: Effect of plant population density on *A. annua* plant height.

Site 3.

Height (cm)								
	30DAT		60DAT		90DAT		120DAT	
Treatment	I Year	II Year	I Year	II Year	I Year	II Year	I Year	II Year
T1	60.3	62.5	95.3	96.3	160.3	162.3	210.3	215.6
T2	60.5	65.3	101.2	103.6	165.4	169.2	233.6	240.3
T3	67.3	69.3	110.3	115.3	174.3	179.6	260.1	269.2
T4	59.2	62.3	119.6	124.6	185.3	191.3	280.3	291.3
T5	65.2	68.9	168.3	171.6	240.3	248.7	315.3	329.8
T6	69.3	70.2	166.9	170.3	230.3	240.6	305.2	315.4
S.Em±	1.98	2.56	2.78	3.95	3.38	1.66	3.67	2.26
C.D. (5%)	6.24	8.06	8.77	12.44	10.66	5.22	11.57	7.12

Table 3.12: Effect of plant population density on *A. annua* plant height.

ii. Yield and artemisinin content

Site 1: Fresh herb yield, dry stem yield and dry leaves yield was found to be maximum in T5. Artemisinin content was ranging from 0.79% to 0.90, highest content was found in T6. The standard error was 0.31 & 0.35 with a critical difference of 0.98 and 1.10.

Dry leaf/stem ratio was maximum in T3 and T6 followed by T2. Leaves harvest index was seen highest in T6 while artemisinin yield was found highest in T5. Site 2: Fresh herb yield, dry stem yield and dry leaves yield was found to be maximum in T5. Artemisinin content varied from 0.81 to 0.89%, highest found in T3.Dry leaf/stem ratio and leaves harvest index was found maximum in T6. Artemisinin yield was found low in T1 and high in T5. Site 3: Fresh herb yield, dry stem yield and dry leaves yield were maximum in T5. Artemisinin content was more in T1 followed by T2. Dry leaf/stem ratio and leaves harvest index was highest in T5 followed by T6. Artemisinin yield was maximum in T5 as compared with other densities. The standard error was 0.67 & 0.53 with a critical difference of 2.11 and 1.68.

Site 1.

i.Biomass and Artemisinin content

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	7.95	8.20	1.96	1.95	1.03	1.01	0.86	0.81
T2	12.32	13.10	3.17	3.17	1.67	1.45	0.86	0.79
T3	13.80	13.90	4.40	4.40	2.36	2.14	0.82	0.85
T4	23.90	24.60	5.20	5.19	2.71	2.72	0.85	0.84
T5	29.63	30.80	7.95	7.95	4.15	4.40	0.84	0.86
T6	26.76	27.70	6.34	6.34	3.41	3.71	0.83	0.87
Mean	19.06	19.72	4.48	4.83	2.56	2.21	0.84	0.84
S.Em±	0.31	0.35	0.31	0.31	0.12	0.13	0.07	0.05
C.D.(5%)	0.98	1.10	0.98	0.99	0.39	0.42	NS	NS

Table 3.13. Effect of plant population density on *A. annua* yield & Artemisinin % performance

ii.Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	II Year
T1	0.53	0.53	34.45	34.56	8.86	8.34
T2	0.53	0.53	34.50	34.50	14.36	13.19
T3	0.54	0.54	34.91	34.91	19.35	20.06
T4	0.52	0.52	34.26	34.39	23.04	22.85
T5	0.52	0.52	34.30	34.30	34.86	35.69
T6	0.54	0.54	34.97	34.97	28.30	29.67

S.Em±	0.04	0.04	0.45	0.47	0.67	0.53
C.D.(5%)	NS	0.12	NS	1.49	2.11	1.68

Table 3.14. Effect of plant population density on quality parameters & Artemisinin yield performance.

Site 2.

i.Biomass and Artemisinin content

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	13.95	13.51	2.18	2.35	1.09	1.30	0.89	0.83
T2	21.90	22.42	3.35	3.25	1.87	2.01	0.86	0.85
T3	22.50	22.86	4.55	4.32	2.44	2.65	0.85	0.89
T4	41.40	40.60	5.84	5.65	2.95	3.21	0.87	0.81
T5	48.90	43.20	8.19	7.16	4.48	4.12	0.84	0.85
T6	44.55	41.80	6.49	6.10	3.59	3.62	0.83	0.86
S.Em±	0.36	0.26	0.10	0.19	0.14	0.15	0.02	0.05
C.D. (5%)	0.51	0.37	0.13	0.27	0.20	0.21	0.03	0.07

Table 3.15. Effect of plant population density on yield and artemisinin %

ii.Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	0.50	0.55	33.33	35.62	9.70	10.79
T2	0.56	0.62	35.82	38.21	16.08	17.08
T3	0.54	0.61	34.91	38.02	20.74	23.58
T4	0.51	0.57	33.56	36.23	25.67	26.00
T5	0.55	0.58	35.36	36.52	37.63	35.02
T6	0.55	0.59	35.62	37.24	29.80	31.13
S.Em±	0.04	0.06	1.02	0.67	0.24	0.42
C.D.(5%)	0.05	0.09	1.45	0.95	0.34	0.60

Table 3.16. Effect of plant population density on quality parameters & Artemisinin yield performance.

Site 3.

i.Biomass and Artemisinin content

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	13.95	14.25	2.18	2.22	1.09	1.12	0.82	0.90
T2	21.9	21.60	3.35	3.43	1.87	1.09	0.80	0.88
T3	22.5	23.55	4.55	4.66	2.44	2.53	0.86	0.84
T4	41.4	42.15	5.84	5.93	2.94	3.01	0.83	0.86
T5	48.9	49.8	8.19	8.23	4.48	4.62	0.87	0.80

T6	44.5	45.6	6.48	6.57	3.58	3.64	0.85	0.84
S.Em±	0.23	0.14	0.16	0.20	0.12	0.20	0.17	0.15
C.D. (5%)	0.41	0.38	0.13	0.23	0.34	0.21	0.11	0.11

Table 3.17.Effect of plant population density on *A. annua* leaves yield & Artemisinin % performance.

ii.Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
T1	0.50	0.50	33.33	33.53	8.94	10.08
T2	0.56	0.32	35.82	24.12	14.96	9.59
T3	0.54	0.54	34.91	35.19	20.98	21.25
T4	0.50	0.51	33.49	33.67	24.40	25.89
T5	0.55	0.56	35.36	35.81	38.98	36.96
T6	0.55	0.55	35.59	35.65	30.43	30.58
S.Em±	0.21	0.22	0.45	0.41	0.39	0.32
C.D.(5%)	0.14	0.11	0.25	0.22	0.18	0.20

Table 3.18. Effect of plant population density on quality parameters & artemisinin yield performance.

Plant height was recorded for plant population density of *A.annua*. Location Ratlam had maximum height of 320.6 cm at T5 (30x30cm), Vadodara had maximum height 315.3 cm at T5 (30x30) and Dehradun showed highest height 329.8 cm at T5. The spacing in T5 can be concluded to be the most appropriate for maximum height in short span of time.

Effect of plant population density on quality parameters like dry leaf/stem ratio and leaf harvest index was found maximum in 50x30 plots while artemisinin yield kg/ha was maximum (35.69 kg/ha) in 30x30 plot at Ratlam (table). At Vadodara, dry leaf/stem ratio was maximum at T2 (75x75 cm), leaves harvest index was maximum at T6 while artemisinin yield was more in T5. Dehradun had dry leaf/stem ratio, leaves harvest index and artemisinin yield more in T5 (30x30cm).

Fresh herb yield, dry stem yield, dry leaves yield and %AMS were evaluated for observing the effect of plant population density. In Ratlam, Vadodara and Dehradun fresh herb yield, Dry stem yield and Dry leaves yield were found maximum in T5.

Percent artemisinin content was different at different locations. It was maximum in T5 (30x30cm) in Ratlam, in T2 (75x75cm) in Vadodara and T1 (100x100cm) in Dehradun.

Density had significant effect on plant height in *A.annua*. Initial height at 30DAT in Ratlam was maximum in T6 plants which was more than T5 plants. At 60DAT, the height of T5 plants increases. 90 DAT and 120 DAT also the height of T5 plants is more as compared to other plots. Vadodara location had similar observations where initial height at 30DAT is more in T2 plants followed by T3 plants and T6 plants. After 60DAT all T5 plants were found to gain more height. Dehradun location had a different pattern, where T6 plants had maximum height at 30DAT followed by T5 and T3 while 60 to 120DAT had maximum values in T5 plants.

Dry leaf/stem ratio was found maximum in T6 (50x30) at Ratlam, in T2 at Vadodara and in T2 & T5 at Dehradun. Leaves harvest index was more in T6 at Ratlam and Dehradun while in Vadodara, it was found maximum in T2. Artemisinin yield was found maximum in T5 at all the three locations.

Density had significant effects on grain yield, plant height, number of kernels/ear, the number of grains row, number of grains/ear row, harvest index, number of cobs/plant, cob length, ear diameter and stem diameter. (Sharifi, Sedghi and Gholipouri, 2009). Here also plant density significantly increased the plant height.

The effect of plant spacing and number of seedlings per hill in rice was significant in 1% probability level and highest grain yield was found from 15x15 cm treatment (Bozorgi *et al* 2011). Plant density is one of the most important cultural practices determining grain yield, as well as other important agronomic attributes of this crop. Stand density affects plant architecture, alters growth and developmental patterns and influences carbohydrate production and partition (Casal, 1985). Maize is more sensitive to variations in plant density than other members of the grass family (Almeida & Sangoi, 1996). The spacial distribution of plants in a crop community is an important determinant of yield. However, the mechanisms responsible for the observed responses to changing plant density are not well understood. Plant densities providing interplant competition, the rate of yield increase was reduced. Yield of the indeterminate cultivar increased as plant density increased above the density required for 95% isolation interception at growth stage R5 (Egli, 1988). It was found that the grain yield of the late maturing hybrid was highest under the low plant density and that of an early maturing hybrid was highest under the high density. The superiority of low density in the late maturing hybrid and of high density in the early maturing hybrid was also consistent regarding yield per plant, number of heads per plant and number of grains per panicle. The highest yield in the experiment was obtained with the earliest maturing hybrid planted at the densest plant population.

Experiment. 3

Effect of growth regulators and stress on *A. annua* growth.

i.Plant height

Site 1.

Height (cms)								
	30DAT		**60DAT**		**90DAT**		**120DAT**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	31.86	25.30	99.33	88.60	169.47	175.90	210.30	215.30
Gibberlic Acid	33.13	36.20	90.06	86.29	164.27	171.20	208.60	218.60
Gibberlic Acid+ Indole Acetic Acid	30.53	36.50	96.40	90.40	163.13	172.50	212.30	225.30
Chitosan	30.53	31.20	91.60	92.27	169.20	178.90	218.90	216.20
Methyl Jasmonate	28.95	29.20	93.06	96.20	168.20	176.20	225.60	236.30
Acetyl Salicylic Acid	30.53	27.30	95.73	94.30	163.73	170.20	220.30	222.30

Lead acetate	32.60	29.30	97.26	99.50	171.06	174.20	212.80	218.60
Sodium Chloride	30.26	26.90	96.33	97.50	174.20	180.20	218.60	217.90
Nanozime	31.60	33.50	99.66	96.30	171.06	174.20	222.70	219.30
Pushak	30.73	34.20	92.93	98.30	152.20	169.20	217.30	210.60
Fantac	29.93	35.30	94.33	97.10	153.13	170.60	214.40	215.30
Control	31.13	28.10	93.40	85.00	167.53	173.90	207.30	209.3
S.Em±	0.67	0.48	1.99	1.44	1.85	1.14	1.23	2.43
C.D.(5%)	NS	1.52	NS	4.53	5.83	1.31	4.62	7.67

Table 3.19.Effect of growth regulators and stress on *A.annua* growth

At 30 DAT, height was seen to increase in treatment with Gibberellic acid and lead acetate, while other were either had similar values as that of control or showed slow growth in height. Abcissic acid, chitosan, methyl jasmonate, acetyl salicylic acid, sodium chloride treatment showed less height than in control. The standard error was 0.67& 0.48 with a critical difference of 1.52. At 60 DAT, the height increased in treatment of abcissic acid, gibberellic acid+indole acetic acid, methyl jasmonate, acetyl salicylic acid, lead acetate, sodium chloride, nanozime, pushak and fantac while in others, the height was less as compared to control. The standard error was 1.99 & 1.44. At 90DAT, abcissic acid, chitosan, sodium chloride and lead acetate had maximum height. At 120 DAT, height in all treatments increased when compared to control, with the maximum height with methyl jasmonate treatment. The standard error was 1.23& 2.43 with a critical difference of 4.62 and 7.67, which was significant.

Site 1.

ii. Biomass and Artemisinin content

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	25.68	26.20	9.83	10.20	2.74	2.60	0.75	0.79
Gibberellic Acid	28.97	29.60	11.30	11.60	3.31	3.50	0.73	0.78
Gibberlic Acid+ Indole	28.15	29.40	8.96	8.50	2.70	2.80	0.75	0.81

Acetic Acid								
Chitosan	28.31	29.10	9.58	9.80	3.24	3.60	0.71	0.74
Methyl Jasmonate	26.83	28.00	9.69	10.24	3.47	3.80	0.84	0.90
Acetyl Salicylic Acid	25.02	25.80	9.59	9.80	3.29	3.40	0.74	0.78
Lead acetate	26.34	27.10	9.32	9.20	3.13	3.20	0.81	0.83
Sodium Chloride	28.31	28.90	8.91	9.30	2.99	3.20	0.78	0.81
Nanozime	25.02	25.30	9.70	9.60	2.89	2.70	0.79	0.70
Pushak	22.71	23.20	8.39	8.60	2.69	2.90	0.68	0.71
Fantac	21.23	22.10	7.07	8.90	2.47	2.50	0.73	0.69
Control	19.75	20.30	6.98	8.50	2.22	2.30	0.66	0.65
S.Em±	0.56	0.41	0.29	0.23	0.33	0.20	0.05	0.05
C.D.(5%)	1.77	NS	0.91	0.73	1.04	0.64	0.16	0.16

Table 3.20.Effect growth regulators and stress on *A.annua* leaves yield & Artemisinin % performance.

Fresh herb yield was found maximum in plants treated with gibberellic acid+indole acetic acid. The standard error was 0.56 & 0.4.Dry stem yield was more in plants treated with gibberellic acid followed by methyl jasmonate and abscisic acid. The standard error was 0.29 & 0.23 with a critical difference of 0.91 and 0.73. Dry leaves yield was seen more in chitosan, gibberellic acid and acetyl salicylic acid. Artemisinin content was more in all treatments in comparison to control and was highest in plants treated with methyl jasmonate. The standard error was 0.05 with a critical difference of 0.16.

iii.Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	0.28	0.25	21.80	20.31	20.56	20.54
Gibberlic Acid	0.29	0.30	22.66	23.18	24.17	27.30
Gibberlic Acid+ Indole Acetic Acid	0.30	0.33	23.16	24.78	20.28	22.68
Chitosan	0.34	0.37	25.27	26.87	22.98	26.64
Methyl Jasmonate	0.36	0.37	26.37	27.14	29.15	34.20

Acetyl Salicylic Acid	0.34	0.35	25.54	25.76	24.34	26.52
Lead acetate	0.34	0.35	25.14	25.81	25.33	26.56
Sodium Chloride	0.34	0.34	25.13	25.60	23.34	25.92
Nanozime	0.30	0.28	22.95	21.95	22.81	18.90
Pushak	0.32	0.34	24.69	25.22	18.29	20.59
Fantac	0.35	0.28	25.86	21.93	18.05	17.25
Control	0.32	0.27	24.13	21.30	14.75	14.95
S.Em±	0.03	0.03	0.04	0.87	0.72	0.40
C.D.(5%)	0.08	NS	1.25	NS	2.27	1.25

Table 3.21.Effect of growth regulators and stress on quality parameters & Artemisinin yield performance

Dry leaf/stem ratio was highest was low in abscisic acid, gibberellic acid, nanozime and GA+IAA treatment than in control while it was more in all other treatments. Leaf harvest index was maximum in methyl jasmonate followed by acetyl salicylic acid, lead acetate and sodium chloride. Artemisinin yield increased in all treatments, with the highest in methyl jasmonate treated plants.

i.Plant height

Site 2.

