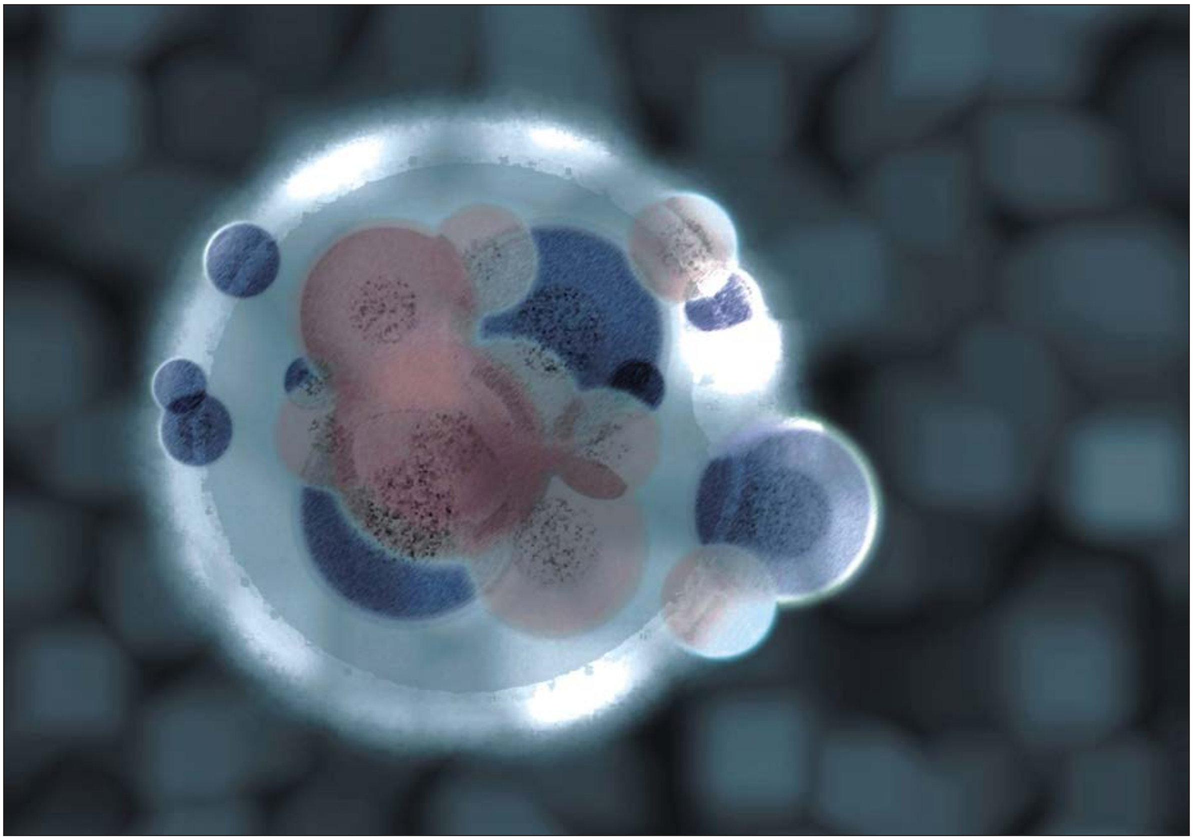

Journal of Biological Sciences

ISSN 1727-3048

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 13 (6): 499-506, 2013
ISSN 1727-3048 / DOI: 10.3923/jbs.2013.499.506

An Attempt to Enhance Select Secondary Metabolite of *Artemisia annua* L.

[1]Afaq Ahmad Malik, [1]Sanghmitra Suryapani, [1]Javed Ahmad,
[1]Shahid Umar, [2]M.Z. Abdin and [3]S.R. Mir
[1]Department of Botany,
[2]Centre for Transgenic Plant Development,
[3]Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi 110 062, India

Abstract: Artemisinin and its derivatives are potent anti-malarials and have become essential components of Artemisinin Based Combination Therapies (ACTs) for malarial treatment. Over 1 million deaths and 300-500 million cases of malaria are reported annually. Production of artemisinin is less as regards its world demand and access to ACTs is still limited in worst hit countries. Economics of artemisinin yield from *A. annua* crops determines the cost and availability of ACTs worldwide. Artemisinin yield from *Artemisia annua* depends on a number of factors including improved agricultural practices. An experiment was conducted in this regard to assess the impact of organic residues from urban areas and industries on *A. annua* L. Enhancement in artemisinin content, artemisinin yield, herb dry yield and plant characteristics were observed by the application of industrial wastes. Highest artemisinin content in leaf (0.53%) was found at flower initiation stage and highest artemisinin yield (29.97 kg ha^{-1}) was obtained from sewage sludge biosolid treatment. The study reveals that organic waste amendments enhance herbage yield and artemisinin content so these, can be utilized for the commercial production of *A. annua* with additional benefits of mitigation of environmental pollution caused by the accretion of these large quantities.

