BIOCHEM CELL ARCH Vol. 16, Supplement 1, pp. 306-310, 2016 | ISSN 0972-5075

Cultivation of *Artemisia annua* in North Indian Gangetic Plains for Antimalarial Artemisinin

Sugandha Tiwari
Department of Botany, D.G.P.G. College, C.S.J.M. University, Kanpur
e-mail: sugandhatiwari7@gmail.com

ABSTRACT

Artemisia annua L. (family Asteraceae) is native to China and Vietnam but is now cultivated in many parts of the world, including India. This plant was traditionally used in China to cure fevers. Ethno-pharmacological applications led to the isolation of the active principle , artemisinin which is a sesquiterpene lactone with an endoperoxide group. Several artemisinin derived drugs namely artemether, arteether and artesunate obtained as methylether, ethylether and hemisuccinate esters of dihydroartemisinin have proved to be effective against multidrug resistant *Plasmodium falciparum* malaria and used in artemisinin based combination therapy.

Artemisia annua is a cross pollinated and determinate species. The plants die after flowering and seed set. Therefore economically it is essential to select high artemisinin containing genotypes in the vegetative stage and to improve the yield by multiple harvests. The present study was undertaken to cultivate *A. annua* and to determine the developmental stage of *Artemisia annua* plants having highest artemisinin content in North Indian Gangetic plains. The seeds of *A. annua* variety Asha, Suraksha and Jeevanraksha were sown in raised nursery beds in the month of December. Two month old seedlings were transplanted in the experimental plots in the month of February. No pesticide and fertilizers were applied .The experiment was laid out in a randomized block design with four replications. Samples of *Artemisia annua* plants var. Asha, Suraksha and Jeevanraksha were harvested in the months of June, July and August when the plants were 180, 210 and 240 days old respectively for the estimation of artemisinin. Highest artemisinin content was found in the samples harvested in the month of July when the plants were 210 days old with plant density of 41,650 plants / hectare and plant to plant distance of 50cmx30cm. From the results it is also observed that there is only 0.008% decrease in the artemisinin content in the samples harvested in the month of August, so we can maximize artemisinin yield by three harvests, first in the months of June, second in July and a third harvest in the month of August to increase the economics of artemisinin production in North Indian Gangetic plains.

Keywords : Artemisia annua, artemisinin, North Indian Gangetic plains, cultivation

INTRODUCTION:

Artemisia annua is an indigenous medicinal plant of China and Vietnam, used to treat fevers. It is mentioned in the pharmacopoeia of People's Republic of China (Tang W. & Eisenbrand G.,1992) . A sesquiterpene lactone , artemisinin was isolated as the plant's active principle. Artemisinin has proved to be a safe and effective antimalarial agent against multidrug resistant *Plasmodium falciparum* malaria which is the cause of 300-500 million clinical attacks and 1-2 million deaths each year. It is the fourth leading cause of death and the parasite has become resistant to conventional antimalarials like Chloroquine, Quinine, Mefloquine, Pyrimethamine etc. According to WHO artemisinin based combination therapy (ACT) is the only

reliable treatment option for multidrug resistant *Plasmodium falciparum* malaria and to control the spread of the disease under its global malaria eradication programmes. Artemisinin based combination therapies (ACTs) involving two or three drugs are recommended by WHO to control multidrug resistant malaria (WHO 2003,2005). Artemisinin and its semisynthetic derivatives are believed to kill asexual and sexual intra erythrocytic stages of *Plasmodium* by interacting with the heme discarded by the proteolysis of ingested hemoglobin (Jefford, 2001). Artemisinin and its derivatives are also found to be effective against schistosomiasis caused by parasite *Schistosoma japonica, S. mansoni* and *S. haematobia* causing 1.0 -1.5 million disabilities each year (Shuhua, *et al*., 2002). Oral administration of artemisinin has also been found to prevent and delay the development of 7, 12-dimethylbenz (a) anthracene (DMBA) induced breast cancer in rat (Lai and Singh, 2006). Total synthesis of artemisinin that have been worked out are not economical for commercial production (Schmidt and Hofheinz, 1983), therefore, the crops of high artemisinin containing genotypes of *A. annua* remain the only practical source of artemisinin. Artemisinin content varies during different developmental stages of the plant and ranges between 0.01% to 1.5%.