Height (cm)								
	30DAT		**60DAT**		**90DAT**		**120DAT**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	23.40	25.30	79.60	76.30	148.90	155.30	259.30	248.30
Gibberlic Acid	34.50	26.30	73.60	74.20	150.30	169.20	263.10	252.40
Gibberlic Acid+ Indole Acetic Acid	24.90	24.90	77.60	77.90	169.20	164.20	269.20	255.10
Chitosan	29.30	26.60	72.70	73.10	165.30	166.30	265.10	251.60
Methyl	35.00	28.40	89.50	83.60	179.20	174.20	275.10	260.80

Jasmonate								
Acetyl Salicylic Acid	31.20	29.30	84.30	73.20	159.60	169.20	262.30	268.30
Lead acetate	33.40	25.90	71.20	76.00	142.30	155.30	248.50	250.60
Sodium Chloride	32.50	26.70	76.50	72.30	146.88	161.30	249.90	260.30
Nanozime	28.60	27.20	77.00	73.20	144.10	165.30	241.70	259.20
Pushak	26.50	25.30	69.30	73.20	140.30	169.30	238.50	272.00
Fantac	25.30	25.80	67.20	74.20	135.20	163.20	225.10	265.10
Control	20.30	24.80	61.00	69.20	120.60	139.30	215.80	237.90
S.Em±	0.77	0.41	2.98	2.61	3.53	1.98	1.30	0.67
C.D. (5%)	0.08	1.31	NS	NS	NS	NS	NS	NS

Table 3.22. Effect of growth regulators and stress on *A.annua* growth

At all the stages, height of the plant was distinctly maximum in methyl jasmonate. Other treatments which increased the height of the plant were abscisic acid, gibberellic acid, chitosan, acetyl salicylic acid, lead aceatate, sodium chloride and nanozime.

Site 2.

ii.Biomass and Artemisinin content

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	26.70	26.90	10.80	11.20	3.74	3.60	0.73	0.72
Gibberlic Acid	29.60	28.60	12.20	12.60	3.80	3.30	0.79	0.75
Gibberlic Acid+ Indole Acetic Acid	29.30	28.10	9.90	10.30	3.10	3.50	0.75	0.70
Chitosan	29.50	27.90	10.80	11.10	3.90	3.40	0.79	0.79
Methyl Jasmonate	28.90	29.30	10.50	12.30	4.20	3.80	0.92	0.90
Acetyl Salicylic Acid	26.50	27.60	10.20	11.30	3.60	3.00	0.80	0.81

Lead acetate	27.20	26.20	9.80	10.60	3.60	2.80	0.72	0.76
Sodium Chloride	28.60	26.30	9.40	10.70	3.40	3.10	0.76	0.71
Nanozime	26.00	26.50	10.20	10.10	3.20	3.30	0.68	0.69
Pushak	23.60	24.20	9.20	9.90	3.10	3.20	0.68	0.70
Fantac	22.90	23.60	8.90	8.80	2.90	2.90	0.65	0.67
Control	22.50	23.20	7.60	8.10	2.60	2.60	0.61	0.65
S.Em±	0.30	0.36	0.18	0.20	0.14	0.17	0.04	0.03
C.D. (5%)	NS	NS	0.57	0.64	0.43	0.54	0.13	0.09

Table 3.23. Effect growth regulators and stress on *A. annua* leaves yield & Artemisinin % performance.

Fresh herb yield was found maximum in methyl jasmonate, gibberellic acid and chitosan. Dry stem yield was also maximum in methyl jasmonate followed by chitosan and acetyl salicylic acid.

iii.Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	I Year	II Year	I Year	II Year	I Year	II Year
Abscisic Acid	0.35	0.32	25.72	24.32	27.30	25.92
Gibberlic Acid	0.31	0.26	23.75	20.75	30.02	24.75
Gibberlic Acid+ Indole Acetic Acid	0.31	0.34	23.85	25.36	23.25	24.50
Chitosan	0.36	0.31	26.53	23.45	30.81	26.86
Methyl Jasmonate	0.40	0.38	28.57	27.60	38.64	34.20
Acetyl Salicylic Acid	0.35	0.27	26.09	20.98	28.80	24.30
Lead acetate	0.37	0.26	26.87	20.90	25.92	21.28
Sodium Chloride	0.36	0.29	26.56	22.46	25.84	22.01
Nanozime	0.31	0.33	23.88	24.63	21.76	22.77
Pushak	0.34	0.32	25.20	24.43	21.08	22.40
Fantac	0.33	0.33	24.58	24.79	18.85	19.43
Control	0.34	0.32	25.49	24.30	15.86	16.90
S.Em±	0.03	0.02	0.39	0.46	0.52	0.07
C.D.(5%)	NS	NS	NS	NS	1.64	0.21

Table 3.24.Effect of growth regulators and stress on quality parameters & Artemisinin yield performance

Dry leaf/stem ratio and leaves harvest index was maximum in methyl jasmonate while the lowest values were found in gibberellic acid. Artemisinin yield was also highest in methyl jasmonate.

i.Plant height

Site 3.

At 30DAT, height of the plant was maximum in methyl jasmonate followed by acetyl salicylic acid. At 60, 90 and 120 DAT also height was seen to increase in Methyl jasmonate. All other treatments were also found effective in increasing the height of the plant.

Fresh herb yield was found more in Gibberellic acid, Gibberellic acid+indole acetic acid and sodium chloride. Dry stem yield was also maximum in Gibberellic acid treated plants. But dry leaves yields were found more in methyl jasmonate and chitosan. Artemisinin content ranged from 0.65 to 0.89, with the maximum content in methyl jasmonate treatment.

Height (cm)								
	30DAT		**60DAT**		**90DAT**		**120DAT**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	25.3	27.8	82.3	85.2	151.7	158.5	268.1	272.3
Gibberlic Acid	36.4	34.3	80.2	86.1	158.2	162.4	271.2	275.2
Gibberlic Acid+ Indole Acetic Acid	26.4	29.2	81.1	84.2	175.1	182.2	278.5	281.6
Chitosan	32.1	35.1	79.0	83.1	169.3	171.2	277.2	283.1
Methyl	38.2	40.2	92.3	95.5	184.3	183.4	281.3	290.3

Jasmonate								
Acetyl Salicylic Acid	35.3	40.1	89.3	90.4	163.2	170.1	275.3	282.1
Lead acetate	31.2	35.4	75.2	81.2	150.1	155.0	255.2	265.3
Sodium Chloride	30.3	33.2	79.1	86.3	149.4	154.3	251.1	265.3
Nanozime	27.9	32.1	83.1	88.2	155.3	159.3	255.2	268.3
Pushak	29.3	35.2	70.3	77.5	148.1	152.1	238.3	250.3
Fantac	27.3	30.5	71.1	76.1	145.3	150.3	235.1	245.1
Control	22.3	28.1	68.2	74.2	125.2	130.2	225.9	230.3
S.Em±	0.54	1.2	0.21	0.51	0.61	2.2	2.5	0.4
C.D.(5%)	2.1	1.3	0.2	1.4	0.9	1.1	0.42	1.6

Table 3.25.Effect of growth regulators and stress on *A.annua* growth

Site 3.

ii.Biomass and Artemisinin content

Dry leaf/stem ratio and leaves harvest index was highest in methyl jasmonate. Artemisinin yield was maximum in methyl jasmonate and chitosan. Other treatments also had a positive effect in increasing the artemisinin yield**.**

	Fresh Herb yield T/ha		**Dry stem yield T/ha**		**Dry leaves yield T/ha**		**% AMS**	
Treatment	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**	**I Year**	**II Year**
Abscisic Acid	28.3	28.6	11.1	11.3	3.9	3.82	0.79	0.76
Gibberlic Acid	31.9	31.5	13.1	12.8	3.7	3.72	0.75	0.73
Gibberlic Acid+ Indole Acetic Acid	31.0	30.9	10.2	10.1	3.3	3.6	0.71	0.72
Chitosan	31.1	30.7	11.4	11.2	4.0	4.1	0.80	0.83
Methyl Jasmonate	30.4	31.3	10.6	10.5	4.2	4.5	0.85	0.89
Acetyl Salicylic Acid	27.5	28.6	10.4	10.44	3.8	3.72	0.81	0.85
Lead	28.9	28.2	10.0	10.2	3.6	3.71	0.69	0.70

acetate								
Sodium Chloride	31.3	30.9	9.5	9.4	3.3	3.5	0.70	0.73
Nanozime	27.7	28.1	10.3	10.4	3.4	3.5	0.73	0.71
Pushak	25.0	26.2	9.3	9.5	3.2	3.0	0.71	0.72
Fantac	23.2	23.4	9.2	9.3	3.0	3.1	0.70	0.71
Control	21.6	22.0	8.1	8.3	2.9	2.85	0.65	0.69
S.Em±	0.22	0.15	0.41	0.5	0.61	0.5	0.24	0.21
C.D.(5%)	1.2	1.4	1.6	1.5	2.1	1.4	0.5	1.1

Table 3.26. Effect growth regulators and stress on *A.annua* leaves yield & Artemisinin % performance

iii.Yield

	Dry leaf/stem ratio		**Leaves harvest index**		**Artemisinin yield Kg/ha**	
Treatment	I Year	II Year	I Year	II Year	I Year	II Year
Abscisic Acid	0.35	0.34	26.0	25.26	30.81	29.03
Gibberlic Acid	0.28	0.29	22.02	22.52	27.75	27.16
Gibberlic Acid+ Indole Acetic Acid	0.32	0.36	24.44	26.28	23.43	25.92
Chitosan	0.35	0.37	25.97	26.80	32.0	34.03
Methyl Jasmonate	0.40	0.43	28.38	3.0	35.7	40.05
Acetyl Salicylic Acid	0.37	0.36	26.76	26.67	30.78	31.62
Lead acetate	0.36	0.36	26.58	26.67	24.98	25.97
Sodium Chloride	0.35	0.37	25.78	27.13	23.1	25.55
Nanozime	0.31	0.34	23.70	235.18	23.36	24.85
Pushak	0.32	0.32	24.39	24.0	21.30	21.60
Fantac	0.32	0.33	23.97	25.0	20.30	22.01
Control	0.32	0.34	24.30	25.56	16.90	19.38
S.Em±	0.5	0.12	0.5	0.32	0.62	0.31
C.D.(5%)	1.2	1.5	0.5	0.13	2.1	1.1

Table 3.27.Effect of growth regulators and stress on quality parameters & Artemisinin yield performance

Effect of growth regulators like abscisic acid, GA3, indole acetic acid, chitosan, methyl jasmonate, and acetyl salicylic acid was recorded on different parameters of *A.annua* growth. At Ratlam, height was maximum in plants treated with abscisic acid at 30, 60 DAT while at 90 DAT height raised in chitosan treated plants. At 120 DAT Methyl jasmonate treated plants were found with maximum height (table 3.19, 3.22 & 3.25). At Vadodara, height was maximum in methyl jasmonate treated plants at 30DAT, 60DAT, 90DAT and 120DAT. Dehradun had maximum height in Gibberlic acid + indole acetic acid 30DAT while at 60DAT & 90DAT the plant height increased in abscisic acid and sodium chloride respectively.

Fresh herb yield at Ratlam was found highest in gibberlic acid, followed by a combination of gibberlic acid and indole acetic as compared to control. At Vadodara the yield was more than Ratlam, in plants treated with methyl jasmonate having the maximum yield followed by gibberlic acid and acetyl salicylic acid. In Dehradun also the yield was more in plants treated with methyl jasmonate (table 3.20, 3.23 & 3.26).

Dry stem yield in Ratlam, Vadodara and Dehradun was more in gibberlic acid treated plants while dry leaves yield was found more in methyl jasmonate treated plants. This shows gibberlic acid promotes stem growth and methyl jasmonate promotes leaf growth. As artemisinin is found in trichomes present on leaves, dry leaves yield is directly proportion to the artemisinin yield. Thus percent artemisinin was also found more in methyl jasmonate treated plants at all three locations. In Ratlam it ranged 0.71 to 0.90%, 0.70 to 0.90% in Vadodara and in Dehradun 0.70 to 0.89%.

Plants are challenged by a variety of biotic stresses like fungal, bacterial, or viral infections. This lead to a great loss of plant yield. There are various options available for the farmers to protect their crop from the disease. The better understanding of plant signalling pathways has led to the discovery of natural and synthetic compounds called elicitors that induce similar defence responses in plants as inducted by the pathogen infection (Gómez-Vásquez *et al*., 2004).

Different types of elicitors have been characterized, including carbohydrate polymers, lipids, glycopeptides and glycoprotein. In plants, a complex array of defence response is induced after detection of microorganism via recognition of elicitor molecules released during plant pathogen interaction. Originally the term elicitor was used for molecules capable of inducing the production of phytoalexins but it is now commonly used for compounds stimulating any type of plant defense (Eder & Cosio, 1994).This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors) (Boller, 1994).

iii. Micromorphological studies

The leaves of treated plants were examined for micromorphological studies. The glandular trichomes of leaves from *A.annua* were examined by light and scanning electron microscopy. The study was conducted to know the impact of different treatments on trichome density as these trichomes are sites of artemisinin synthesis. Artemisinin content (%) was determined through HPTLC and was correlated with trichome index.

A.annua species possess two types of trichomes – Glandular and non glandular trichomes (Fig 3.9) .The large filamentous non glandular trichome, which does not produces essential oil are found abundant on leaf (Fig 3.9). The other is glandular trichome found sparsely throughout the aerial tissue of the plant. The trichome is indented in the epidermis; the cuticle surrounding the apex of the biseriate trichome detaches and forms a sac-like reservoir for essential oil excreted by the apical cells. These trichomes have a bicellular head and a short, cylindrical stalk of different length and features.

