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## **Volatiles of *Artemisia annua* L. as Influenced by Soil Application of Organic Residues**

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### **ABSTRACT**

India with its copiousness of exuberant flora is one of the richest countries as regards the genetic resources of medicinal plants and occupies unquestionably a premier position in the use of herbal drugs but in this era of high-tech wizardry, urbanization and industrialization has created needless disturbance in the vulnerably delicate ecosystems due to the accretion of large quantities of municipal wastes and industrial residues. On the other hand, continued applications of chemical fertilizers resulted in the contamination of surface and ground waters and have adversely affected the biological, physical and chemical properties of soil. It was therefore, suggested to find and evaluate methods which can help solve serious environmental and plant nutritional problems. An experiment was conducted in this regard at the research field of Jamia Hamdard, New Delhi, India to assess the impact of sewage sludge biosolid, composted sugarcane pressmud and farmyard manure on volatile oil content and composition of *Artemisia annua* Linn., an important aromatic-antibacterial herb. The oil is quite useful pharmaceutically and is used in perfumery, cosmetics, aromatherapy and dermatology as an antimicrobial agent having fungicidal properties. We undertook the present study with a view to maintain the ecosystems of the soil and mitigate environmental pollution. The principal findings revealed significant variations in the oil content and percentage occurrence of different constituents therefore, paving the way for enhanced production of volatiles in this industrial crop as well as emerging as an alternative control measure for mitigation of environmental pollution.

**Key words:** *Artemisia annua*, farmyard manure, sewage sludge biosolid, sugarcane pressmud

### **INTRODUCTION**

Due to rapid urban development and industrial boom, large quantities of municipal wastes and unexploited organic by-products or residues from various industries are produced. To avoid environmental and economic costs of disposal and taking up space in landfills, their use in the agricultural sector has been suggested. On the other hand, unbalanced application of chemical fertilizers coupled with inadequate land management practices posed a serious threat to soil health in terms of decline in soil organic matter (Saha *et al.*, 2007; Qazi *et al.*, 2009). Due to rapid increase in the prices of chemical fertilizers and also with a view to maintain the ecosystem of soils, it

has become, necessary to minimize the use of chemical fertilizers (Kumar *et al.*, 2009). Addition of soil organic matter with different origins to the soil is one of the most common rehabilitation practices to improve soil properties (Darwish *et al.*, 1995; Mbah *et al.*, 2007).

An alternative source of organic matter is Sewage Sludge Biosolid (SSB). SSBs are the stabilized organic residues from domestic and industrial wastewater treatment (Bright and Healey, 2003). They consist of multi element organic wastes that are used commonly as manures (Otobbang *et al.*, 1997). SSB can substitute for commercial fertilizers and organic matter if applied in the right amounts to soil (Yamur *et al.*, 2005). SSBs are rich in N, P, macro-and micro-nutrients and organic matter, thus making them suitable for land applications where it is expected to increase the soil organic content and improve soil properties like soil stability, porosity and water filtration rates (Asagi *et al.*, 2007; Hussein, 2009) and promote recycling of nutrients (Gilani and Bahmanyar, 2008). In addition, sewage biosolid decreases soil acidification if applied to soil in the right amounts (Bengston and Cornette, 1973) and increases beneficial soil organisms, improving soil physical and biological properties. Plant nutrient uptake also improves (Kavitha and Subramanian, 2007). All these effects are advantageous for plant health (Pinamonti and Zorzi, 1996).

During the manufacture of white sugar, pressmud and molasses are produced as by-products. These waste products cause disposal and pollution problems. Pressmud is rich in potassium with considerable quantity of other nutrients and could be converted into nutrient rich organic manure or composted sugarcane pressmud (CPM). Conversion of waste products into rich organic manures not only solves the problems of disposal and pollution but also reduces the fertilizer cost (Rakkiyappan *et al.*, 2001) besides improving soil properties and crop yield (Kalaivanan and Omar Hattab, 2008).

Farmyard manure (FYM) is a potential source of organic fertilizer. Use of FYM is important for improving soil quality and crop yield (Ashiono *et al.*, 2006). Organic fertilizers, in addition to supplying nutrients and increasing yield, improve the physico-chemical condition of soils, enhance nutrient cycling and build the soil organic-matter capital (Palm, 1995; Reeves, 1997; Nakhro and Dkhar, 2010). Organic fertilizers have been reported to improve growth characteristics and yield in both medicinal as well as field crops (Abd El-Lattief, 2011; Abdelhamid *et al.*, 2011; Affendy *et al.*, 2011; Aslam *et al.*, 2011).