Key words: Artemisinin, malaria, organic farming, waste management

INTRODUCTION

Artemisia annua (Asteraceae), commonly known as sweet or annual wormwood, originally native to Eastern Europe and China, is now cultivated in China, India, Kenya, Romania, Tanzania and Vietnam (WHO, 2006). It is a strongly fragrant annual herb and has a great medicinal value due to the presence of artemisinin, a sesquiterpene lactone, extracted from its aerial parts especially the leaves. Other sesquiterpene lactones isolated from *A. annua* and related to artemisinin are arteannuic acid (qinghao acid, artemisic acid, artemisinic acid), 6,7-dehydroarteannuic acid, arteannuin B, epi-deoxyarteannuin B, artemisitene and deoxyartemisinin. Artemisinin and its related compounds are more efficient, quicker and less toxic than the conventional chloroquine in treating malaria (Klayman, 1985; Abdin *et al*., 2003). In addition, they are also effective against hepatitis B, some infectious diseases and have also been shown to possess potent anti-cancerous properties (Efferth *et al*., 2001; Romero *et al*., 2005; Sen *et al*., 2007).

The demand of artemisinin outpaces supply as it is extracted from *A. annua* only (Weathers *et al*., 2006). Scientists across the globe have attempted to increase artemisinin production through chemical and biological synthesis and genetic engineering in *A. annua* (Avery *et al*., 1987; Van Geldre *et al*., 1997; Ro *et al*., 2006). Results obtained are not satisfactory either because of poor yields or because of the high cost in chemical synthesis or complex nature of the gene regulation and expression in artemisinin biosynthesis. Some viable approaches include (1) its collection from wild, (2) agro-technology to improve biomass as well as drug component and (3) cropping of *A. annua* at large scale. Commercial cultivation of *A. annua*, to maximize artemisinin yields, has been studied (Nguyen *et al*., 2011). However, artemisinin yield depends on the above ground biomass of the plant which is largely influenced by the soil characteristics. Earlier we have reported that *A. annua* responds well to chemical, organic and bio-fertilizers in terms of oil quality (Malik *et al*., 2009, 2012). The present study was proposed to assess the impact of Sewage Sludge Biosolid (SSB), Composted Sugarcane Pressmud (CPM) and Farmyard Manure (FYM) on growth characteristics, artemisinin content and yield of *A. annua* with a view to (1) Increase artemisinin production, (2) Maintain the ecosystem of the soils which has been adversely affected by the continuous use of chemical fertilizers and (3) Mitigate environmental pollution caused by the accretion of large quantities of municipal wastes and organic industrial residues.

Corresponding Author: Javed Ahmad, Department of Botany, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110 062, India

Sewage sludge biosolids are the stabilized organic residues from domestic and industrial wastewater treatment. They contain a number of elements like nitrogen, phosphorus, macro-and micro-nutrients and organic matter, thus making them suitable for land applications (Pinamonti, 1998). During manufacture of white sugar, pressmud and molasses are produced as by-products. These waste products cause disposal and pollution problems. Composted Sugarcane Pressmud (CPM) has considerable quantity of various nutrients particularly potassium. Potassium enrichment has been found to reduce the requirement of other fertilizers in crop plants (Suryapani *et al*., 2013). Farmyard Manure (FYM) is another potential source of organic fertilizer. Use of FYM is important for improving soil quality including organic carbon and total nitrogen (Whalen *et al*., 2001; Khorsandi and Nourbakhsh, 2007). Organic fertilizers, in addition to supplying nutrients, improve the physico-chemical condition of soils, enhance nutrient cycling and build the soil organic-matter resources (Palm, 1995).

MATERIALS AND METHODS

Plant material and field experiments: Seeds of *A. annua*, obtained from the Herbal Garden, Jamia Hamdard (Hamdard University), New Delhi, India, were sown in 1×1 m nursery beds in the middle of December, 2008. Two-month old seedlings were transplanted to the main field in a randomized block design. Sixteen plants were transplanted in each block of 2×2 m size with plant to plant and row to row spacing of 50 cm. The soil of the experimental field was sandy loam with neutral pH. The organic carbon content of the soil was 0.29% (w/w). The soil had 127 kg ha^{-1} available nitrogen, 24 kg ha^{-1} available phosphorus and 119 kg ha^{-1} available potassium. Three treatments consisting of SSB, CPM and FYM (each at the rate of 20 tons ha^{-1}) were applied at the time of transplantation. The crop without any treatment was taken as control. At the time of transplantation, the treatments were applied. Each treatment was replicated five times. The crop beds were irrigated on alternate days for the first ten days and then throughout the whole process of growth and development, irrigation was carried out at times dependent upon the rainfall. In the case of long-term drought, watering was carried out.

Growth characteristics: Sampling for growth characteristics were carried out at three stages of plant development i.e., 120 DAS (days after sowing), 150 DAS and 180 DAS stages. Plants were cut at ground level after taking shoot length with the help of a meter scale and then leaves were separated from stem. The separated plant parts were weighed on fresh weight (FW) basis and then dried separately under shade. After getting constant weight, dried plant parts were weighed again to obtain dry weight (DW). Weight of the samples was recorded with the help of an electronic balance (Sartorius-GE 412).

