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Impact of planting date on growth, biomass and artemisinin yield of *Artemisia annua* L. in Malwa plateau agro-climatic zone of Madhya Pradesh, India

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Abstract
Artemisa annua L. is an annual aromatic herb with effective antimalarial properties due to the presence of artemisinin. The intention of the present study was to establish plant survival, growth attributes and yield attributes of *A. annua* cv CIM–Arogya with different transplanting months in two cropping seasons under sub-tropical climatic conditions of Madhya Pradesh, India. During study it was observed that the artemisinin yield was maximum (34.03 T/ha) in the plants which were transplanted in month of December and minimum (17.93 T/ha) in the February transplanted plants. It is observed that the crop transplanted in the December is more profitable, as it has more artemisinin content. December transplanting of *A.annua* is suitable for Malwa region of Madhya Pradesh, and it also opens new areas for cultivation of this crop in the country. *A.annua* contractual cultivation is already adopted by the farmers in Uttar Pradesh, Uttarakhand and other states.

Keywords: artemisia, artemisinin, planting date, sub-tropical region, yield

Introduction

Artemisia is one of the largest and most widely distributed genus of 100 genera in the tribe Anthemideae of Asteraceae. Plant is annual, aromatic, green, glaborous or with scattered, small hairs. It is a traditional medicinal herb,native to China and widely cultivated in Asia, America and Europe (Ozguven *et al*., 2008) [3]. *A. annua* contains many biologically active compounds; the most important is a sesquiterpene lactone with an endoperoxide bridge called artemisinin. Together with its semi-synthetic derivatives such as arteether, antemether, artesunate and dihydroartemisinin, this compound has been established as the most potent antimalarial drug and possesses activity against drug resistant strains of the malarial parasite (*Plasmodium falciparum*). Currently, artemether is recommended by the World Health Organization (WHO) for resistant and cerebral malaria (Wyk and Wink, 2004) [5, 6].

The foliage and inflorescence of *A. annua* plants also yield an essential oil, which has potential to be used in perfumery, cosmetics and aromatherapy and has also been reported to possess antifungal and antimicrobial activities (Woerdenbag *et al*., 1993) [9]. As the synthetic production of artemisinin is not feasible the only viable source of artemisinin is from the plant, *A. annua*. Artemisinin in the leaves of *A.annua* ranges from 0.01% to 1.4% (Wright, 2002) [10]. The content are affected by numerous factors such as geographical conditions, harvesting time, temperature, fertilizer application, population density and age of harvesting (Wright, 2002; Delabays, Darbellay and Galland, 2002) [10, 2]. There is a large variation in biomass, artemisinin and essential oil content of plant harvested at different age (Wright, 2002) [10].

There is no research effort to determine the effect of planting date on growth, biomass and artemisinin yield of *A.annua*. Hence the experiment was designed with the objective of determining the appropriate time of planting.

Material and Methods

The field experiment was conducted at the Ipca Laboratories Ltd, Ratlam, and Madhya Pradesh, India in the year 2015-2017. The treatments consisted of date of transplanting of *A.annua* at monthly intervals over a period of different cropping seasons. The seeds of the cultivar were obtained from CIMAP, Lucknow India. Seedlings were transplanted when they were 45 days old. Transplanting was done in plots of 3m x 2m replicated thrice in a randomized blocked design at a spacing of 50cm x 30cm. the recommended dose of compound fertilizers, N:P:K at 50:50:50 kg/ha, respectively was applied. Data collection for field experiment was carried out by taking random sample of net plots, ten plants were randomly selected. These selected plants were tagged as per treatment. All pre harvest observation was recorded from these selected plants. Height of ten randomly selected plants for each treatment was measured from base of the plant to tip of the plants. Average of height was worked out from the observations. This observation was recorded at 30, 60, 90 and 120 days after transplanting. The AMS% (Artemisinin) was checked at regular intervals of 15 days. It was extracted with 20 ml Hexane fraction pooled and concentrated under reduced pressure. Residues were dissolved in1 ml of methanol. Scanning and quantification of spots was performed at 540 nm in absorbance/ reflectance mode with Camag TLC. Methanolic extracts of each sample were applied on a TLC plate. The TLC plate was developed and analyzed for artemisinin present in the sample solutions. All determination was performed in triplicate. Content of artemisinin was expressed as % on dry weight basis.

Conclusion

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) (Figure 1). 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) (Figure 2). 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% (Figure 3) 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) (Figure 4).

Minimum and maximum temperatures in different months of transplantation of *A. annua* were found fluctuating (Figure 5). 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 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.

A. annua used in Chinese traditional medicine for centuries has become an important part of the solution where malaria has become resistant to other medicines. Artemisinin based combination therapies have been recommended by WHO since 2001 in all countries. Like in every medicinal plant, artemisinin content and efficacy are subject to climatic, geographical and environmental conditions. High temperatures can damage the quality of plant.