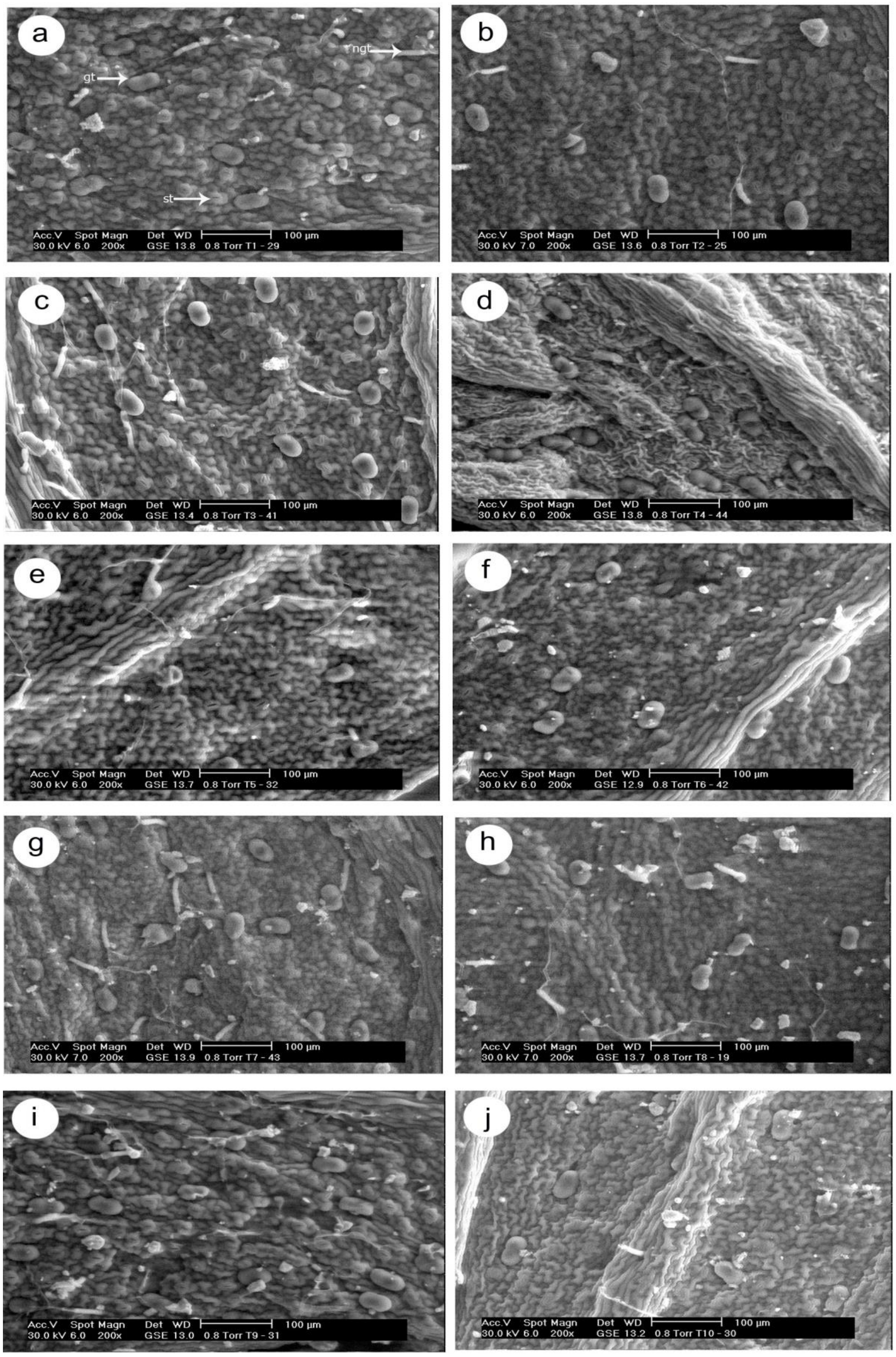


Fig: 3.9: (a-h) Ventral leaf surface with glandular and non-glandular trichomes. a-T1- treatment with absisic acid; b- T2- treatment with GA3; c-T3- treatment with GA3+IAA; d-T4- treatment with chitosan; e-T5- treatment with methtl jasmonate; f- T6- treatment with acetyl salicylic acid, g- T7- treatment with lead acetate; h-T8- treatment with NaCl; i- T9- treatment with Nanozime; j-T10- control.

Site 1.

Treatments	Chemicals	Conc. (mg/l)	Artemisinin content (%)	Trichome index

T1	Abscisic acid	10μM/lit	0.81	0.070
T2	GA3	10μM/lit	0.80	0.055
T3	GA3+ IAA	10μM/lit+10μM/l it	0.80	0.066
T4	Chitosan	150 mg/lit	0.81	0.062
T5	Methyl jasmonate	4.8 μM/lit	0.92	0.115
T6	Acetyl salicylic acid	1mM	0.80	0.064
T7	Lead acetate	500 μM/lit	0.78	0.051
T8	NaCl	160mM	0.82	0.065
T9	Nanozime	1 ml/lit	0.75	0.050
T10	Control	No spray	0.74	0.050

Table 3.28: Effect of growth regulators on artemisinin content and trichome index of A.*annua.*

All the treatments had a positive effect in increasing the trichome index and consequently increasing the artemisinin content. However, the maximum artemisinin content was observed in methyl jasmonate treatment along with the high trichome index. Lowest trichome index was found in nanozime with low artemisinin content. (Table 3.28).

Site 2.

Treatments	**Chemicals**	**Conc. (mg/l)**	**Artemisinin content (%)**	**Trichome index**
T1	Absicissic acid	10μM/lit	0.88	0.073
T2	GA3	10μM/lit	0.81	0.053
T3	GA3+ IAA	10μM/lit+10μM/l it	0.86	0.070
T4	Chitosan	150 mg/lit	0.81	0.055
T5	Methyl jasmonate	4.8 μM/lit	0.94	0.128
T6	Acetyl salicylic acid	1mM	0.80	0.052
T7	Lead acetate	500 μM/lit	0.81	0.059
T8	NaCl	160mM	0.80	0.060
T9	Nanozime	1 ml/lit	0.81	0.055
T10	Control	No spray	0.78	0.049

Table 3.29: Effect of growth regulators on artemisinin content and trichome index of *A.annua*.

The maximum trichome index was observed in the samples treated with methyl jasmonate (0.128) and minimum in control (0.049). Treatments of GA3, Chitosan, Lead acetate and Nanozime showed very less difference. Whereas treatments with acetyl salicylic acid and sodium chloride recorded the lowest trichome index over control. (Table 3.29).

HPTLC analyses for Artemisinin (%) content also followed the same pattern, where samples treated with methyl jasmonate were having higher artemisinin content followed by the treatments of Abscisic acid and GA3+IAA.Treatments of GA3, Chitosan, Lead acetate, Nanozime, Acetyl salicylic acid and sodium chloride were with comparatively low percentage.

Site 3.

Treatments	**Chemicals**	**Conc. (mg/l)**	**Artemisinin content (%)**	**Trichome index**
T1	Abscisic acid	10µM/lit	0.85	0.075
T2	GA3	10µM/lit	0.75	0.060
T3	GA3+ IAA	10µM/lit+10µM/lit	0.82	0.071
T4	Chitosan	150 mg/lit	0.80	0.062
T5	Methyl jasmonate	4.8 µM/lit	0.99	0.134
T6	Acetyl salicylic acid	1mM	0.80	0.056
T7	Lead acetate	500 µM/lit	0.83	0.074
T8	NaCl	160mM	0.86	0.068
T9	Nanozime	1 ml/lit	0.84	0.058
T10	Control	No spray	0.79	0.052

Table 3.30: Effect of growth regulators on artemisinin content and trichome index of *A.annua.*

All the treatments showed increased levels of artemisinin content and high trichome index. But the maximum effect was seen in the methyl jasmonate treated plants, where the artemisinin content (0.99%) and trichome index (0.134) was exceptionally high. Abscisic acid and sodium chloride also had a fair amount of artemisinin content (Table 3.30).

All three sites of the study showed positive results, when treated with elicitors. Both trichome index as well as artemisinin was found to increase when treated with these elicitors. Methyl jasmonate was the most effective among all the treatments. Site 3 had highest artemisinin content (0.99%) while site 2 had (0.94%) and site 1 (0.92%) in methyl jasmonate treated plants. Similarly trichome index was also more in site 3 (0.134) followed by site 2 (0.128) and site 1 (0.115).

Although some authors reported artemisinin being highest during preflowering stages (Woerdenbag *et al*. 1994), others reported artemisinin reaching its peak during flowering (Singh *et al*. 1988; Laughlin 1995). Reports on the distribution of artemisinin throughout the plant have been inconsistent. During the life cycle of a leaf, artemisinin was always present. Quantities were low at leaf appearance and increased steadily. The number of mature, capitate trichomes on the adaxial leaf side increased after leaf appearance until the end of leaf expansion, and then decreased, probably due to collapse of trichomes

Artemisinin production thus (also) occurred when trichomes were collapsing. Later formed leaves achieved higher concentrations of artemisinin than earlier formed leaves, because of a higher trichome density and a higher capacity per trichome.

Plant trichome initiation depends upon the diverse developmental and environmental conditions. In this study, all treatments promoted trichome initiation. As it is known that the artemisinin is secreted in glandular trichome, the trichome density directly corresponds to the percent artemisinin content. So, higher the trichome number, higher will be the artemisinin content.

We studied the effect of all exogenous treatment on leaf trichome formation and found the general ability of phytohormones to impinge on trichome initiation. The most effective phytohormone found was methyl jasmonate which activated the formation of large number of glandular trichomes.

Elicitors are natural compounds that have shown far reaching effects on the growth and development of plants even at low concentration (Arshad and Frankenberger, 1998). Plant growth regulators are known to affect growth,flowering and assimilate translocation in plants (Hayat *et al*., 2001)Auxins, gibberillins (GA3) and kinetin being well known plant growth promoting hormones have shown to be involved in a variety of plant growth and development processes (Frankenberger and Arshad, 1995). Attempts to increase artemisinin production by adjusting the nutrients, hormones, growth conditions, elicitors, and stresses are reported. (Qian *et al*., 2007; Wang & Tan, 2002) .Previous research has identified an important role for the hormone gibberellin, in constitutive trichome production in Arabidopsis.(Perazza *et al*.,1998). Phytohormones are also known to modulate epidermal differentiation programs (Maes, L. *et al*., 2008)

Traw & Bergelson (2003), demonstrated, both NaCl and lead induce oxidative stress in *A.annua* plants and that of the stress is proportional to the concentration of the stressor. The oxidative stress in turn alters artemisinin concentration and yield. But here the artemisinin content and trichome density has a minimum effect after applying NaCl. Studies on salicylic acid show reduction in trichome density and number and when treated with jasmonic acid it showed large increase in trichome density and number. Similar results were found in the present study. Also the application of GA3 was effective in the increasing the content and trichome density as compared to control. According to Farooqi et.al. (1994) a positive effect of GA3 on secondary product production of alkaloids and diosgenin has been reported.

The substantial increase in plant height, fresh & dry yield and artemisinin yield following the applications of elicitors to plants after 20 weeks transplanting may be attributed to the favourable effect of these chemicals and growth regulators on the growth and trichome formation.

Experiment 4.

Effect of planting time on growth, biomass and artemisinin yield.

The purpose of the study was to analyse the most suitable time for transplanting the sapling in different studied regions to get higher values of all the studied parameters. The three regions were tested for different month starting from September to February.

Site 1.

Sr. no.	Treatment	Plant height at harvest (cm)	Fresh biomass (T/ha)	Dry leaves (T/ha)	Dry stem (T/ha)	Leaf stem ratio	Artemisinin %	Artemisinin yield (T/ha)
1	T1	208.9	21.66	3.70	7.64	0.42	0.74	30.66
2	T2	220.2	29.51	3.23	7.94	0.53	0.72	30.45
3	T3	210.8	31.04	3.85	8.1	0.47	0.75	28.87
4	T4	212.1	39.84	4.1	9.32	0.43	0.83	34.03
5	T5	191.5	28.51	3.66	8.23	0.44	0.78	28.54
6	T6	149.6	12.73	2.94	3.92	0.75	0.61	17.93

Table 3.31: Effect of planting time on growth, biomass and Artemisinin yield.

(T1 sept, T2 Oct, T3 Nov, T4 dec, T5 Jan, T6 feb)

The plant height throughout the year ranged from 149.6 cm to 220.2 cm. The maximum plant height at harvest (220.2 cm) was recorded in plants transplanted in T2 while the plants transplanted in T6 had minimum plant height (149.6 cm). Fresh biomass was maximum in T4 transplanted plants (39.84 T/ha) and minimum in February transplanted plants (12.73 T/ha). The dry leaves and dry stem yield (T/ha) was also highest in T4 transplanted plants (4.1 T/ha and 9.32 T/ha respectively). Leaf stem ratio was highest (0.75) in plant transplanted in T6 while it was found lowest (0.42) in plant transplanted in September. Artemisinin% (fig 3.9) was maximum in T4 (0.83%) and minimum in T6 (0.61%) along with artemisinin yield more in T4 (34.03 T/ha) and less in T6 (17.93) (Table 3.31).

Minimum and maximum temperature in different months of transplantation of *A.annua* has been anlaysed. The temperature in December ranged from minimum 12.8 to maximum 34.8 ˚C, which seems to be suitable temperature for the plant growth attributes. Also it favored good growth of the plant which was responsible for high biomass consecutively giving high amount of dry leaves and dry stem yield. Plant height was found more in October, where the temperature ranged from minimum 20.1 to maximum 38˚ C**.**

Site 2.

Sr. no.	**Treat ment**	**Plant height at harvest (cm)**	**Fresh biomass (T/ha)**	**Dry leaves (T/ha)**	**Dry stem (T/ha)**	**Leaf stem ratio**	**Artemis inin %**	**Artemisinin yield (T/ha)**
1	T1	201.3	24.12	2.50	7.51	0.38	0.72	29.79
2	T2	211.5	26.55	4.21	8.10	0.51	0.72	32.50
3	T3	203.4	37.15	4.90	8.60	0.46	0.75	29.27
4	T4	210.1	41.65	4.15	9.21	0.40	0.82	33.45
5	T5	195.1	31.55	3.84	7.96	0.42	0.75	27.21
6	T6	152.4	14.11	3.11	3.85	0.73	0.60	18.25

Table 3.32: Effect of planting time on growth, biomass and Artemisinin yield.

Height of the plant and Leaf stem ratio was found maximum in T2, but the fresh biomass and dry stem yield was found highest in T4. Dry leaves yield was maximum in T3. Artemisinin % and yield was maximum in T4.

Site 3.

Sr. no.	Treat ment	Plant height at harvest (cm)	Fresh biomass (T/ha)	Dry leaves (T/ha)	Dry stem (T/ha)	Leaf stem ratio	Artemis inin %	Artemisinin yield (T/ha)
1	T1	210.5	21.66	3.70	7.64	0.42	0.74	30.66
2	T2	223.5	29.51	3.23	7.94	0.53	0.72	30.45
3	T3	216.2	31.04	3.85	8.1	0.47	0.75	28.87
4	T4	196.5	28.51	3.66	8.23	0.44	0.78	28.54
5	T5	214.8	39.84	4.1	9.32	0.43	0.83	34.03
6	T6	159.2	12.73	2.94	3.92	0.75	0.61	17.93

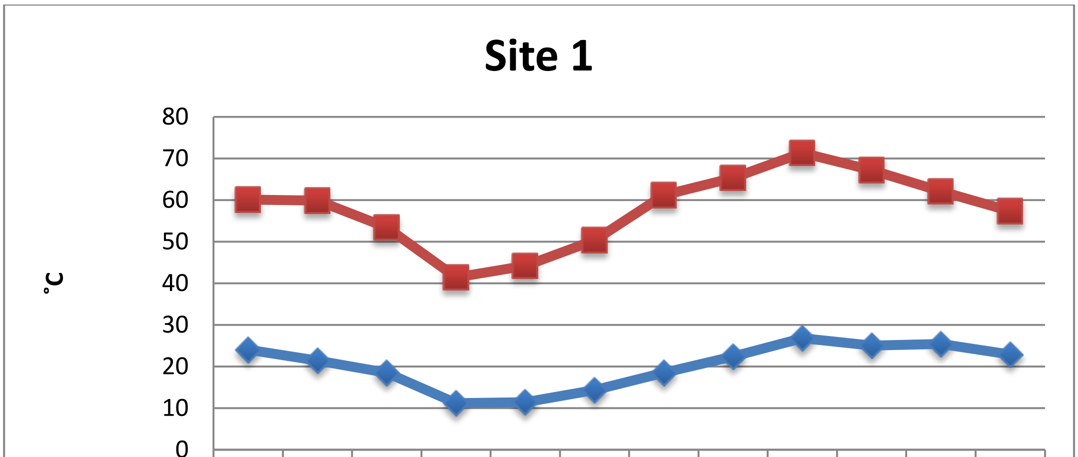
Table 3.33: Effect of planting time on growth, biomass and Artemisinin yield.