Detection of artemisinin: Standard artemisinin was purchased from Sigma Aldrich (USA). HPLC grade water, petroleum ether, methanol, sodium hydroxide, glacial acetic acid, di-potassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from E. Merck Limited (Mumbai, India). All chemicals were of analytical grade. Detection of artemisinin in stem and leaves of *A. annua* was performed by the method of Zhao and Zeng (1986). As artemisinin lacks any chromophore for UV detection in HPLC hence it was chemically modified to a compound Q_{260}. Stem and leaves of *A. annua* plants were collected at three sampling stages and dried to constant weight under shade. The dried stem and leaves were ground to powder. Accurately weighed powder samples (1 g) were added to an extraction bottle containing 20 mL of petroleum ether (boiling range 30-60°C) and extracted overnight on rotary shaker. The process was repeated 3 times. The extracts were pooled and evaporated to dryness. The residue was dissolved in 1 mL methanol and centrifuged at 12000 rpm for 10 min to precipitate the un-dissolved components. The supernatant containing the artemisinin extract was used for HPLC detection after derivatization. For derivatization, 100 μL aliquot of each sample was taken and to this 4 mL of 0.3% NaOH was added. The samples were incubated in shaking water bath at 50°C for 30 min, so that a derivatized product with UV maxima at 260 nm could be obtained. After cooling at room temperature, the solution was neutralised with glacial acetic acid (0.1M in 20% MeOH). The pH of the solution was maintained at 6.8. Derivatized artemisinin was filtered through a Millipore filter (0.45 μm) before HPLC analysis.

HPLC (Waters system with UV detector-2487 and binary pump) was performed using a C18 reverse-phase column (4.6×250 mm, 5 μm). A 10 min isocratic program was used. The mobile phase used was methanol and 0.01M potassium-phosphate buffer (pH 6.5) in the ratio of 60:40, at constant flow rate of 1 mL min^{-1}, with the detector set at 260 nm. The injection volume was 20 μL. The retention time of artemisinin reference was about

5 min under these conditions. Artemisinin was quantified by comparing the peak areas with the help of calibration curve prepared using HPLC. Calibration curve was prepared using 1 mg of standard artemisinin dissolved in 1 mL of HPLC-grade methanol to make the stock solution. The artemisinin standard solutions with concentrations of 10, 20, 30, 40 and 50 µg mL^{-1} were prepared by serial dilution method.

Yield parameters: The experiment includes estimation of yield of secondary metabolites (kg ha^{-1}) as well as dry yield (kg ha^{-1}) of stem and leaf separately. Sampling for yield parameters was carried out at three stages of plant development i.e., 120 DAS, 150 DAS and 180 DAS stages. Plants in five replicates from each treatment were harvested at sampling stages and cut into stem and leaves. The dry stem and leaf yield of five plants was estimated on kg ha^{-1} basis.

Statistical analysis: Each plot was treated as one replicate and all the treatments were replicated five times. Data were statistically analyzed using the statistical software SPSS (Statistical Procedure for Social Sciences, ver. 11.0 Inc., Chicago, USA). Mean values were statistically compared by Duncan's Multiple Range test (DMRT) using different letters.

RESULTS

Plant characteristics: All growth characteristics were significantly ($p<0.05$) affected by organic soil amendments. Highest increment in plant height was observed in SSB amended soil followed by FYM treatment. CPM also influenced plant height markedly, although slightly lower than FYM. Application of organic residues proved effective in increasing the number of leaves at all stages of plant growth and development. The mean values for number of branches and number of leaves in organic treatments were higher than control variant (Table 1). Fresh and dry weight of each plant organ as well as total (weight of stem and leaf taken together) fresh and dry weight were positively influenced by the application of organic residues (Table 2).

Table 1: Effect of organic soil amendments on plant characteristics of *A. annua* at three growth stages

		Treatments			
Parameter	Stages	Control	SSB	CPM	FYM
Plant height (cm plant^{-1})	120 DAS	68±3.47^{c}	152±4.99^{a}	121±5.57^{b}	127±4.71^{b}
	150 DAS	135±6.81^{c}	294±8.89^{a}	253±12.35^{b}	269±8.41^{b}
	180 DAS	139±7.88^{a}	299±10.21^{a}	259±13.32^{a}	277±10.26^{a}
No. of branches (plant^{-1})	120 DAS	19±1.20^{c}	36±1.53^{a}	30±1.73^{b}	35±1.45^{a}
	150 DAS	24±1.86^{c}	53±2.31^{a}	41±2.65^{b}	45±2.03^{ab}
	180 DAS	23±1.45^{c}	50±2.08^{a}	39±2.40^{b}	44±1.76^{b}
No. of leaves (plant^{-1})	120 DAS	429±12.45^{b}	602±15.49^{a}	573±16.52^{a}	590±14.43^{a}
	150 DAS	598±13.01^{d}	1108±18.35^{a}	926±20.58^{c}	1030±17.70^{b}
	180 DAS	325±13.23^{c}	579±14.95^{a}	506±15.72^{b}	537±13.35^{ab}

Data are given as means of five replicates±SE, Means within a row followed by the same letter are not significantly different ($p\leq0.05$), SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: Farmyard manure

Table 2: Effect of organic soil amendments on weight of different organs of *A. annua* at three growth stages