MATERIALS AND METHODS:

A. annua is a an annual determinate shrub and dies after seed set. Height ranges between 0.75 metres to upto 2.0 metres in difeerent varieties. Branches arise alternately and are spreading. Leaves are dissected, aromatic and contain biseriate glandular trichomes that sequester artemisinin and essential oil. After about five months of sowing, the plants enter reproductive phase. The inflorescence is capitulum, typical for family Asteraceae. Capitula arise in loose panicles bearing numerous central bisexual disc florets and marginal pistillate ray florets. Biseriate glandular trichomes are present on both receptacle and florets. In India *A. Annua* plant was introduced by Central Institute of Medicinal and Aromatic Plants in 1986 and adopted for cultivation in Kashmir valley for its essential oil. Out of the introduced material, a variety called 'Asha' was developed and released. *A. annua* being cross pollinated, the variety "Asha" was highly heterozygous and the yield of artemisinin content of the plants varied from 0.001 to 0.2%. Therefore the use of this variety for commercial production of artemisinin appeared unviable. Recurrent selection principle was adopted to improve artemisinin content of the population . Repeated cycles of selection were carried out. Superior genotypes having high artemisinin were identified and selected. Plants containing higher artemisinin were intermated and improved progeny selected (Sugandha Tiwari, 2011). The variety "Suraksha" was the product of recurrent selection. Variety "Jeevanraksha" represented an isolated population of *A. annua* selected for high artemisinin content and is late flowering (Sushil Kumar et al., 1999) .

A field experiment was conducted in semi arid subtropical climate of North Indian Indo Gangetic plains for the cultivation of *Artemisia annua*. In the subtropical climate *A. annua* is sown in winters and completes its vegetative growth in 5-6 months. The plant requires 12- 16 hours of photoperiod for flowering and completes its reproductive phase in summer season.

Propagation:

The plant is propagated by seeds. The seeds of *A. annua* plants were collected during sunny days and dried in shade in well aerated place. The seeds were stored in paper bags or air tight glass containers till the time of sowing.

Sowing of the seeds: Raised nursery beds were prepared with water channels in between . Soil consisted of sandy loam with a pH ranging between 6.8 – 8.1. The chemical composition of soil was : 0.50% organic matter, 0.25% total Nitrogen, 13.5% available Phosphorus and 110 Kg per hectare exchangeable Potassium.

The seeds of *A. annua* variety Asha, Suraksha and Jeevanraksha were sown in raised nursery beds in the month of December. The nursery beds were kept moist for first four to five days by sprinkling with water and later by flood irrigations.

Transplantation :

Two month old seedlings of variety Asha, Suraksha and Jeevanraksha were transplanted in the experimental plots in the month of February. No pesticide and fertilizers were applied .The experiment was laid out in a randomized block design with four replications. The treatment consisted of five planting densities of 12500, 20825, 41650,62500 and 83300 plants per hectare with plant to plant spacing of 100x80, 50x80, 50x30, 50x20 and 25cmx 30cm respectively. Following a light irrigation at the time of planting, flood irrigation was done four to five times whenever required. Weeding was done two to three times during the growth of the crop. The plants of *Artemisia annua* were hardy and were generally not infected with pathogens. The crop matured in six to seven months time.

Harvesting:

Artemisia annua plants were harvested three times- in vegetative stage, pre anthesis and post anthesis stage. Plants were harvested in the months of June, July and August when the plants were 180, 210 and 240 days old respectively for the artemisinin. Maximum herb yield, artemisinin content and oil yield is obtained in the plants at the time of anthesis. It is advisable to harvest the plant during sunny days and during noon when the moisture content in the atmosphere is minimum.

Post harvest storage :

The harvested leaves, inflorescence and shoots were shade dried in well aerated place and stored in airtight containers until extraction.

Extraction of artemisinin and essential oil :

For the estimation of artemisinin, TLC densitometry method was utilized. 1.0 g of dried sample was crushed in 5 ml n- hexane, filtered, evaporated in microwave and re-dissolved in 0.5 ml n-hexane. 5µl of the extract solution was spotted on 0.25 mm thick 60 F 254 silica gel plate (Merck- Germany). Mobile phase consisted of n-hexane: diethyl ether in the ratio of 1:1.Artemisinin spots were visualized by immersing the plates in a freshly prepared mix of glacial acetic acid: conc. sulphuric acid: anisaldehyde (50: 1.0: 0.5) followed by heating of the plate at 105°C for 10 minutes to visualize the pink colour of artemisinin (Gupta et al.,1996) .

Estimation of essential oil

For the estimation of essential oil 200 g fresh leaf samples were cut into small pieces and put in 2 litre capacity round bottom flask with 700 ml water.The herb was hydrodistilled for about two and a half hours in the Clevenger's apparatus and oil collected.