Height of the plant was highest in T2. Fresh biomass, dry leaves and stem yield was more in T4. Artemisinin content and yield were also found maximum in T4.

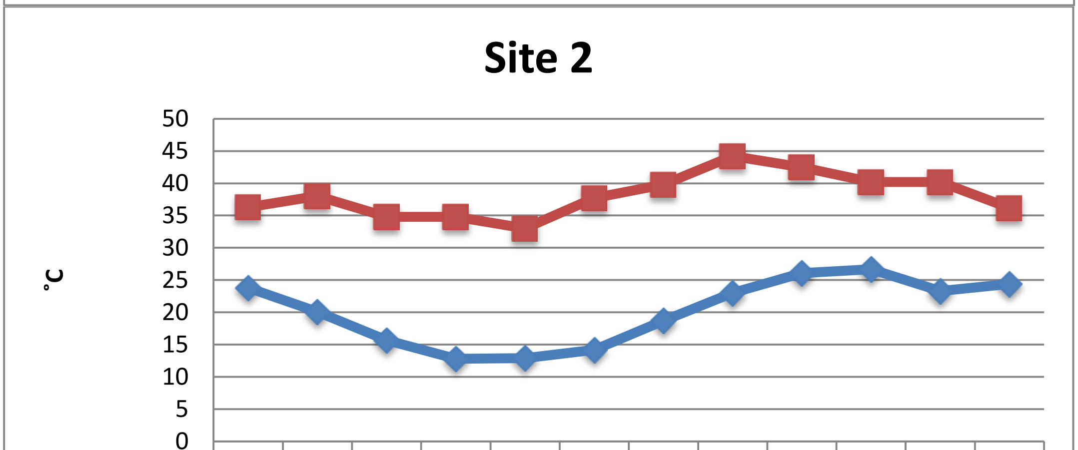
The most suitable time for transplantation for all sites was T4 for more artemisinin content and high yield. T2 transplanted plants had more height and biomass.

The influence of planting time on *A.annua* was very evident. The plant height throughout the year ranged from 149.6 cm to 220.2 cm. The maximum plant height at harvest (220.2 cm) was recorded in plants transplanted in October while the plants transplanted in February had minimum plant height (149.6 cm) (Fig 3.10 A). Fresh biomass was maximum in December transplanted plants (39.84 T/ha) and minimum in February transplanted plants (12.73 T/ha). The dry leaves and dry stem yield (T/ha) was also highest in December transplanted plants (4.1 T/ha and 9.32 T/ha respectively) (Fig 3.10). Leaf stem ratio was highest (0.75) in plant transplanted in February while it was found lowest (0.42) in plant transplanted in September. Artemisinin% (Fig 3.10) was maximum in December (0.83%) and minimum in February (0.61%) along with artemisinin yield more in December (34.03 T/ha) and less in February (17.93) (Fig 3.10).

Minimum and maximum temperatures in different months of transplantation of *A.annua* were found fluctuating. The temperature in December ranged from minimum 12.8 to maximum 34.8 C, which seems to be suitable temperature for the plant to produce artemisinin. Also it favoured good growth of the plant which was responsible for high biomass consecutively giving high amount of dry leaves and dry stem yield. Plant height was found more in October, where the temperature ranged from minimum 20.1 to maximum 38 ˚C.



	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Max (˚C)	36.1	38.5	34.9	30.2	32.8	36	42.7	42.9	44.5	42.2	36.7	34.4
Min (˚C)	24	21.4	18.5	11.2	11.4	14.3	18.5	22.4	26.8	25	25.4	22.9



	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Min (˚C)	23.8	20.1	15.7	12.8	12.9	14.2	18.7	23	26.1	26.7	23.3	24.4
Max (˚C)	36.3	38	34.8	34.8	33	37.7	39.8	44.2	42.5	40.2	40.2	36.2

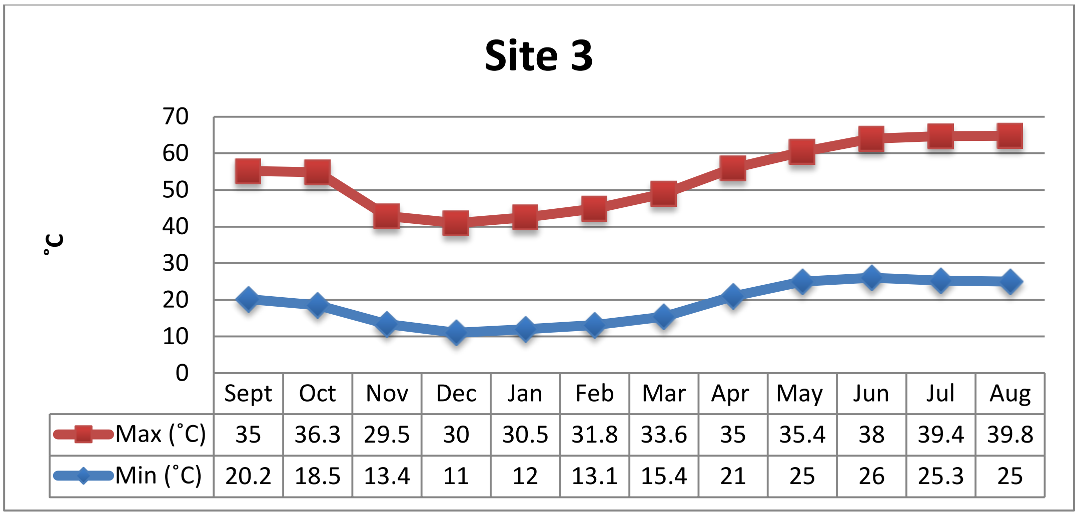


Figure 3.10: Minimum and maximum temperatures in different months.

Agricultural, environmental and genetic aspects may be useful in the successful large scale cultivation of *A.annua*. It also includes geographic aspects (latitude and altitude), which will help make decisions about crop establishment in tropical countries.

Report (Bolina et al 2013) suggested, the artemisinin levels plants with a smaller canopy volume and a smaller number of branches should be selected which will lead to higher artemisinin levels, but in this case increase in the production of the active principle occurs at the expense of the primary metabolism which decreases biomass production and as a result the artemisinin yield per planted area will be smaller.

The effect of variation in date of transplanting of seedlings, date of harvest and number of harvests on the yield of artemisinin and related characters was examined in the field grown crops of *A.annua* cv Jeevanraksha, over several cropping seasons in the subtropical agroclimatic environment of Indo-Gangetic plains.

Study examined the possibility of introducing *A. annua* germplasm into the agricultural landscape of the lowland humid tropics in field experiments and identified promising accessions that are capable of producing high leaf biomass and artemisinin yields. Differences amongst seed origin, planting season and soil moisture availability had highly significant ($P < 0.01$) effects on most of the agronomic characteristics evaluated, suggesting that these are very critical factors when considering the cultivation of *A. annua* in the humid tropics.

There are many problems in trying to introduce cultivation of a plant to areas where it is not widely grown or known. These are compounded in the case of *Artemisia* which until recently had not been cultivated but was essentially a wild crop. In East Africa the principal challenges have been weed control harvesting and drying at a time when drenching rains are possible.

A study conducted by Thu *et al*., reported the variation observed in plant growth and artemisinin contents were due to temperature effects, where the cold weather was suitable for planting of *A.annua* as opposed to the tropical weather. The present study clearly indicated that the temperature affected the growth and artemisinin content of *A.aanua*. Also the vegetative growths of the plants at higher temperatures were affected.

It was probably the high temperature and hot weather which resulted in strong transpiration, and more water loss in the *A.annua* plants (Wang et al 2007). Environmental variations, such as light, temperature and availabilities of water and salt were reported to alter the artemisinin yield (Weathers et al. 1994).

II. Post harvest studies

Experiment 5: Effect of shade drying and oven drying on artemisinin content of *A.annua*

The post harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active principles in the product sold. Drying has been one of the most important processes in pre-processing of agricultural products. Aromatic plants are often dried before extraction to reduce moisture content. The aim of drying is to reduce the moisture content of the product from actively growing in the field to a level that prevents deterioration of the product and allows storage in a stable condition. Proper drying of medicinal plants is fundamental to the achievement of a high quality product.

An experiment was conducted in Vadodara (Gujarat), to study the variation in content of artemisinin extracted from the leaves which were subjected to (i) shade drying and (ii) drying in hot air oven (60˚C for 24hrs). The results obtained from the studies are represented below.

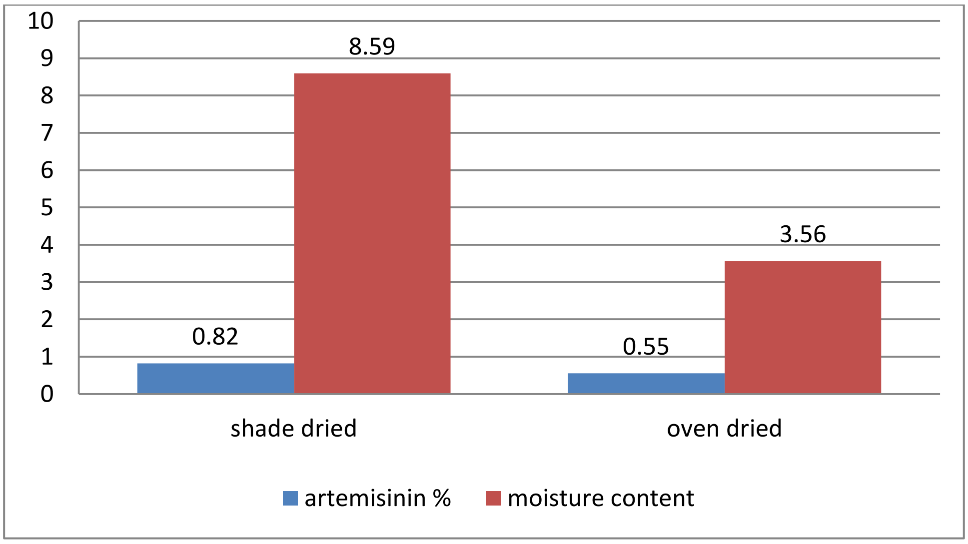


Fig 3.11: Comparison of artemisinin % and moisture content in shade dried and oven dried plants.

From the figure, it is evident that the artemisinin content decreased when the plants were oven dried while in shade dried plants the content is much more. *A.annua* is an aromatic plant, which loses its active compound artemisinin at higher temperature. So shade drying is the effective measure for retaining the artemisinin content.

The main purpose of drying medicinal plants is to extend product shelf life, minimize packaging requirements and reduce shipping weights (Sellami *et al*., 2011). Drying is used to stop the growth of microorganisms and preserve the quality of agricultural products (Lin *et al*., 2011). Drying techniques have been applied to reduce the moisture content of some medicinal and aromatic plants and their effects on yield and composition of essential oil have been studied by other researchers such *as Matricaria recutita* (Azizi *et al*., 2009), *Ocimum basilicum* (Ebadi *et al*., 2013), *Laurus nobilis* (Sellami *et al*., 2011) and *Mentha longifolia* (Asekun *et al*., 2007)

Oladele and Aborisade (2009), reported the effects of drying methods on nutrient retention in a leaf vegetable during storage. Ca and Mg were found to increased in shade dried leaves during storage. Vyankatrao (2014), studied effective drying method for retention of nutrients in leafy vegetables. Five different methods of drying were taken up, out of which ascorbic acid content was found maximum in treatment of oven 90˚C as compared to shade dry which was comparatively less but water soluble reducing sugars were more in shade dried plants. Alakali *et al*., (2015) reported that *Moringa* leaves on shade drying had more protein content, fat content, Beta carotene and vitamin C than in oven dried plants.

Ambrose *et al*., (2013) studied the effect of drying on the yield of volatile oil of Patchouli. The herbage was dried under different drying condtions and they found the volatile oil content of sample dried at 40˚C was 2.46% while in shade dried sample it was 2.40%. In a study by Khorshidi *et al*., (2009) maximum essential oil percentage was obtained in shade dried leaf samples of rosemary while minimum essential oil percentage was obtained in stem sample of oven dried plants.

Many workers have reported that drying method has a significant effect on oil content and composition of aromatic plants (Okoh *et al*., 2008; Ahmadi *et al*., 2008 and Asekun *et al*., 2007). Also duration of essential oil extraction affected on the quantity and quality of essential oil. Jamshidi *et al* (2009) reported that essential oil percentage and essential oil component of fennel were affected by duration of essential oil extraction. Ebadi *et al*., (2013) studied the influence of four drying methods (shade drying, freeze drying, oven drying and vaccum drying at 40, 50 and 60˚C) on the essential oil content and composition of lemon verbena. Higest amount of limonene and 1,8-cineole were determined in vaccum dried (60˚C) leaves. In present study, shade drying was much more beneficial than oven drying in all aspects.

Experiment 6: GC-MS analysis data from different samples of *Artemisia* leaves

GC-MS analysis of *n*-hexane extract samples, of different geographical locations, revealed presence of many components but only major components present in the extract samples. Therefore, only five major components, in each geographical area, have been analyzed for their mass-spectrometric fragmentation pattern for identification of major components present in extract samples (fig 3.12). Details of GC-MS analysis like, sample I.D., peak area %, retention time and mass fragmentation have been shown in Table 3.34. Total ion chromatograms of all the collected samples are shown in figure 3.13 and 3.14.

Sample I.D.	Major peak no.	Peak area, %	Retention time (minutes)	Mass fragmentation
A-1 (Uttar Pradesh)	1	20.32	22.903	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 166, 167, 178, 192, 193, 209, 211, 212, 223, 239, 282, 284, 309, 327, 341, 386 and 399.
	2	13.55	24.260	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 122, 137, 149, 150, 151, 166, 179, 204, 222, 223, 224, 248, 264, 283, 295, 308, 332, 368, 376, 424, 437 and 468.
	3	11.20	21.858	43 (100%), 43, 55, 67, 69, 81, 93, 95, 107, 123, 137, 151, 166, 178, 180, 208, 234, 236, 237, 238, 250, 279, 292, 322, 349, 354, 432, 438.
	4	8.71	22.62	43 (100%), 55, 67, 79, 81, 93, 124, 135, 150, 151, 164, 165, 166, 179, 195, 210, 224, 225, 266, 273, 295, 327, 350, 385, 394, 442, and 490.
	5	6.46	39.77	41, 55, 69, 81, 95, 109, 122, 133, 135, 136, 147, 149, 163, 189, 203, 218 (100%), 219, 245, 257, 271, 298, 313, 315, 339, 368, 381, 409, 424, 425, 426, 474, 501, 536, 579, and 584.
A-2 (Jaora, Madhya Pradesh)	1	14.67	22.903	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 166, 167, 178, 192, 193, 209, 211, 212, 223, 239, 282, 284, 309, 327, 341, 386 and 399.
	2	8.40	21.858	41, 43 (100%), 55, 67, 69, 81, 95, 122, 123, 137, 151, 166, 180, 193, 208, 209, 234, 236, 237 and 238.
	3	7.58	22.628	41, 43, 55, 67, 79, 81, 93, 95, 107, 124, 135, 150, 151, 164, 165 (100%), 166, 179, 195, 210, 224, 225, 266, 275, 295, 306, 327, 355, 413, 484 and 487.
	4	7.32	24.241	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 137, 150, 151, 166, 179, 204, 222, 223, 224.
	5	6.30	20.042	41, 43, 55, 67, 79, 81, 93, 95, 107, 109, 133, 147, 162 (100), 163, 164, 175, 218, 236, 250, 294, 309, 346, 381, 394 and 489.
A-3 (Uttarakhand)	1	15.63	22.866	41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 165, 166, 167, 178, 192, 193, 211, 222, 223, 239, 282, 284, 306, 343, 346, 379, 414, 428, 472 and 477.
	2	13.62	20.043	41, 43, 55, 67, 77, 79, 91, 107, 121, 133, 147, 162 (100), 163, 175, 190, 218, 219, 236, 264, 294, 297, 331, 363, 367, 382, 432, 464 and 476.
	3	9.85	24.205	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 137, 150, 151, 166, 179, 204, 222, 223, 224.
	4	9.06	22.609	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 195, 210, 224, 225, 266, 273, 312, 319, 369, 394, 396, 417 and 461.
	5	8.59	21.784	41, 43 (100%), 55, 67, 69, 81, 95, 107, 123, 135, 151, 166, 178, 180, 193, 208, 218, 219, 236, 237, 264, 281, 314, 341, 415, 426, 439, 464 and 475.