*Artemisia annua* Linn. (annual wormwood) is a vigorous growing annual and aromatic herb, belonging to family Asteraceae. Chinese physicians used the plant for hundreds of years (Perazzo *et al.*, 2003) externally for nosebleeds, bleeding rashes and sores and internally to treat heat stroke and malarial fever symptoms (Hsu, 2006). The plant produces a range of secondary compounds consisting volatile and non-volatile constituents (Simon *et al.*, 1990), including terpenes. It produces an aromatic essential oil upon hydrodistillation which is used in ointments and is rich in monoterpenes (Charles *et al.*, 1991; Ahmad and Misra, 1994). The oil is quite useful pharmaceutically because of its dermatological and fungicidal properties. It is used in perfumery, cosmetics, aromatherapy and dermatology as an antimicrobial agent (Woerdenbag *et al.*, 1992; Laughlin, 1994). The essential oil is produced in its trichomes present on leaves and flowers (Duke and Paul, 1993). Significant variations in the percentage occurrence of different constituents have been reported in our earlier reports (Ali and Siddiqui, 2000; Mukhtar *et al.*, 2007; Malik *et al.*, 2009). We undertook the present study with a view to

maintain the ecosystems of the soil and mitigate environmental pollution as well as solve the nutrition requirements of the very important medicinal and aromatic crop, *A. annua*.

## **MATERIALS AND METHODS**

**Plant material and field experiments:** Seeds of *A. annua*, obtained from the Herbal Garden, Jamia Hamdard, New Delhi, were sown in 1×1 m nursery beds in the middle of December 2007. 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<sup>-1</sup> available N, 24 kg ha<sup>-1</sup> available P and 119 kg ha<sup>-1</sup> available K. At the time of transplantation, the treatments were applied. Each treatment was replicated three times. Three treatments consisting of SSB, CPM and FYM each at the rate of 20 tons ha<sup>-1</sup>, were applied. The crop without any treatment was taken as control. The crop was 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.

**Isolation of essential oil:** For the isolation of essential oil of aerial parts, the plants were harvested at full bloom stage of development as recommended by Gupta *et al.* (2002). Aerial parts of each sample (500 g) was collected and subjected to hydro-distillation in a Clevenger-type apparatus (Clevenger, 1928) for 4 h. The oil after extraction was collected in screw capped glass vials and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The oil collected from the three replicates of a sample was pooled (composite sample) and analyzed by GC and GC/MS.

**Instrumentation and analysis of oil:** GC analyses were carried out on a Shimadzu-17A gas chromatograph equipped with a Flame Ionization Detector (FID) and a DB-5 capillary column packed with 5% phenyl polysiloxane (30 m, 0.25 mm i.d.; 0.25 μm film thickness). The oven temperature was held at 90°C for 1 min then programmed to rise at 7°C min<sup>-1</sup> to 230°C, held for 20 min. Other operating conditions were as follows: carrier gas, helium at a flow rate of 30 mL min<sup>-1</sup>; oxidant, oxygen at a flow rate of 300 mL min<sup>-1</sup>; fuel, hydrogen at a flow rate of 30 mL min<sup>-1</sup>; injector temperature, 240°C; detector temperature, 260°C, injection volume was 0.1 μL.

GC/MS analyses were performed on a HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary column packed with fused silica (60 m, 0.25 mm i.d.; 0.25 μm film thickness). The oven temperature program was initiated at 40°C, held for 1 min then raised at 3°C min<sup>-1</sup> to 250°C, held for 20 min. Other operating conditions were as follows: carrier gas, helium at a flow rate of 1 mL min<sup>-1</sup>; injector temperature, 250°C; split ratio, 1:50; injection volume was 0.1 μL. Mass spectra were recorded at 70 eV. Mass range was from m/z 20 to 500 amu.

**Identification of essential oil constituents:** The components of the essential oils were identified by comparing their retention indices and mass spectra fragmentation patterns with the literature values (Andersen and Falcone, 1969; Jennings and Shibamoto, 1980; Swigar and Silverstein, 1981; Libey, 1991; Ali, 2001; Adams, 2007).