Result and discussion:

Artemisia annua is cross pollinated and determinate species. The plants die after flowering and seed set. In North Indian Gangetic plains, seeds must be sown in the winter months between October to December to get a summer crop of *Artemisia annua*. Economically, it is essential to select high artemisinin containing genotypes in the vegetative stage and to improve the yield by multiple harvests. Samples can be harvested in vegetative stage, pre anthesis and post anthesis stage for better yield of artemisinin. Highest artemisinin content was found in the samples harvested in the month of July when the plants were 210 days old and in flowering stage. Plant density of 41,650 plants / hectare and plant to plant distance of 50cmx30cm

favoured plant growth and artemisinin yield. From the results it is also observed that there is only 0.008% decrease in the artemisinin content in the samples harvested in the month of August, so we can maximize artemisinin yield by three harvests, first in the months of June, second in July and a third harvest in the month of August to increase the economics of artemisinin production in North Indian Gangetic plains. Maximum artemisinin content was recorded in the variety Jeevanraksha and ranged between 0.5 % to 1.0 %. This variety can be adopted for commercial cultivation by farmers for higher artemisinin content and good profit. The average essential oil content isolated from inflorescence of this variety was also highest (0.6%) as compared to Asha (0.3%) and Suraksha (0.3%).

Table : Comparison between *A. annua* variety Asha, Suraksha and Jeevanraksha

Artemisia annua Variety	Flowering	Minimum artemisinin content (%)	Maximum artemisinin content (%)	Average Essential oil %	
				Leaves	inflorescence
Asha	Early	0.001	0.18	0.2	0.3
Suraksha	Early	0.01	0.33	0.2	0.3
Jeevanraksha	Late	0.5	1.00	0.2	0.6

(a) Nursery bed

(b) Plants transplanted in the field

(c) *A. annua* variety Suraksha

(d) *A. annua* variety Jeevanraksha

(e) *A. annua* Leaf with trichomes

(f) *A. annua* Inflorescence

References :

1. Charles W. Jefford. 2001. Why Artemisinin and Certain Synthetic Peroxides are Potent Antimalarials. Implications for the Mode of Action. *Current Medicinal Chemistry*, **8(15)** : 1803-1826.
2. Gupta, M.M., D.C.Jain, R.K. Verma and A.P. Gupta. 1996. A rapid analytical method for the estimation of artemisinin in *Artemisia annua*. *Journal of Medicinal and Aromatic Plant Sciences* , **18** : 5-6.
3. Lai H, Singh NP. 2006. Oral artemisinin prevents and delays the development of 7, 12 dimethylbenz(a) anthracene (DMBA) induced breast cancer in the rat. *Cancer Letters* , Jan 8, **231(1)** :43 8.
4. Schmid, G., and W. Hofheinz. 1983. Total synthesis of qinghaosu. *J. Amer. Chem. Soc.* , **105** : 624-625.
5. Shuhua, X. *et al*. 2002.Recent investigation of arteetther, a novel agent for the prevention of Schistosomiasis japonica, mansoni & haematobia. *Acta Trop.*, **82** : 175-181.
6. Sugandha Tiwari. 2011. Effect of developmental stage and plant spacing on artemisinin and essential oil yield in Artemisia annua plants. *Trends in Biosciences*, **4** (2) : 221-223.
7. Sushil Kumar , Suchitra Banerjee , Sugandha Dwivedi, M.M. Gupta, R.K. Verma, D.C. Jain, S.P.S. Khanuja, A.K. Mathur, G.D. Bagchi, Mehar Zehra, V.K. Mehta, A.A. Naqvi, Shilpi Paul, Gobind Ram, Muni Ram, D. Saikia, R.S. Sangwan, T.R. Shanta Kumar, A.K. Shasany, M.P. Darokar, A.K. Singh and Archana Singh.1999.Registration of Jeevanraksha and Suraksha varieties of antimalarial medicinal plant *Artemisia annua. Journal of Medicinal and Aromatic Plant Sciences*, **21** : 47-48.
8. Tang W. & Eisenbrand G.1992. Chinese drugs of plant origin, Springer- Verlag. Berlin, pp 159-174.
9. WHO document. 2003. Access to antimalarial medicines: Improving the affordability & financing of artemisinin based combination therapies, WHO document.
10. World Malaria Report. 2005. Prepared by Roll Back Malaria, World Health Organization, UNICEF.