Sample I.D.	Major peak no.	Peak area, %	Retention time (minutes)	Mass fragmentation
A-4 (Baroda, Gujarat)	1	17.70	22.866	41, 43, 55, 67, 69, 79, 96, 109, 137 (100%), 165, 166, 167, 192, 193, 211, 222, 239, 254, 282, 295, 305, 413, 467 and 487.
	2	10.02	24.204	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 122, 150, 151, 166, 179, 204, 222, 223, 236, 264, 283, 295, 329, 341, 361, 379, 417, 420, 433, 450 and 477.
	3	8.85	21.784	41, 43 (100%), 55, 67, 69, 81, 93, 95, 107, 123, 135, 151, 166, 178, 180, 193, 208, 218, 219, 236, 237, 250, 281, 297, 334, 346, 376, 400, 426, 435, 466 and 475 and 480.
	4	8.56	22.609	41, 43 (100%), 55, 67, 79, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 177, 195, 210, 224, 225, 238, 266, 282, 296, 318, 344, 364, 387, 446, 450 and 483.
	5	6.66	20.006	41, 53, 55, 67, 77, 79, 91, 93, 107, 121, 133, 147, 162 (100), 163, 164, 175, 218 (M^+-2), 236, 265, 281, 298, 345, 352, 368, 399, 414, 459, 464, 469 and 499.
A-5 (Andhra Pradesh)	1	13.84	22.59	41, 43 (100%), 55, 67, 79, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 177, 195, 210, 224, 225, 238, 266, 283, 303, 319, 345, 364, 394, 412, 450, 466, 469, and 474
	2	10.61	39.68	41, 43, 55, 67, 69, 81, 93, 95, 109, 135, 136, 147, 149, 163, 189, 203, 218 (100%), 219, 245, 257, 271, 311, 313, 314, 341, 381, 406, 409, 425, 426, 465, and 475.
	3	10.52	21.381	41, 43, 55, 60, 71, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 213, 227, 228, 256, 257, 276, 314, 327, 352, 376, 406, 415, 419, 464, 495.
	4	9.08	22.83	41, 43, 55, 67, 69, 81, 96, 109, 137 (100%), 166, 167, 192, 193, 211, 222, 232, 264, 282, 305, 319, 364, 376, 414, 426, 464 and 472.
	5	7.01	38.28	41, 43, 55, 67, 69, 81, 93, 95, 109, 119, 121, 135, 137, 147, 149, 175, 189, 203, 218 (100%), 219, 231, 257, 271, 285, 299, 325, 339, 355, 406, 425, 426, 476, and 484.
A-6 (Kharkhedi, Gujarat)	1	8.85	22.848	41, 43, 55, 67, 69, 81, 95, 96, 109, 137 (100%), 165, 166, 167, 193, 211, 232, 239, 282, 304, 319, 345, 350, 376, 411 and 447.
	2	8.11	21.784	41, 43 (100%), 55, 67, 69, 81, 95, 107, 123, 135, 151, 166, 178, 180, 208, 218, 236, 237, 250, 281, 286, 341, 355, 388, 407, 415, 436, 452 and 477.
	3	7.96	22.609	41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 124, 135, 150, 151, 164, 165, 166, 167, 195, 196, 224, 225, 238, 266, 283, 307, 312, 355, 385, 400 and 415.
	4	7.19	24.186	41, 43 (100%), 55, 56, 67, 79, 81, 93, 95, 122, 150, 151, 166, 179, 204, 222, 223, 224, 264, 283, 295, 320, 332, 365, 391, 408, 433, 450 and 489.
	5	6.98	20.024	41, 43, 55, 67, 79, 81, 93, 95, 107, 109, 133, 147, 162 (100), 163, 164, 175, 218, 236, 250, 294, 309, 346, 381,

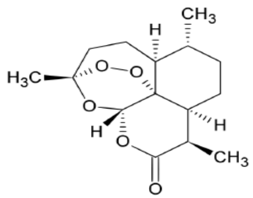
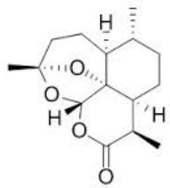
Sample I.D.	Major peak no.	Peak area, %	Retention time (minutes)	Mass fragmentation
				394 and 489.

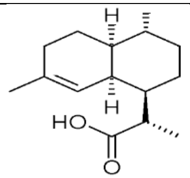
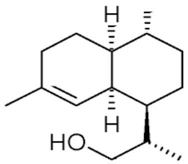
Table 3.34: GC-MS Analysis data of *n*-hexane extract of *A. annua* (Leaves) collected from different locations of India

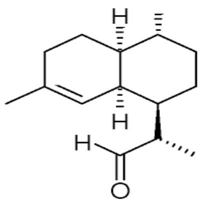
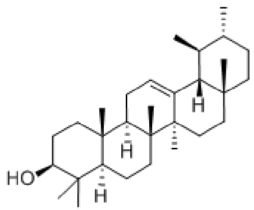
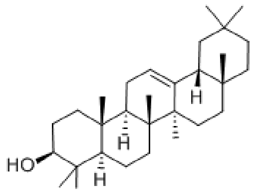
Systematic investigation of mass spectrometric fragmentations of major detected components and their retention times (in minutes) indicated that almost all samples are chemically similar, except A-5, but they differs in the %age contents of components present.

Total 8 major components present in the leaves were identified, in all six samples collected from different geographical area, with the help of hits (NIST and NBS library) provided with GC-MS analysis, retention times and fragmentation patterns, which are given in Table 3.35. GC/EI-MS of the identified compounds are shown in figure 3.14 and 3.15.

Total eight major components (constituents or compounds) were present in all six samples collected from different geographical area. The comparison of samples was based only on the chemical constituents of higher quantity considering their retention time and percentage area. Every sample differed in their chemical constituents quantitatively, as indicated from area percentage value. Sample collected from Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3) and Baroda, Gujarat (A-4) were almost chemically similar. All the samples showed the presence of Artemisinin (average retention time is 22.87 minutes) and deoxyartemisinin (average retention time is 22.61 minutes). In spite of unanimous presence of Artemisinin and deoxyartemisinin, both the compounds differed quantitatively in every sample. Dihydroartemisinic acid, dihydroartemisinic alcohol, artemisinin and deoxyartemisinin were present in extracts of the samples collected from Uttar Pradesh (A-1), Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3), Baroda, Gujarat (A-4) and Kharkhedi, Gujarat (A-6), in addition extract from Uttar Pradesh (A-1) showed the presence of α-amyrin, while extracts collected from Jaora, Madhya Pradesh (A-2), Uttarakhand (A-3) and Baroda, Gujarat (A-4) showed dihyroartemisinic aldehyde, and extract of sample collected Kharkhedi, Gujarat (A-6) showed dihydroartemisinic acid. Comparatively, samples collected from Andhra Pradesh (A-5) and Kharkhedi, Gujarat (A-6) showed clear deviation in chemical constituents from rest of samples. α-amyrin was present in two samples (A-1 and A-5) while β-amyrin is present only in one sample (A-5) which was collected from Andhra Pradesh. Artemisinin content was highest in sample collected from Uttar Pradesh.

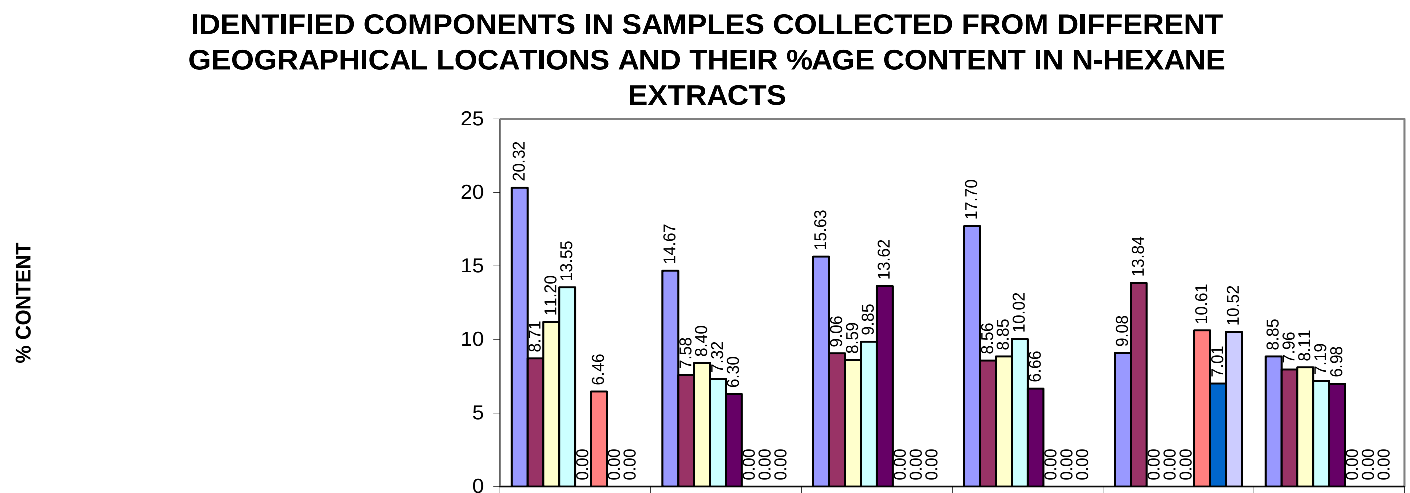
Components identified	Average peak area, %	Average retention time (minutes)	Compound name and structure	Molecular formula	Molecular weight	Mass fragmentation	Ref.
Artemisinin	**14.375**	**22.87**		$C_{15}H_{22}O_5$	**282**	**41, 43, 55, 67, 69, 81, 93, 95, 96, 109, 123, 137 (100%), 151, 166, 167, 178, 192, 193, 209, 211, 212, 223, 239, 282 (M^+), 284, 309, 327, 341, 386 and 399.**	**14**
Deoxyartemisinin	**9.29**	**22.61**		$C_{15}H_{22}O_4$	**266**	**43 (100%), 55, 67, 79, 81, 93, 124, 135, 150, 151, 164, 165, 166, 179, 195, 210, 224, 225, 266 (M^+), 273, 295, 327, 350, 385, 394, 442, and 490.**	**15**
Dihydroartemisinic acid	**9.03**	**21.81**		$C_{15}H_{24}O_2$	**236**	**43 (100%), 43, 55, 67, 69,**	**16**

						**81, 93, 95, 107, 123, 137, 151, 166, 178, 180, 208, 234, 236 (M^+), 237 (M^++1), 238 (M^++2), 250, 279, 292, 322, 349, 354, 432, 438.**	
Dihydroartemisinic alcohol	**9.59**	**24.22**		**$C_{16}H_{26}O$**	**222**	**41, 43 (100%), 55, 67, 79, 81, 93, 95, 107, 122, 137, 149, 150, 151, 166, 179, 204, 222 (M^+), 223 (M^++1), 224 (M^++2), 248, 264, 283, 295, 308, 332, 368, 376, 424, 437 and 468.**	**16**

Dihydroartemisinic aldehyde	**8.39**	**20.03**		**$C_{15}H_{24}O$**	**220**	**41, 43, 55, 67, 79, 81, 93, 95, 107, 109, 133, 147, 162 (100), 163, 164, 175, 218 (M^+-2), 236, 250, 294, 309, 346, 381, 394 and 489.**	**16**
α-Amyrin	**8.54**	**39.72**		**$C_{30}H_{50}O$**	**426**	**41, 43, 55, 67, 69, 81, 93, 95, 109, 135, 136, 147, 149, 163, 189, 203, 218 (100%), 219, 245, 257, 271, 311, 313, 314, 341, 381, 406, 409, 425 (M^+-1), 426 (M^+), 465, and 475.**	**17**
β-Amyrin	**7.01**	**32.29**		**$C_{30}H_{50}O$**	**426**	**41, 43, 55, 67, 69, 81, 93, 95, 109, 119, 121, 135, 137, 147, 149, 175, 189, 203, 218**	**17**

						(100%), 219, 231, 257, 271, 285, 299, 325, 339, 355, 406, 425 (M^+-1), 426 (M^+), 476, and 484.	
Tritetracontanol	**10.52**	**21.38**	**CH_3-$(CH_2)_{32}$-CH_2OH**	**$C_{34}H_{70}O$**	**494**	**41, 43, 55, 60, 71, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 213, 227, 228, 256, 257, 276, 314, 327, 352, 376, 406, 415, 419, 464 (M^++H-C_2H_5OH), 495 (M^++H).**	**--**

Table 3.35: Major components identified through GC-MS



	A-1 (Uttar Pradesh)	A-2 (Jaora, Madhya Pradesh)	A-3 (Uttarakhand)	A-4 (Baroda, Gujarat)	A-5 (Andhra Pradesh)	A-6 (Kharkhedi, Gujarat)
Artemisinin (Av. RT = 22.87 min.)	20.32	14.67	15.63	17.7	9.08	8.85
Deoxyartemisinin (Av. RT = 22.61 min.)	8.71	7.58	9.06	8.56	13.84	7.96
Dihydroartemisinic acid (Av. RT = 21.81 min.)	11.2	8.4	8.59	8.85	0	8.11
Dihydroartemisinic alcohol (Av. RT =24.22 min.)	13.55	7.32	9.85	10.02	0	7.19
Dihydroartemisinic aldehyde (Av. RT =20.03 min.)	0	6.3	13.62	6.66	0	6.98
Alpha-Amyrin (Av. RT = 39.72 min.)	6.46	0	0	0	10.61	0
Beta-Amyrin (Av. RT = 38.29 min.)	0	0	0	0	7.01	0
Tritetracontanol (Av. RT = 21.38 min.)	0	0	0	0	10.52	0

EXTRACTS OF SAMPLE COLLECTED FROM DIFFERENT GEOGRAPHICAL REGIONS

Figure 3.12: Identified components in the samples collected from different geographical area and their percentage content in extracts