		Treatments			
Parameter	Stages	Control	SSB	CPM	FYM
Stem FW (g plant^{-1})	120 DAS	46±1.96^{d}	180±5.78^{a}	127±6.15^{c}	148±3.78^{b}
	150 DAS	121±2.86^{d}	316±6.11^{a}	259±6.77^{c}	282±5.05^{b}
	180 DAS	98±2.76^{d}	281±5.94^{a}	224±6.60^{c}	247±4.25^{b}
Leaf FW (g plant^{-1})	120 DAS	45±2.64^{d}	160±3.64^{a}	120±3.95^{c}	142±2.99^{b}
	150 DAS	124±3.63^{d}	327±4.56^{a}	269±4.98^{c}	286±3.89^{b}
	180 DAS	35±2.82^{d}	158±4.65^{a}	90±4.39^{c}	112±4.14^{b}
Total FW (g plant^{-1})	120 DAS	91±0.85^{d}	339±8.66^{a}	247±9.79^{c}	291±6.68^{b}
	150 DAS	246±1.65^{d}	643±10.66^{a}	528±11.70^{c}	569±4.85^{b}
	180 DAS	133±4.86^{d}	439±8.78^{a}	315±4.15^{c}	359±7.73^{b}
Stem DW (g plant^{-1})	120 DAS	15±1.63^{c}	61±2.58^{a}	42±2.81^{b}	50±2.28^{b}
	150 DAS	72±2.49^{d}	187±3.10^{a}	154±3.50^{c}	167±2.84^{b}
	180 DAS	62±2.42^{d}	176±3.07^{a}	140±3.14^{c}	155±2.76^{b}
Leaf DW (g plant^{-1})	120 DAS	13±0.57^{d}	46±1.11^{a}	34±1.39^{c}	41±0.67^{b}
	150 DAS	42±1.53^{c}	110±2.17^{a}	89±2.72^{b}	96±1.71^{b}
	180 DAS	23±0.72^{d}	104±1.62^{a}	60±2.17^{c}	72±1.07^{b}
Total DW (g plant^{-1})	120 DAS	28±1.31^{d}	106±3.59^{a}	76±4.04^{c}	90±2.74^{b}
	150 DAS	113±1.68^{d}	297±5.27^{a}	243±6.04^{c}	263±2.70^{b}
	180 DAS	85±3.03^{d}	279±4.35^{a}	200±1.01^{c}	227±3.75^{b}

Data are given as means of five replicates±SE, Means within a row followed by the same letter are not significantly different ($p\leq0.05$), SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: Farmyard manure

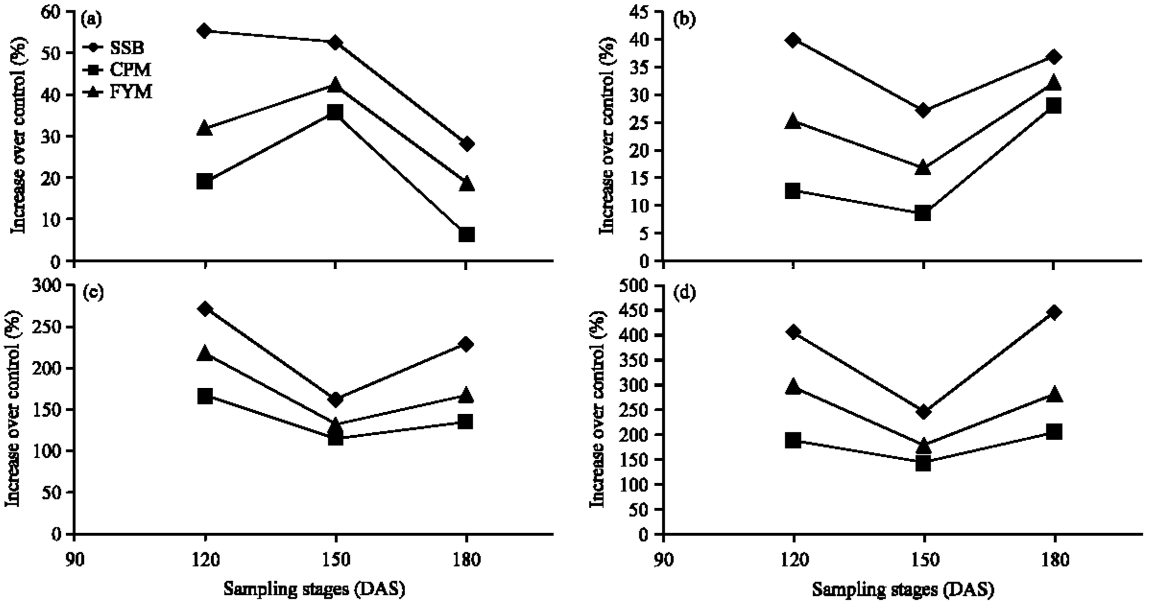

Fig. 1(a-d): Percent increase over control in (a) Stem artemisinin content, (b) Leaf artemisinin content, (c) Dry herbage yield and (d) Total artemisinin yield of *A. annua* at three sampling stages after applying various organic wastes, SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: farmyard manure, DAS: Days after sowing