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) [1] 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 *A. annua* 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.*, (2011) [4] 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. annua*. 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) [7]. Environmental variations, such as light, temperature and availabilities of water and salt were reported to alter the artemisinin yield (Weathers *et al.*, 1994) [8].

From the above study, it can be concluded that December is the best month for cultivation of *A. annua* in M.P region. Artemisinin content and yield are comparatively high in plants transplanted in December.

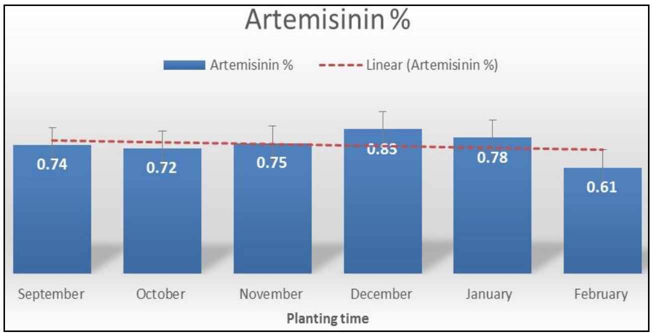

Fig 1: Artemisinin % in leaves of the plants transplanted in different months.

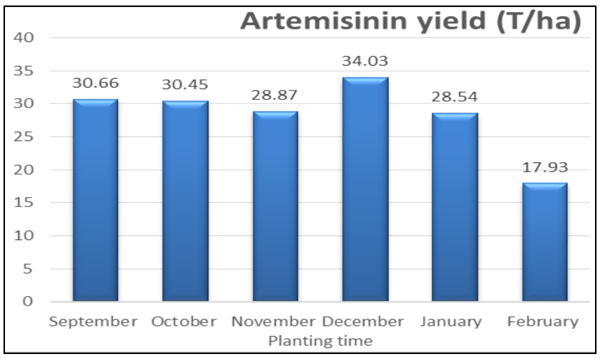

Fig 2: Artemisinin yield (T/ha) in leaves of the plants transplanted in different months.

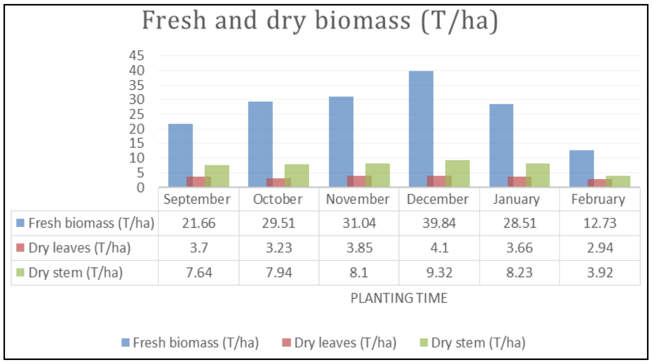

Fig 3: Fresh and dry biomass in leaves of the plants transplanted in different months.

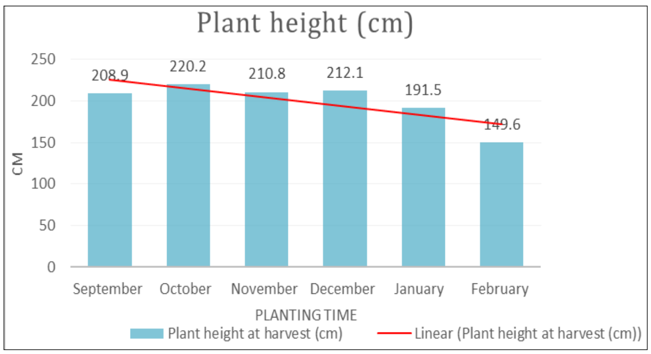

Fig 4: Height of the plants transplanted in different months.

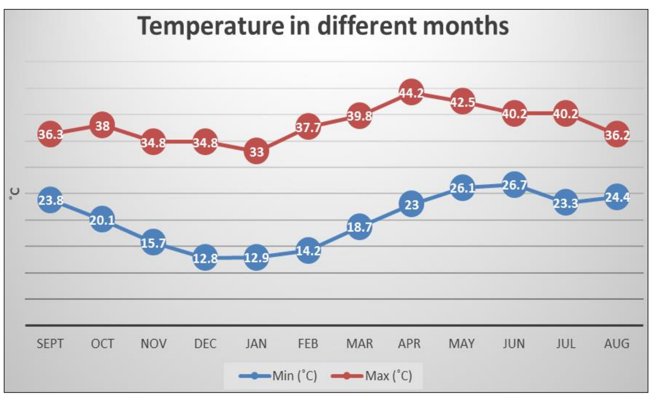

Fig 5: Minimum and maximum temperatures in different months.

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