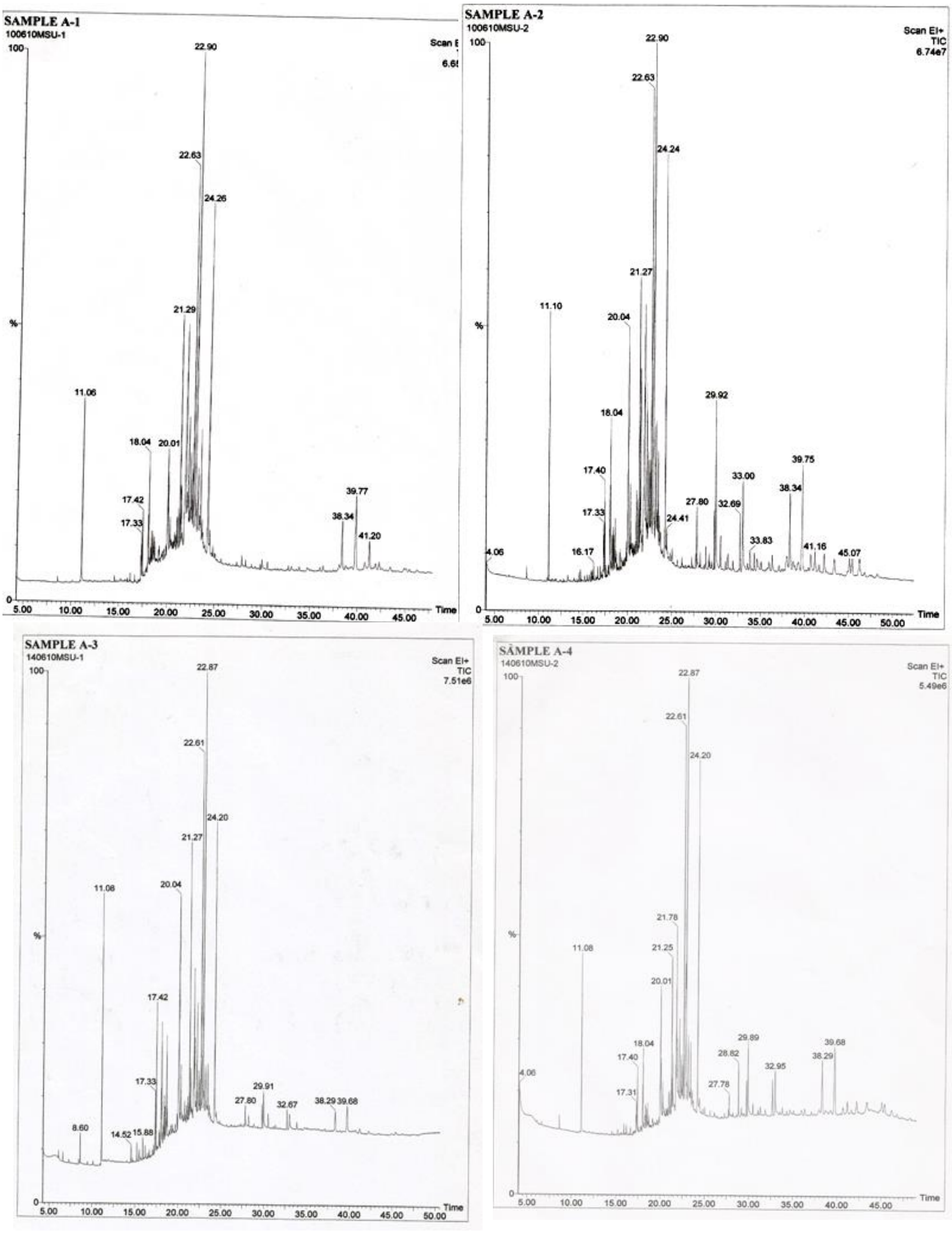


Fig 3.13: Total ion chromatogram of sample A1, A2, A3 and A4

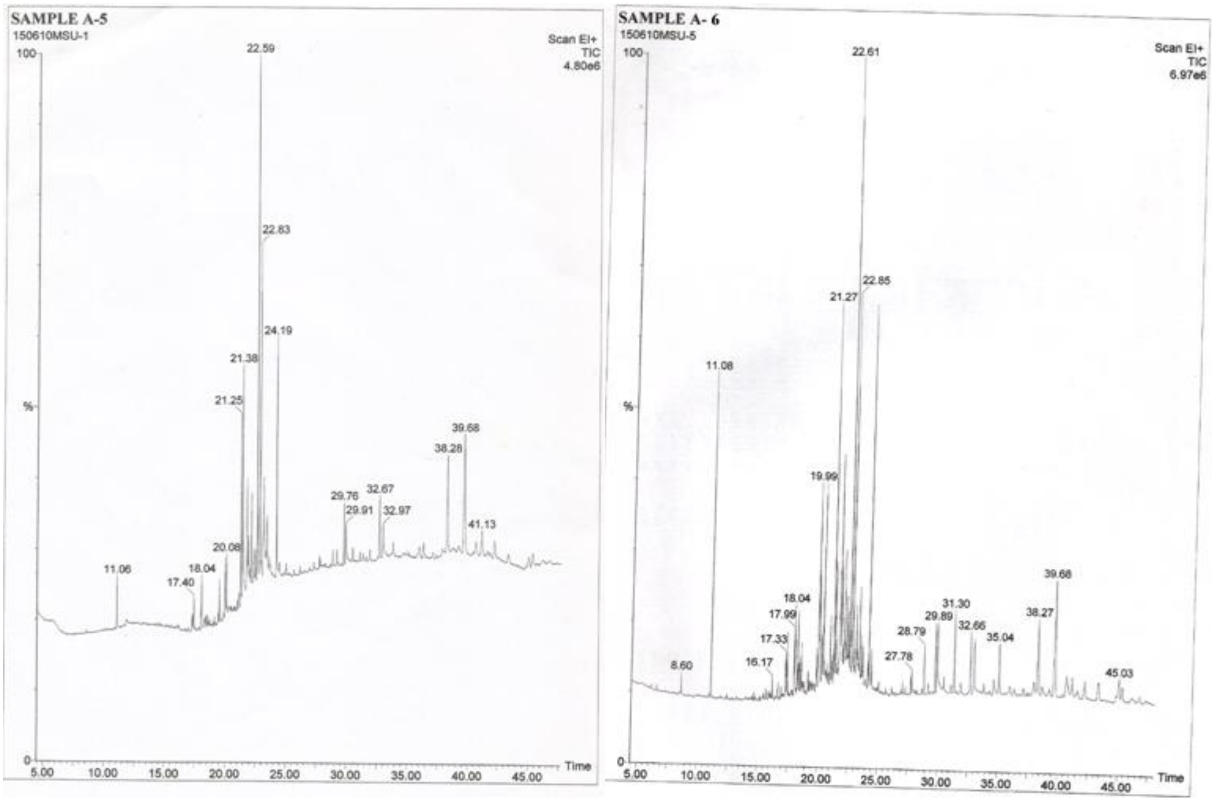


Fig 3.14: Total ion chromatogram of sample A5 and A6

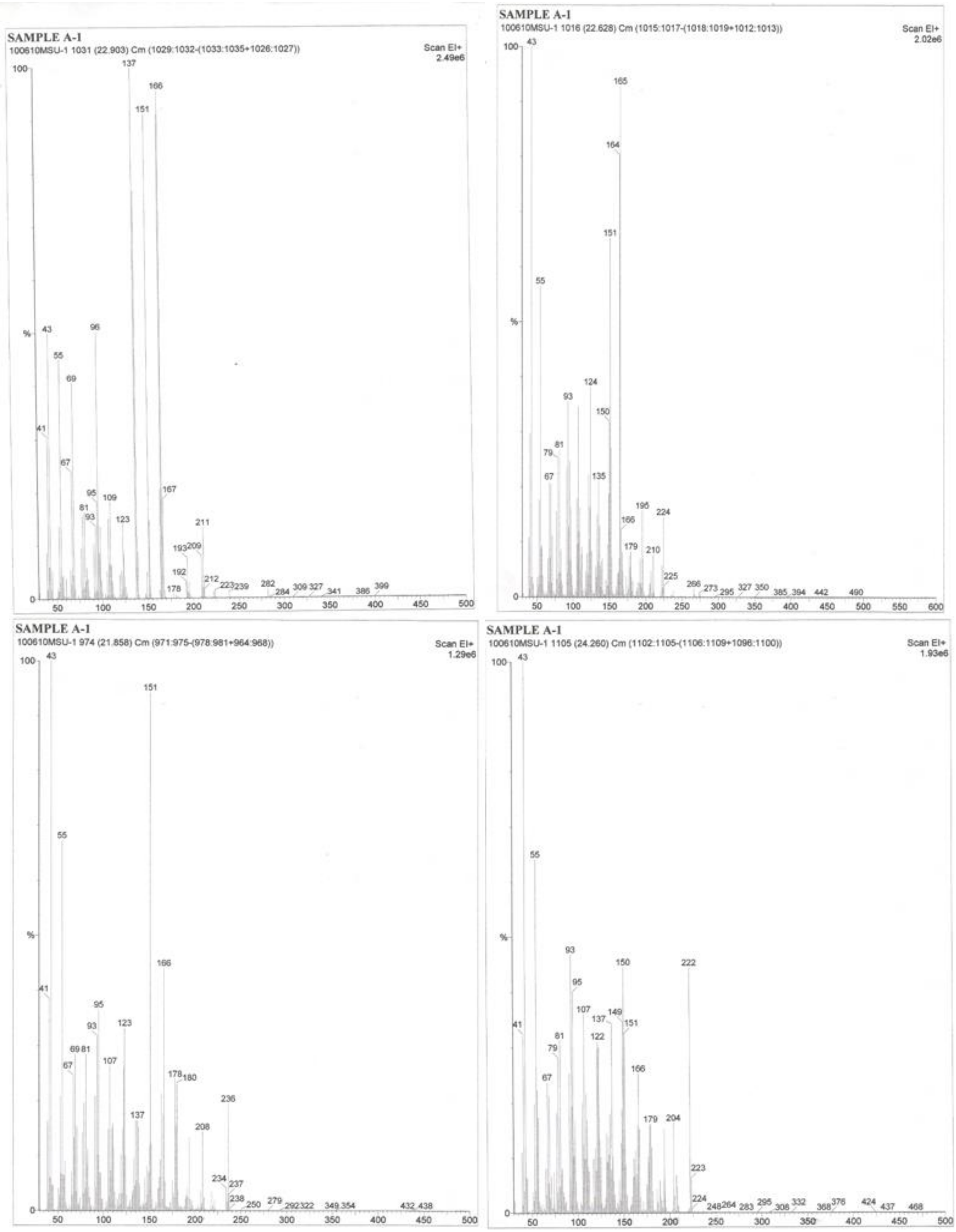


Fig 3.15: GC/EI-MS of (A) Artemisinin (B) Deoxyartemisinin, (C) Dihydroartemisinic acid, (D) Dihydroartemisinic alcohol

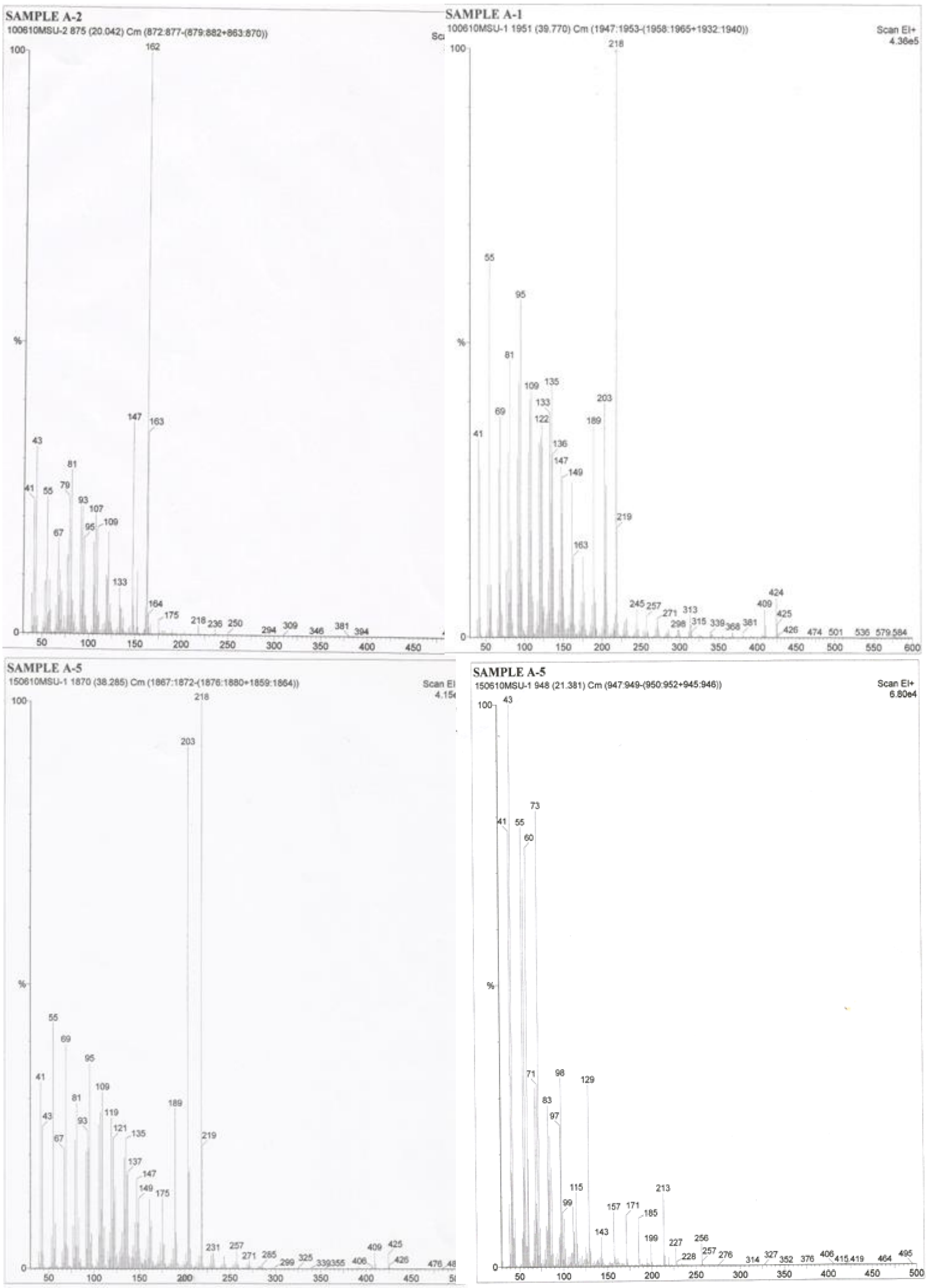


Fig3.16: GC/EI-MS of (A) Dihydroartemisinic aldehyde (B) α Amyrin , (C) β-Amyrin, (D) Tritetracontanol

Experiment 7. Studies on utilizing waste

i. Briquette making

As it is known from the earlier studies, dry leaves of *A. annua* are used for artemisinin extraction. The waste which is left after the extraction can be utilized in various ways. In commercial industries tons of coal is used to run boilers. The waste of *A. annua* can be utilized here, which can be the replacement for coal.

Ipca Labs followed the experiment on utilizing waste. After extraction of artemisinin from the leaves of *A. annua*, the waste obtained was used in making briquettes. This reduces the cost of boiler operation. Also it eliminates the waste and saves coal & energy. Every year about 30 tons of extracted leaves are obtained at Ipca labs which are useless. Everyday 22 tons coal is required to run the boiler.

Analysis of data

Sr. No.	Month	Extracted *Artemisia* leaves (tons/month)	Output from *Artemisia* leaves through new extraction plant	Use
1	November	35.27	300	Unused
2	December	42.56	300	Unused
3	January	43.61	300	Unused

Table 3.36: Quantity of extracted *Artemisia* leaves generated

Sr. No.	Month	Coal (tons/month)	Cost (Rs/Kg)	Cost
1	September	22	7.89	1,73,580
2	October	22	7.89	1,73,580
3	November	22	7.89	1,73,580

Table 3.37: Quantity of coal used in industry

Fig 3.17: a- Brick machine, b- dry artemisia powder, c- brick of Artemisia powder and Boiler ash (70:30), d- brick of Artemisia powder and soyabean waste(70:30) e- brick of artemisia waste (100%)

Many of the developing countries produce huge quantities of agro residues but they ate used inefficiently causing extensive pollution to the environment. The major residues are rice husk, coffee husk, coir pith, jute sticks, groundnut shells, mustard stalks and cotton staks (Grover, 1996; Khoa, 1999). Briquetting of the husk could mitigate these pollution problems while at the same times making use of this important industrial/ domestic energy resource. The briquettes can be used for domestic purposes and industrial purposes in both rural and urban areas (Singh, 1996).