Table 3: Effect of organic soil amendments on artemisinin content (% on dry weight basis) in stem and leaf of *A. annua* at three growth stages

		Treatments			
Parameter	Stages	Control	SSB	CPM	FYM
Stem artemisinin content	120 DAS	0.047±0.001^{a}	0.073±0.001^{a}	0.056±0.001^{a}	0.062±0.001^{a}
	150 DAS	0.059±0.001^{a}	0.090±0.002^{a}	0.080±0.001^{a}	0.084±0.001^{a}
	180 DAS	0.032±0.001^{a}	0.041±0.001^{a}	0.034±0.001^{a}	0.038±0.001^{a}
Leaf artemisinin content	120 DAS	0.363±0.003^{c}	0.508±0.003^{a}	0.409±0.003^{bc}	0.455±0.002^{ab}
	150 DAS	0.415±0.004^{a}	0.528±0.003^{a}	0.451±0.001^{a}	0.485±0.002^{a}
	180 DAS	0.239±0.002^{a}	0.327±0.001^{a}	0.306±0.001^{a}	0.316±0.002^{a}

Data are given as means of five replicates±SE, Means within a row followed by the same letter are not significantly different ($p \leq 0.05$), SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: Farmyard manure

Artemisinin content: Artemisinin content increased generally over time until a maximum was reached just at the onset of flowering (which coincided with the second sampling i.e., 150 DAS). After that, artemisinin content started decreasing (Table 3). Leaf artemisinin content exhibited a percent variation of 39.94, 12.67 and 25.34% (120 DAS), 27.23, 8.67 and 16.87% (150 DAS) and 36.82, 28.03 and 32.22% (180 DAS) with SSB, CPM and FYM treatments respectively, over the control (Fig. 1a, b). Percent increase in stem artemisinin content with the organic amendments was highest at 150 DAS stage whereas, in case of leaf, it was lowest at this stage.

Yield characteristics: The addition of organic wastes improved all yield characteristics significantly ($p<0.05$) over control. At 150 DAS stage, the lowest dry stem yield (2861.70 kg ha^{-1}) was obtained in unfertilized control variant whereas, the highest dry stem yield (7481.00 kg ha^{-1}) was obtained with SSB application, followed by FYM (6696.00 kg ha^{-1}) and the CPM (6156.70 kg ha^{-1}). Highest leaf dry yield of 4397.70 kg ha^{-1} in this experiment was obtained in SSB amended soil at 150 DAS stage, a percent variation of 162.60 % over the control variant (Table 4). Because herbage yield and artemisinin content increased by the same treatment, artemisinin yield followed the same pattern. Highest stem artemisinin yield (6.757 kg ha^{-1}) was obtained with SSB at 150 DAS stage. All organic amendments produced a significant effect on leaf artemisinin yield at all sampling stages. The maximum value (23.210 kg ha^{-1}) in this experiment was obtained at 150 DAS stage in SSB treated plots. Total artemisinin yield in this study reached up to 29.96 kg ha^{-1} (Table 5).

Dry herbage yield exhibited least percent variation among different treatments and control at 150 DAS stage and was 161.86, 114.5 and 131.94% higher with SSB, CPM

Table 4: Effect of organic soil amendments on dry herbage yield of *A. annua* at three growth stages

		Treatments			
Parameter	Stages	Control	SSB	CPM	FYM
Stem dry yield (kg ha^{-1})	120 DAS	619±64.97^{c}	2422±103.39^{a}	1661±112.12^{b}	1983±91.04^{b}
	150 DAS	2862±99.48^{d}	7481±123.70^{a}	6157±140.05^{c}	6696±13.85^{b}
	180 DAS	2467±97.03^{d}	7021±122.70^{a}	5605±125.57^{c}	6185±110.14^{b}
Leaf dry yield (kg ha^{-1})	120 DAS	521±22.66^{d}	1826±44.30^{a}	1380±55.73^{c}	1635±26.85^{b}
	150 DAS	1675±61.35^{c}	4398±86.74^{a}	3574±109.17^{b}	3825±68.22^{b}
	180 DAS	925±28.58^{d}	4156±64.63^{a}	2384±86.50^{c}	2897±42.58^{b}
Total dry yield (kg ha^{-1})	120 DAS	1140±52.16^{d}	4248±143.49^{a}	3041±161.60^{c}	3617±109.45^{b}
	150 DAS	4536±67.10^{d}	11879±210.9^{a}	9730±241.52^{c}	10522±108.10^{b}
	180 DAS	3392±121.09^{d}	11178±174.3^{a}	7989±40.39^{c}	9082±149.75^{b}

Data are given as means of five replicates±SE, Means within a row followed by the same letter are not significantly different ($p \leq 0.05$), SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: Farmyard manure

Table 5: Effect of organic soil amendments on artemisinin yield in stem and leaf of *A. annua* at three growth stages