Billions of tons of agricultural residue are generated each year in the developing and developed countries. This volume of biodegradable wastes can be converted to an enormous amount of energy and raw materials. Agricultural biomass waste converted to energy can substantially displace fossil fuel, reduce emissions of greenhouse gases and provide renewable energy to people in developing countries, which still lack access to electricity (Quartey,). As raw materials, biomass wastes have attractive potentials for large-scale industries and community-level enterprises. This study aims at providing biomass as an alternative to wood charcoal. Using agricultural wastes converted into charcoal briquettes to provide much needed source of cheap fuel that is cleaner in burning. It is also intended to create awareness of agricultural wastes briquettes technology in India and to make use of the technology by small scale entrepreneurs. Agricultural residue includes all leaves, straw and husks left in the field after harvest, hulls and shells removed during processing of crop at the mills.

Briquetting or pelletizing is the process to improve the characteristics of biomass as a renewable energy resource by densification. Densification means less volume needed for the same amount of energy output. Several studies exist regarding the use of compacted biomass as an energy source. Many of these studies are focused on comparing the economical-environmental impact of compacted biomass as a substitute for traditional fuel material with emphasis on the effect of greenhouse gases in which the value of biomass briquettes are highlighted as a cost-effective option to reduce CO and CO2 (Peterson, 2006;Ericsson, 2003; Gustavsson, 1995;Hektor 1998; Schmidt *et al*, 2007). Briquetting often leads to a volume reduction of more than 10 times. For flammable dust, briquetting has been an effective way to reduce the risk of explosions and fires.

Results: Using waste *Artemisia* extracted leaves and other waste, briquettes were made by briquetting machine (fig. 3.17).

Month	**Extracted *Artemisia* leaves (Kg)**	**Other waste (Kg)**	**Briquette output (Kg)**
October	41186	4600	18000
November	35270	2600	56750
January	43618	2700	84600

Table 3.38: Production of briquette using extracted Artemisia leaves and other waste

Along with *Artemisia* leaf waste, soybean waste, boiler ash and coal waste were used to make briquettes. Analyses were done for comparing the Kcal/Kg calculation of coal with bio briquettes.

Boiler input	**GCV Kcal/Kg**	**Daily requirement for running the boiler (MT) ***	**Cost (Rs/Kg)**	**Daily requirement for running the boiler**	**Everyday Savings (Rs.)**
Coal	5000	22	7.89	1,73,580	

Soybean waste (100%)	3641	30.21	4.50	1,35,945	37635
Extracted *Artemisia* leaves	3484	31.57	1.62	51143	122437
Extracted *Artemisia* leaves: Soybean waste (70:30)	3651	30.13	1.88	56544	117036
Extracted *Artemisia* leaves: Coal waste (70:30)	3615	30.43	1.21	36820	136360

Table 3.39: Comparative cost of fuel used in boiler

Costing of briquettes from :

Artemisia extracted leaf waste: 1.62/Kg

Soybean waste :2.50/Kg

Coal waste: 0.25/Kg

The cost of coal per day to run the boiler: Rs. 1,73,580.00

Extracted leaves per day to run the boiler cost: Rs. 51143.00

The savings in everyday : Rs. 1,22,437.00

Savings on the availability of extracted leaves everyday

Extraction plant	**Availability of extracted leaves every year**	**Boiler running by leaves 100%**	**Boiler running by leaves (70:30)**	**Coal**	**Cost of waste**	**Savings**
Extraction plant	365	12 days	17.38 days	3016820	888865	2127955 (Achiev

capacity						ed)
New extraction plant	3600	120 days	171 days	2968 2180	8745453	20936727 (proposed)
Leaves and other waste	10950	-	365 days	6335 6700	18667195	44689505 (proposed)

Table 3.40: Estimated annual saving

Material	GCV Kcal/Kg	Cost (Rs/Kg)	Consumption (Kg)	Costing (Rs)
Soybean waste (100%)	3641	4.08	12451	50,800
***Artemisia* stem waste (100%)**	3641	1.62	1245	20170
Saving	-	-		30629

Saving : 4.47+0.31

Total saving: 4.78 crore/ year

Table 3.41: Comparison of calorific value and cost saving

Sr.no.	Description	Amount (Rs.)
1	Power consumed 36.25 Kwh@ 5.84 Kwh	211.70
2	Man power consumed 4 nos. @ 190	760.00
3	Cooling water expenses 7M3/hr flow	77.00
4	Maintenance cost of machine (considering Rs 75000 year/ 350 MT manufacturing)	214.00
5	Shifting charges from XV to briquette machine	200
6	Shifting charges from machine to boiler	112.00
7	Sale price of artemisinin leaves	50.00
8	Total cost of briquette Rs/Ton	1624.70
9	Cost of briquette Rs/ Kg	160

Further when the production will increase the cost will come down for Sr. 2, 4, 5 & 6.

Table 3.42: Cost of Briquette/Ton with Briquette machine

About 70 biomass briquetting machines were installed in India by 1995. By 2007 the number of briquetting plants increased to 250. As the technology is locally mastered and economically viable, the number is increasing annually. Two biomass briquetting technologies dominate the Indian market: the ram and die machine and the screw machine. These two machines use different processes to densify sawdust and agricultural waste, and the end products also have different densities and shapes. The two types of machines are locally manufactured. A third kind of press, the hydraulic press has not been used in India and is considered unsuitable for Indian raw materials. The most common raw materials for heated-die screw-press briquetting machines are saw dust and rice husk.

Unlike wood charcoal, this briquette charcoal is a smokeless fuel, due to the fact that during carbonization its smoke disappears. The smoke produced by wood charcoal fires in an indoor cooking environment can lead to multiple respiratory illnesses [6,7,8]. It is also one means of getting rid of solid wastes which is hazardous for health. Chopped agricultural wastes cannot be used as a fuel directly, because it produces a lot of smoke. Also it requires a very high expenditure of energy to compress. On the other hand, it is well known that organic matter can be charred. The char briquettes are more efficient in burning with no smoke at all.

Instead of burning agricultural wastes in the fields, using the wastes as a fuel source slows the advance of deforestation by eliminating the need to cut down trees for fuel wood. The other advantage is that since there is no smoke while burning, smoke pollution is reduced for the environment.

Briquette charcoal is viewed as an advanced fuel because of its clean burning nature and the fact it can be stored for long periods of time without degradation. Therefore, a micro enterprise can be formed. Any entrepreneur can create briquette from agricultural wastes and sell them in a local market for personal income. In this way, more money stays within the community rather than being exported for foreign fuels. By turning something that was previously unused into a means by which to produce income, the wealth of individual entrepreneurs and the country in general is increased.

ii. Scopoletin

Plants have a long history as therapeutic tools in the treatment of human diseases and have been used as a source of medicines for ages. In search of new biologically active natural products, many plants and herbs used in traditional medicine are screened for natural products with pharmacological activity. Qinghao is a traditional Chinese medicine prepared from the aerial parts of *A. annua* L. which belongs to Compositae family. It is used as an anti-parasitic and fever relieving agent. Artemisinin was isolated from Qinghao as a major bioactive constituent and has been used to treat malaria. Besides artemisinin, the chemical constituents of Qinghao include volatile oil, terpenes, flavonoids and coumarins. Scopoletin-derived sesquiterpene ethers Sesquiterpene derivatives of 7-hydroxy-6-methoxycoumarin (Scopoletin) are not so common. In this study we estimated the Scopletin using TLC. The estimation was done in the commercial sample of *A. annua* L. which were previously extracted (extracted for Artemisinin) again with solvent. The Scopoletin content of the leaves were calculated from the area calibration curve by this method was found to be 0.04477% w/w (plant dry weight basis). This HP-TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for this plant or its derived products.

The *A. annua* extract (5 µl), when subjected to TLC showed the presence of scopoletin (fig 3.18). A comparison of the spectral characteristics of the peak for standard compound and that of the sample further confirmed the presence of scopoletin in the sample (fig 3.19). Good resolution with symmetrical and reproducible peak was obtained (fig 3.20)

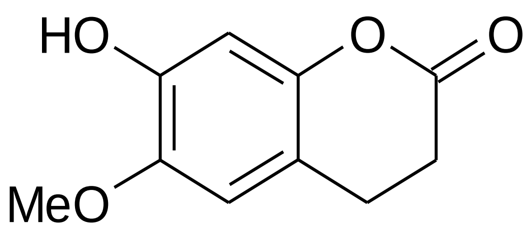


Fig 3.18: Chemical structure of Scopoletin

S. No.	Parameter	Results
1	R_f	0.62
2	Dynamic range (ng spot^{-1})	200 - 700
3	Equation	Y=8549.709+20.799x
4	Slope	20.799
5	Intercept	8549.709
6	Linearity (correlation coefficient)	0.99236

Table 3.43: Validation parameters for quantification of Scopoletin

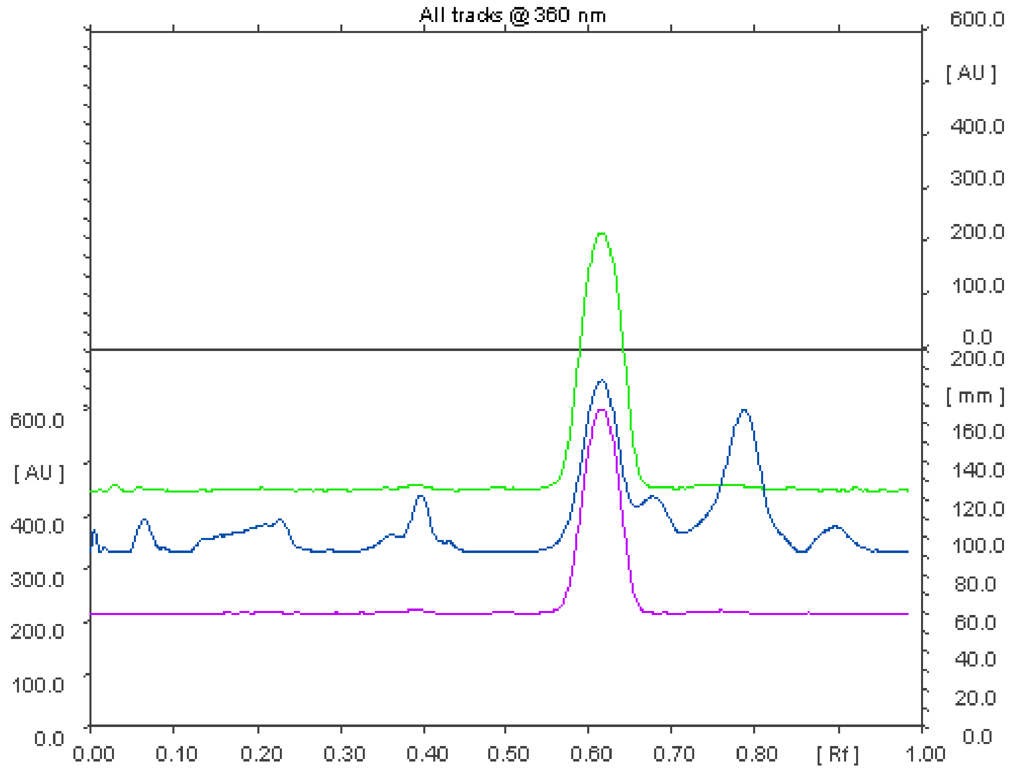


Fig 3.19: ***A. annua*** **extract showing identical peak with standard Scopoletin**

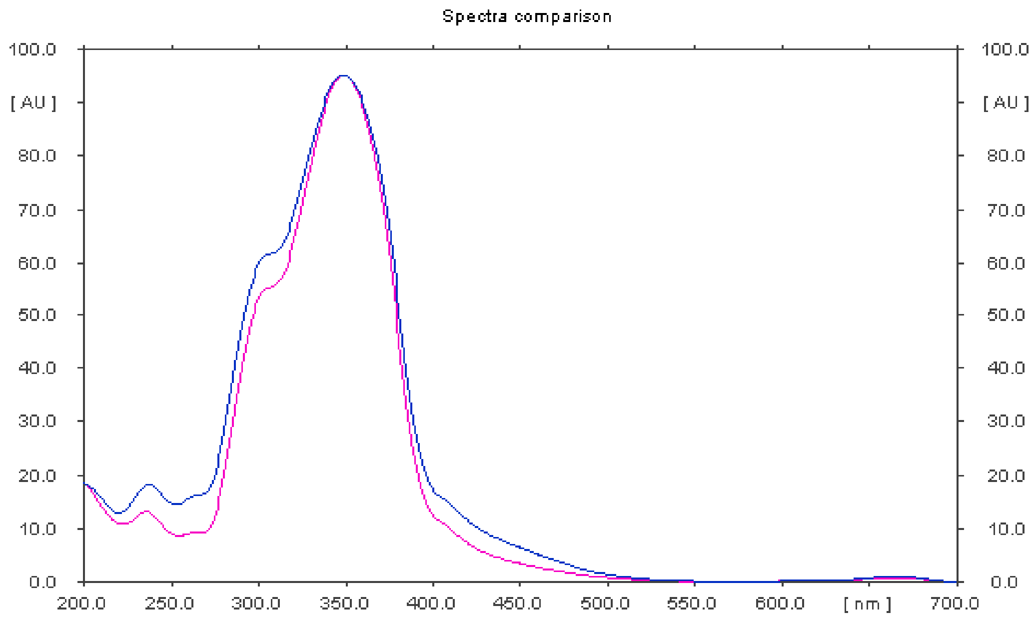


Fig 3.20: Spectral comparison for the peaks of standard scopoletin and *A. annua* extract

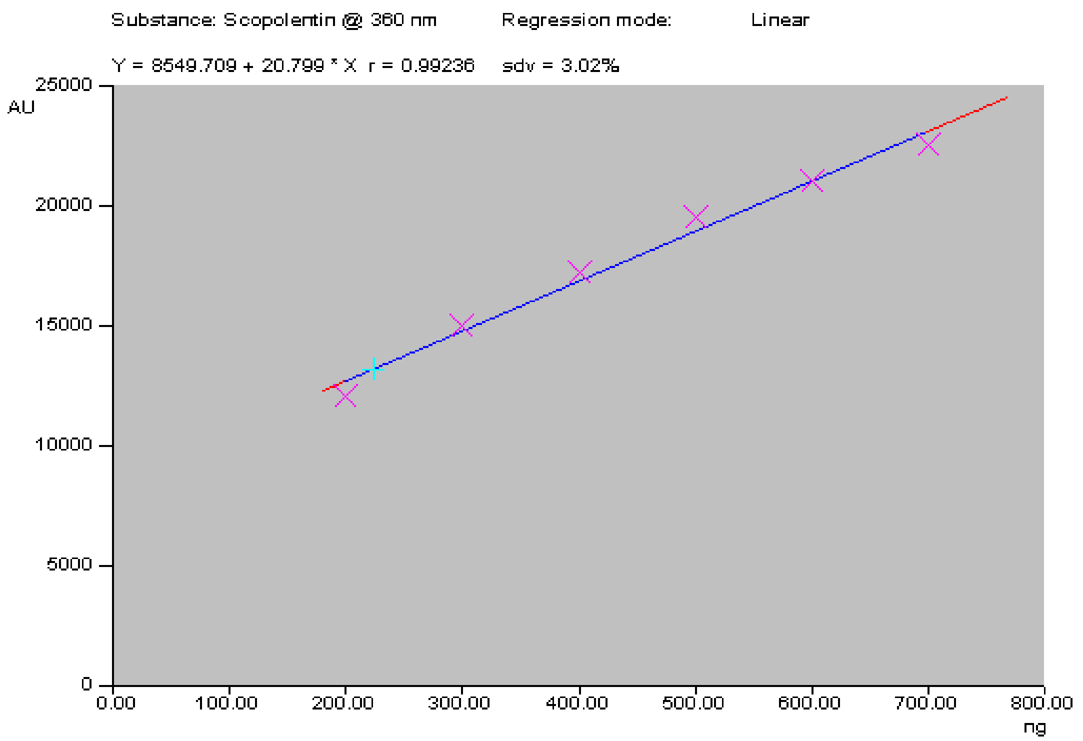


Fig 3.21: Calibration curve of peak area versus concentration for Scopoletin

R_f	Amount/Fraction	Area	X(calc)
0.63	200 ng	12091.80	-
0.62	300 ng	15005.72	-

0.61	**400 ng**	**17225.91**	**-**
0.62	**500 ng**	**19514.45**	**-**
0.61	**600 ng**	**21051.78**	**-**
0.63	**700 ng**	**22565.71**	
0.62	**-**	**13205.57**	**223.85 ng**

Table 3.44: Calibration curve parameters for quantification of Scopoletin in *A. annua* extract

Linearity

The peak area versus concentration plot was found to be linear in the range of 200-700 ng spot^{-1} for scopoletin (fig 3.21). The regression equation and correlation coefficient for scopoletin indicated good linearity (Table 3.43).

Sample analysis

The scopoletin content of the leaves calculated from the area calibration curve (Table 3.44) by this method was found to be 0.04477% w/w (plant dry weight basis). This TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for this plant or its derived products**.**

Discussion

Studies on utilizing the waste obtained from extracting artemisinin from the leaves were also carried out. A compound Scopoletin was detected in the leaves of *A. annua*even after the extraction. Scopoletin (6-methoxy-7-hydroxy coumarin) is one of the coumarins that is present in *Artemisia*. Pure Scopoletin is yellow crystalline powder, the molecular weight is 192 and melting point is 204-206˚C (Vanconcelos, 1998). Ojewole and Adesina (1983) indicated that scopoletin suppressed acetylcholine-induced contractures of the toad rectus abdominis muscle. Cassady *et al*. (1979) demonstrated that scopoletin has anti-tumor activity. Kang *et al*., (1999) showed that scopoletin inhibits the nitric oxide synthesis in a dosedependent manner in murine macrophagelike RAW 264.7 cell stimulated with interferon-y (IFN-y) plus lipo-polysaccharide. Scopoletin is considered as an antiinflammatory medicine (Erazo *et al*., 1997) and as a Hevea hytoalesin (Churngchow *et al*., 2001; Minamikawa, 1963). Some studies evidenced that scopoletin has antifungal synergistic activity (Jurd *et al*., 1971) and it is an antispasmodic component (Behr *et al*., 1967). However, artificial syntheses of artemisinin and scopoletin are costly and infeasible in comparing with Soxhlet solvent extractions of them from nature materials (Lin *et al*., 2006).

A study on allelopathic effect of *Artemisia* on *Brassica nigra* and *Amaranthus spinosus* showed negative effect on growth of *Amaranthus*. The research demonstrated *Artemisia* dry leaf mulch after extraction of artemisinin showed inhibitory effect on *A.spinosus* by reducing seed germination, delaying of seedling emergence and retarding plant growth at higher concentration. While it showed stimulatory effects on *Brassica* which was seen by increase in chlorophyll content and biomass.(Purohit and Pandya, 2011).

Every year millions of tons of agricultural wastes are generated which are either destroyed or burnt inefficiently in loose form causing air pollution. These wastes can be recycled & can provide a renewable source of energy by converting biomass waste into high density - fuel briquettes without addition of any binder. This recycled fuel is beneficial for the environment as it conserves natural resources. For this the biomass briquetting is the main renewable energy resource (Maninder *et al*., 2010).

Briquettes produced from briquetting of biomass are fairly good substitute for coal, lignite, Firewood and offer numerous advantages. This is one of the alternative methods to save the consumption and dependency on fuel wood. Densities fuels are easy to handle, transport and store. They are uniform in size and quality. The process helps to solve the residual disposal problem. The process assists the reduction of fuel wood and deforestation. It provides additional income to farmers and creates jobs. Briquettes are cheaper than coal, oil or lignite once used cannot be replaced. There is no sulphur in briquettes. There is no fly ash when burning briquettes. Briquettes have a consistent quality, have high burning efficiency, and are ideally sized for complete combustion.

Disadvantages of biomass briquetting. High investment cost and energy consumption input to the process. Undesirable combustion characteristics often observed e.g., poor ignitability, smoking, etc. Tendency of briquettes to loosen when exposed to water or even high humidity weather. III. Tissue culture studies (In vitro)

Experiment 8:- Regeneration of A. annua L. plants

In the present study regeneration of A. annua L. plants through leaf explants (fig 3.22) were used for callus and shoot induction.

1. Callus induction

To optimize the culture medium for high frequency callus induction, the whole leaves were cultured with their adaxial surface touching the callus induction medium containing MS salts, vitamins, sucrose (3%), agar (0.8%) and supplemented with different concentrations and combinations of BAP (0.5-1.5 mg/l), NAA (0.5-1.5 mg/l) and Kn (0.5-1.5 mg/l). The explants were maintained under culture room conditions (temperature, 25± 2ºC; light intensity, 2300 lux; photoperiod, 16/8 h day/ night).

On the basis of color two types of callus were obtained, type 1 was light brown and type 2 was light green as shown in fig 3.23 & 3.24. Calluses were induced on all the combinations of culture medium but high frequency of callus induction was observed with the concentration of MS + BAP (1.5 mg/l) + NAA (0.5 mg/l) + Kn (1 mg/l).

2. Shoot regeneration

After proper sterilization, shoot tips and leaf explants of high artemisinin yielding variety of A. annua L. were transferred on shoot induction medium (MS with various combinations of BAP, Kin and NAA).

The maximum shoot initiation was observed after 30 days on MS + BAP (1.0 mg/l)

+ NAA (0.05mg/l) (Fig. 8) and very less shoot induction did occur on other combinations of culture medium. Frequency of shoot induction from callus was very less in compare to direct shoot induction from leaf explants (fig.3.24; table 3.45).

3. Shoot multiplication

After obtaining of shoots, the shoots were separated and transferred to shoot multiplication medium (MS + BAP (0.5-2.0 mg/l + NAA (0.01-0.08 mg/l). Highest numbers of shoots were obtained with application BAP (2.0 mg/l) + NAA (0.05 mg/l) after 2 weeks (Fig. 3.25).

4. Shoot elongation and root regeneration

Clusters of shoots were excised from the base of the leaves and transferred on shoot elongation medium. After two weeks, the apical dominance was observed in shoots with many plantlets (Fig. 3.25). After attaining proper shoot length (Fig. 10), the shoots were transferred on rooting medium. The root regeneration percentage was 100% and number of roots per plantlet was 5.6 on MS medium containing 0.5mg/L NAA (fig. 3.26).

5. Hardening of tissue culture raised plantlets of A. annua L. plants.

In vitro rooted plantlets were planted in plastic pots containing mixture of vermiculite, perlite and soilrite (1:1:1) and then transferred to polyhouse for hardening. After 20 days the hardened plants (fig 3.27 A) were transferred to experimental field 12m2 (4m × 3m plot), row to row distance of 50cm and plant to plant distance of 50cm (fig 3.27 B & C). Each plot was supplied with 80 kg N, 40 kg P, 40 kg K and 30 kg S ha-1 and 15 tonnes ha-1 vermicompost. The plants were irrigated once in a week till the harvesting (fig 3.27 D to F).

6. Artemisinin content in leaves of tissue culture raised plants.

Artemisinin contents in the leaves were determined with the help of HPTLC based method at the rooting stage of A. annua L. plants. Artemisinin contents were in the range of 0.03-0.1% on dry weight basis. HPTLC chromatograms are shown in fig. 3.6.

Growth Regulators Conc. (mg/l) Response (%)Y Average No. of shootsXT

BAP 0.25

0.5

0.75

1.0

1.25

1.5 26.5

39.4

57.8

78.9

65.2

42.5 0.7 ± 0.421

0.82 ± 0.562

1.41 ± 0.745

3.2 ± 0.963

2.4 ± 0.832

1.6 ± 0.762

Kin 0.25

0.5

0.75

1.0

1.25

1.5 6.3

19.5

23.4

37.4

5.8

00.0 0.3 ± 0.091

0.52 ± 0.123

0.71 ± 0.212

1.2 ± 0.461

0.4 ± 0.134

-

NAA 0.1

0.2

0.5 32.2

7.6

00.0 0.43 ± 0.132

0.03 ± 0.002

-

BAP/NAA 1.0/0.05

1.0/0.1 78.9

35.5 3.46 ± 0.812

0.63 ± 0.132

Kin/NAA 1.0/0.05

1.0/0.1 37.4

16.3 0.53 ± 0.132

0.13 ± 0.022

LSD 1.533

(XMean ± standard error.Interval of confidence 95%.,YData are mean of 6 replicates.

TMean separation by LSD.,ZRated after 30 days of culture.)

Table 3.45:. Effect of growth regulators of in vitro shoot induction in A. annua L. from leaf explants on MS medium

Fig. 3.22: Leaf explant placed on direct shoot induction medium.

Fig.3.23 : 3 weeks old culture showing brownish callus.

Fig.3.24: 4 weeks old culture showing greenish callus.

Fig 3.25. : Multiple shoots on multiplication medium after 2 weeks.

Fig 3.26: A. annua L. plantlets on elongation medium.

Fig. 3.27 A. Artemisia cultures in lab B. & C. plantlets in polyhouse during hardening. D. & E. plants in field after hardening. F. mature plant

Discussion

Conventional agricultural vegetative propagation methods to improve the plant Artemisinin yield and cross breeding plants from different localities have been practiced and utilized, but these are not enough to meet the world demand at a commercial level. Growing of the plant in vitro in tissue culture coupled with hairy root culture has been harnessed in order to increase production of Artemisinin. A. annua is easily propagated in vitro by standard protocols. Cytokinins increase shoot proliferation but decrease rooting and increase vitrification. A. annua can be grown and propagated by microcuttings in a hormone-free medium. Artemisinin is produced in shoots in vitro and is enhanced by the presence of roots. None or trace levels of artemisinin are found in roots, callus, cells, or cell free medium (Delabays et al., 1993). There is no evidence that in vitro production of artemisinin in tissue culture will ever be commercially feasible. However, researchers have succeeded in producing artemisinic acid (a precursor) in yeast cultures and artemisinin production, through its precursor, in bioreactors might be a future avenue to explore.

To date, plantation of A. annua are the main source of commercial artemisinin. However, the artemisinin content in plants is highly variable ranging from 0.01- 0.42% (mg/g, dry weight) (Ferreira et al., 1997). Unsuccessful attempts have been made to produce artemisinin through callus culture (He et al., 1983) or shoot culture (Fuzele et al., 1991). Micropropagation and organogenesis of different A. annuaspecies have been previously established by using several parts of plants, in order to obtain large number of plants, such as A. scorpia (Aslam et al., 2006); A. vulgaris (Govindaraj et al., 2008); A. mutellina (Mazzetti and Donato, 1998). An efficient in vitro method for multiple bud induction and regeneration has been developed in A. annua, using stem explants (Lualon et al., 2008); or by using young inflorescence segments. Dangash et al., 2015, established an improved protocol for direct shoot regeneration of A.annua L. using leaf explants on MS medium supplemented with BAP and NAA resulting in a rapid and high number of shoots per explants.

In vitro direct organogenesis of different parts of A. annua was investigated, in this research, to obtain a large number of plants true to type. The ultimate goal was the multiplication of the selected clones with high levels of artemisinin and the utilization of the protocol in any future genetic transformation of this species. The first incidence of bud initiation was noted after 8 days in the form of small protuberances, then the meristematic domes of buds were observed after 12 days of culture, the formation of buds was recorded after 15 days and in many cases the developing buds fused together. The number and the size of shoot buds increased over the course of time (fig 3.25.).

The investigations showed that the bud formation occurred on medium MS with (BAP and Kin) at different concentrations were effective to induce morphogenesis in A. annua. The regeneration frequency depended on the kind of explants, source and the cytokinins concentrations. Similar results were obtained on plant propagation of Artemisia vulgaris (Sujatha and Kumari, 2007). The morphogenetic capacity of different kinds of explants was increased with increasing cytokinins concentrations (Table 3.45). A maximum of shooting response was observed for medium containing 1.5 mg/l of BAP, with the highest number of shoots formed per explant among all treatments tested.

The efficiency of shoots formation from different explants (petioles, leaves and internodes) taken off from mother plants was different under various cytokinins (BAP and Kin) concentrations (fig 3.25 and 3.26). The capacity of bud formation of explants varied between different kinds of explants. (table 3.45). A maximum shooting formation 81% from petiole and internodes was observed with the increasing of BAP and Kin in media. The number of shoots formed increased also with the increasing of cytokinins concentrations. Media supplemented with BAP at the same concentrations gave a lesser morphogenetic response than media supplemented with Kin at the same concentrations.

A very good relationship between the age and the source of explants and their morphogenetic capacity was observed. The explants from leaves and petioles of the plants seedlings induced higher regeneration percentage and number of buds compared to explants taken from the three months old mother plants. Shoot obtained from different explants were transferred to multiplication medium (Table 3.45), where further auxiliary shoots were developed. Shoot subcultured after every 4 weeks. The multiplication rate of shoots and the average number of shoot length increased with the number of subcultures (Fig.3.26).

The stimulating effect of subculture on shoot multiplication and elongation might be ascribed to their rejuvenation influence on in vitro culture. Several authors reported the multiplication of A. annua by using high concentration of BAP (Nam-cheol et al., 1992; Geng et al., 2001;

Lee et al., 2003). Similar results were obtained on other species of A. annuasuch A. Scorpia (Aslam et al., 2006), and A ranunculus (Mackay and Kitto, 1988). Liquid medium was used for mass propagation of A. vulgaris, which permit a big number of shoot proliferations during 4 weeks (Govindaraj et al., 2008).

In our case, the vitrification in multiplication medium was frequently observed in A. annua. However it was reduced by transferring the shoots to medium containing half macro elements of MS.

The present study was undertaken to determine the feasibility of employing tissue culture techniques to obtain artemisinin at costs affordable by the people of countries where malaria is endemic. We have been able to induce callus, root, shoot, or whole plant regeneration using explants from stems, leaves, and flower buds (table3.45, fig. 3.23). The method would thus as- sure a constant supply of the plant material for investigative purposes. Preliminary results with various culture systems indicated the presence of artemisinin in roots and rooted plantlets derived from leaf explants or callus cultures. Callus and unrooted shoots did not produce the compound in amounts detectable in our highly sensitive assay system (He et al., 1983; Staba, 1988; Nin et al., 1996). Initial results indicated also that artemisinin is present in the culture fluid from liquid suspension cultures of callus cells. This latter observation indicates the potential of this methodology for large-scale production of this drug. While this micro scale exulting the media and other culture components do not predict the cost-effective conditions to optimize the production of artemisinin in this method. We are also trying to obtain indicate that it is feasible to produce artemisinin more friable callus and to achieve better artemisinin in batch or continuous suspension separation to promote their rate of suspension cultures.

More studies aiming at improving artemisinin production at hairy root culture level (Waraporn et al., 2007) and by genetic manipulation, targeting genes involved in artemisinin production such as a study by Zhang et al. (2008), still continue to increase production of these highly significant compounds. From this, it is significantly that biotechnology indeed is a very important tool in increasing production of important secondary metabolites in plants which are needed for our well-being and that it can be coupled with other techniques from other fields to produced artemisinin.

It can be concluded that results obtained in this study, permit the development of a mass propagation protocol with a good multiplication rate and a high regeneration percentage from different kinds of explants, leaves, petioles, internodes and cotyledons of A. annua. Moreover the findings attained here might be useful in maintain the quality and yield of artemisinin in plants true to type in A. annua.