		Treatments			
Parameter	Stages	Control	SSB	CPM	FYM
Stem artemisinin yield (kg ha^{-1})	120 DAS	0.293±0.033^{d}	1.767±0.069^{a}	0.930±0.055^{c}	1.230±0.076^{b}
	150 DAS	1.700±0.076^{d}	6.757±0.137^{a}	4.927±0.069^{c}	5.613±0.162^{b}
	180 DAS	0.793±0.024^{d}	2.860±0.070^{a}	1.887±0.037^{c}	2.337±0.055^{b}
Leaf artemisinin yield (kg ha^{-1})	120 DAS	1.890±0.093^{d}	9.280±0.263^{a}	5.420±0.184^{c}	7.433±0.150^{b}
	150 DAS	6.943±0.193^{d}	23.210±0.532^{a}	16.127±0.468^{c}	18.547±0.313^{b}
	180 DAS	2.207±0.052^{d}	13.567±0.222^{a}	7.297±0.276^{c}	9.157±0.092^{b}
Total artemisinin yield (kg ha^{-1})	120 DAS	2.187±0.086^{d}	11.047±0.332^{a}	6.347±0.237^{c}	8.667±0.215^{b}
	150 DAS	8.643±0.160^{d}	29.967±0.609^{a}	21.057±0.512^{c}	24.163±0.379^{b}
	180 DAS	3.003±0.067^{d}	16.427±0.292^{a}	9.187±0.242^{c}	11.487±0.087^{b}

Data are given as means of five replicates±SE, Means within a row followed by the same letter are not significantly different ($p \leq 0.05$), SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: Farmyard manure

and FYM treatments respectively, over the control (Fig. 1c). Total artemisinin yield was highest at the same stage i.e., 150 DAS (Table 5) and exhibited least variations among different treatments and control at this stage (Fig. 1d).

DISCUSSION

Plant characteristics: Sludge contains organic matter and various nutrients which are responsible for the increase in plant biomass and yield. Pinamonti (1998) found that grapevine yield was clearly favored by sewage sludge fertilization. The application of SSB to agricultural land has become a common practice as it improves soil fertility, soil aeration and the water holding capacity of the soil and decreases soil acidification if applied to soil in the right amounts (Bengston and Cornette, 1973). All these effects are advantageous for plant health. Similarly, the stimulation effect of CPM and FYM were also significant as compared to control. The improvement effects of organic treatments on vegetative growth characters can be linked to the important role they have on soil properties, moisture retention and better nutrient availability leading to significant improvement in plant growth. Some researchers have pointed out efficacy of organic manures in increasing the growth and yield of medicinal and aromatic plants (Malik *et al*., 2011). The increase might be related to the positive effect of organic amendments in increasing the root surface area per unit of soil volume, water-use efficiency and photosynthetic activity which directly affects the physiological processes and utilization of carbohydrates.

Artemisinin content: Organic residues had a favourable effect on artemisinin content. Artemisinin accumulation increment with SSB treatment reached to the highest values. Land application of sewage sludge enhances microbial activity that may affect soil N cycling and, therefore, plant available N, influencing the metabolism of the plant. Secondary metabolite pathways are responsive to environmental variations as has been established in many plants (Qureshi *et al*., 2007; Gupta *et al*., 2011; Nadim *et al*., 2011; Malik *et al*., 2012). Terpenes, the main secondary metabolites in *A. annua*, are universally synthesized through condensation of the five-carbon compound Isopentenyl Diphosphate (IPP) and its allylic isomer Dimethylallyl Diphosphate (DMAPP). Two pathways have been proposed for the biosynthesis of terpenoids-the cytosolic mevalonate (MVA) pathway or the Methylerythritol Phosphate (MEP) pathway in the plastid (Sangwan *et al*., 2001). Artemisinin is produced using IPP/DMAPP from both the MVA and MEP pathways via Farnesyl Diphosphate (FPP) which is composed of one isoprene unit from the MEP pathway and two isoprene units from the MVA pathway (Towler and Weathers, 2007; Schramek *et al*., 2010). Artemisinin has been reported to increase significantly by different kinds and types of treatments (Shukla *et al*., 1992; Ozguven *et al*., 2008; Jha *et al*., 2011). Application of sewage sludge and other composts improve soil physical, biological and chemical properties (Garcia-Gil *et al*., 2000; Albiach *et al*., 2001) and their promotive effect on artemisinin content may be attributed to the release of nutrients (mainly nitrogen) which favours primary metabolism (growth, photosynthetic pigments and nutrient status) that is in turn linked to the secondary metabolite production.

Percent increase in stem artemisinin content was highest at 150 DAS stage whereas in case of leaf, it was lowest at this stage. The possible reason for this might be that nutrient deprived control plants accumulate more artemisinin in leaves mainly because its translocation towards other organs is hampered due to deficiency of some ions like K^+, hence showing least variation in leaves among different treatments and control at 150 DAS stage.

Yield characteristics: Organic manure applications led to an effect on plant growth and might have provided the required plant nutrients and therefore resulted in yield enhancement. This increase can be attributed to the role of macro-and micronutrients provided by organic treatments in stimulating metabolic processes, promoting growth and increasing the synthesis and accumulation of more metabolites in plant tissues (Hussein *et al*., 2006). In case of medicinal plants, total dry matter is an important criterion of production. Yield and yield components of *A. annua* have previously been reported to respond to environmental variables including low levels of salinity (Prasad *et al*., 1998), different plant densities (Mert *et al*., 2002), different nitrogen doses (Ayanoglu *et al*., 2002), lime and mineral nutrients in a dystrophic soil (Ritchey and Ferreira, 2006) and organic and chemical fertilizers (Jha *et al*., 2011). Concerning the effect of industrial residues and municipal wastes on yield, it is clear that the highest increment in yield was noticed as a result of SSB application. As also reported previously, yield enhancements can be attributed to the role of nutrients provided by organic sources in stimulating metabolic processes, encouraging growth and flowers' yield as well as improving soil structure (Darwish *et al*., 1995; Matsi *et al*., 2003).

Dry herbage yield, artemisinin content and total artemisinin yield was highest at the 150 DAS in this study and exhibited least variations among different treatments and control at this stage. Therefore, for greater herbage yield and artemisinin production, *A. annua* should be harvested at 150 DAS stage (onset of flowering) as it also exhibits fewer variations among different treatments at this stage.

CONCLUSION

The source of organic amendments had a great effect on plant characteristics, crop yield and artemisinin production in *A. annua*. Better plant development, enhanced artemisinin content and yield was favoured by SSB treatment. Total artemisinin yield in this study reached up to 29.96 kg ha^{-1}. It is thus concluded that agricultural recovery of municipal wastes and industrial organic residues is a way to solve the plant nutrition problems of medicinal and aromatic plants and influence their secondary metabolism besides protecting environment and other natural resources from deterioration caused due to accumulation of large quantities of municipal wastes and organic industrial residues.

ACKNOWLEDGMENT

Hamdard National Foundation (HNF), New Delhi, India, is acknowledged for granting the research fellowship to Afaq Ahmad Malik.

REFERENCES

Abdin, M.Z., M. Israr, R.U. Rehman and S.K. Jain, 2003. Artemisinin, a novel antimalarial drug: Biochemical and molecular approaches for enhanced production. Planta Med., 69: 289-299.

Albiach, R., R. Canet, F. Pomares and F. Ingelmo, 2001. Organic matter components, aggregate stability and biological activity in a horticultural soil fertilized with different rates of two sewage sludges during ten years. Bioresour. Technol., 77: 109-114.

Avery, M.A., C. Jennings-White and W.K.M. Chong, 1987. The total synthesis of (+)-artemisinin and (+)-9-desmethyltemesinin. Tetrahedron Lett., 28: 4629-4632.

Ayanoglu, F., A. Mert and S. Kirici, 2002. The effects of different nitrogen doses on *Artemisia annua* L. J. Herbs Spices Med. Plants, 9: 399-404.

Bengston, G.W. and J.J. Cornette, 1973. Disposal of composted municipal waste in a plantation of young slash pine: Effects on soil and trees. J. Environ. Qual., 2: 441-444.

Darwish, O.H., N. Persaud and D.C. Martens, 1995. Effect of long-term application of animal manure on physical properties of three soils. Plant Soil, 176: 289-295.

Efferth, T., H. Dunstan, A. Sauerbrey, H. Miyachi and C.R. Chitambar, 2001. The anti-malarial artesunate is also active against cancer. Int. J. Oncol., 18: 767-773.

Garcia-Gil, J.C., C. Plaza, P. Soler-Rovira and A. Polo, 2000. Long-term effects of municipal waste compost application on soil enzyme activities and microbial biomass. Soil Biol. Biochem., 32: 1907-1913.

Gupta, M., S. Bisht, B. Singh, A. Gulati and R. Tewari, 2011. Enhanced biomass and steviol glycosides in *Stevia rebaudiana* treated with phosphate-solubilizing bacteria and rock phosphate. Plant Growth Regul., 65: 449-457.

Hussein, M.S., S.E. El-Sherbeny, M.Y. Khalil, N.Y. Naguib and S.M. Aly, 2006. Growth characters and chemical constituents of *Dracocephalum moldavica* L. plants in relation to compost fertilizer and planting distance. Sci. Hortic., 108: 322-331.

Jha, P., M. Ram, M.A. Khan, U. Kiran, Mahmooduzzafar and M.Z. Abdin, 2011. Impact of organic manure and chemical fertilizers on artemisinin content and yield in *Artemisia annua* L. Ind. Crops Prod., 33: 296-301.

Khorsandi, N. and F. Nourbakhsh, 2007. Effect of amendment of manure and corn residues on soil N mineralization and enzyme activity. Agron. Sustainable Dev., 27: 139-143.

Klayman, D.L., 1985. Qinghaosu (artemismin): An antimalarial drug from China. Science, 228: 1049-1055.

Malik, A.A., J. Ahmad, S.R. Mir, M. Ali and M.Z. Abdin, 2009. Influence of chemical and biological treatments on volatile oil composition of *Artemisia annua* Linn. Ind. Crops Prod., 30: 380-383.

Malik, A.A., S. Suryapani and J. Ahmad, 2011. Chemical vs. organic cultivation of medicinal and aromatic plants: The choice is clear. Int. J. Med. Arom. Plants, 1: 5-13.

Malik, A.A., J. Ahmad, S. Suryapani, M.Z. Abdin, S.R. Mir and M. Ali, 2012. Volatiles of *Artemisia annua* L. as Influenced by Soil Application of Organic Residues Res. J. Med. Plant, 6: 433-440.

Matsi, T.A., S. Lithourgidis and A.A. Gagianas, 2003. Effects of injected liquid cattle manure on growth and yield of winter wheat and soil characteristics. Agron. J., 95: 592-596.

Mert, A., S. Kirici and F. Ayanoglu, 2002. The effects of different plant densities on yield, yield components and quality of *Artemisia annua* L. ecotypes. J. Herbs Spices Med. Plants, 9: 413-418.

Nadim, M.M., A.A. Malik, J. Ahmad and S.K. Bakshi, 2011. The essential oil composition of *Achillea millefolium* L. cultivated under tropical condition in India. World J. Agric. Sci., 7: 561-565.

Nguyen, K.T., P.R. Arsenault and P.J. Weathers, 2011. Trichomes+roots+ROS = artemisinin: Regulating artemisinin biosynthesis in *Artemisia annua* L. *In vitro* Cell. Dev. Biol.-Plant, 47: 329-338.

Ozguven, M., B. Sener, I. Orhan, N. Sekeroglu and M. Kirpik *et al*., 2008. Effects of varying nitrogen doses on yield, yield components and artemisinin content of *Artemisia annua* L. Indus. Crops Prod., 27: 60-64.

Palm, C.A., 1995. Contributions of Agroforestry trees to nutrient requirements of inter cropped plants. Agrofor. Syst., 30: 105-124.

Pinamonti, F., 1998. Compost mulch effects on soil fertility, nutritional status and performance of grapevine. Nutr. Cycling Agroecosyst., 51: 239-248.

Prasad, A., D. Kumar, M. Anwar, D.V. Singh and D.C. Jain, 1998. Response of *Artemisia annua* L. to soil salinity. J. Herbs Spices Med. Plants, 5: 49-55.

Qureshi, M.I., M.Z. Abdin, S. Qadir and M. Iqbal, 2007. Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Biol. Plant., 51: 121-128.

Ritchey, K.D. and J.F.S. Ferreira, 2006. Short-term response of *Artemisia annua* to lime, P, K and N in a dystrophic soil. J. Herbs Spices Med. Plants, 12: 49-59.

Ro, D.K., E.M. Paradise, M. Quellet, K.J. Fisher and K.L. Newman *et al*., 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature, 440: 940-943.

Romero, M.R., T. Efferth, M.A. Serrano, B. Castano, R.I. Macias, O. Briz and J.J.G. Marin, 2005. Effect of artemisinin/artesunate as inhibitors of hepatitis B virus production in an *in vitro* replicative system. Antiviral Res., 68: 75-83.

Sangwan, N.S., A.H.A. Farooqi, F. Shabih and R.S. Sangwan, 2001. Regulation of essential oil production in plants. Plant Growth Regul., 34: 3-21.

Schramek, N., H. Wang, W. Romisch-Margl, B. Keil and T. Radykewicz *et al*., 2010. Artemisinin biosynthesis in growing plants of *Artemisia annua*. A $^{13}CO_2$ study. Phytochemistry, 71: 179-187.

Sen, R., S. Bandyopadhyay, A. Dutta, G. Mandal, S. Ganguly, P. Saha and M. Chatterjee, 2007. Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. J. Med. Microbiol., 56: 1213-1218.

Shukla, A., A.H.A. Farooqi, Y.N. Shukla and S. Sharma, 1992. Effect of triacontanol and chlormequat on growth, plant hormones and artemisinin yield in *Artemisia annua* L. Plant Growth Regul., 11: 165-171.

Suryapani, S., S. Umar, A.A. Malik and A. Ahmad, 2013. Symbiotic nitrogen fixation by lentil improves biochemical characteristics and yield of intercropped wheat under low fertilizer input. J. Crop Improv., 27: 53-66.

Towler, M.J. and P.J. Weathers, 2007. Evidence of artemisinin production from IPP stemming from both the mevalonate and the nonmevalonate pathways. Plant Cell Rep., 26: 2129-2136.

Van Geldre, E., A. Vergauwe and E. Van den Eeckhout, 1997. State of the art of the production of the antimalarial compound artemisinin in plants. Plant Mol. Biol., 33: 199-209.

WHO, 2006. WHO Monograph on Good Agricultural and Collection Practices (GACP) for *Artemisia annua* L. World Health Organization, Geneva, Switzerland, ISBN-13: 9789241594431, Pages: 49.

Weathers, P.J., S. Elkholy and K.K. Wobbe, 2006. Artemisinin: The biosynthetic pathway and its regulation in *Artemisia annua*, a terpenoid-rich species. *In vitro* Cell. Dev. Biol.-Plant, 42: 309-317.

Whalen, J., C. Chang and B. Olson, 2001. Nitrogen and phosphorus mineralization potentials of soils receiving repeated annual cattle manure applications. Biol. Fertil. Soils, 34: 334-341.

Zhao, S.S. and M.Y. Zeng, 1986. Determination of qinghaosu in *Artemisia annua* L. by high performance liquid chromatography. Anal. Chem., 58: 289-292.