## RESULTS

The relative abundance of volatile components in the aerial parts of *A. annua* after treatment of the plots with organic residues was analyzed by GC and GC/MS. Hydro-distillation of untreated (control) plants yielded 0.28±0.05% essential oil on fresh weight basis. The predominant components in the essential oil were camphor (32.66%), o-cymol (13.76%), caryophyllene (13.38%), germacrene D (10.03%), camphene (5.48%),  $\alpha$ -pinene (4.22%) and artemisia ketone (2.82%). The essential oil distilled from SSB treated *A. annua* amounted to 0.36±0.03% of fresh weight and consisted mainly of camphor (32.95%),  $\alpha$ -pinene (25.43%), caryophyllene (10.62%), germacrene D (6.49%), artemisia ketone (5.95%), o-cymol (3.66%) and camphene (3.30%). The hydrodistillation of CPM treated *A. annua* yielded 0.31±0.04% essential oil on fresh weight basis. The predominant components in its essential oil consisted of germacrene D (21.76%),  $\alpha$ -pinene (15.95%), camphor (13.49%), caryophyllene (12.99%), o-cymol (11.67%), germacrene B (6.13%), camphene (5.24%) and artemisia ketone (2.95%). Essential oil obtained from plants treated with FYM amounted to 0.33±0.03% of fresh weight and consisted mainly of camphor (37.20%), o-cymol (13.18%), germacrene D (11.09%), caryophyllene (8.46%), artemisia ketone (7.57%),  $\alpha$ -pinene (5.91%) and camphene (4.42%) (Table 1).

Table 1: Comparative statement of the volatile oil composition of *A. annua* treated with different organic wastes

Component	RI	Samples (%)			
		Control	SSB	CPM	FYM
$\alpha$ -Pinene	928	4.22	25.43	15.95	5.91
Camphene	939	5.48	3.30	5.24	4.42
$\beta$ -Phellandrene	955	0.95	0.91	1.01	1.16
$\beta$ -Pinene	979	1.18	2.48	1.12	0.72
3,4-Octadiene	985	-	-	0.01	-
$\alpha$ -Terpinene	990	0.22	0.23	0.27	0.32
o-Cymol	998	13.76	3.66	11.67	13.18
Cineole	1003	0.01	0.01	0.01	0.01
Artemisia ketone	1084	2.82	5.95	2.95	7.57
Thujyl alcohol	1100	2.17	1.73	1.49	1.46
Camphor	1135	32.66	32.95	13.49	37.20
3,3-Dimethyl-6-methylenecyclohexene	1340	-	-	-	0.05
$\gamma$ -Elemene	1350	-	-	-	0.09
6- $\alpha$ -Cadina-4,9-diene, (-)	1370	0.16	0.19	0.16	0.19
Carvacrol	1375	0.21	0.07	0.39	0.32
Aromadendrene	1378	-	0.09	-	0.10
$\alpha$ -Copaene	1395	1.70	1.57	1.57	1.39
$\beta$ -Caryophyllene	1403	13.38	10.62	12.99	8.46
$\alpha$ -Caryophyllene	1451	1.63	1.31	1.11	1.21
Germacrene D	1465	10.03	6.49	21.76	11.09
Germacrene B	1560	1.92	2.07	6.13	2.56
Arachidonic acid, ethyl ester	1626	1.06	0.60	1.32	0.89
Lanceol, cis	1650	1.62	0.40	1.32	1.22

RI: Retention index, SSB: Sewage sludge biosolid, CPM: Composted sugarcane pressmud, FYM: Farmyard manure

## DISCUSSION

All organic soil amendments substantially raised essential oil production, represented by increase in oil percentage and fresh weight of leaves of *A. annua* as compared to the control (unfertilized treatment). In all cases, the total yield of leaves on fresh weight basis increased as compared to the control variant. The hydrodistillation of aerial parts of untreated, SSB treated, CPM treated and FYM treated *A. annua* produced 0.28, 0.36, 0.31 and 0.33% essential oil, respectively, calculated on fresh weight basis. Present results established that SSB, CPM and FYM treated *A. annua* showed enhanced oil percentage. This result may be due to the effect of nutrients present in the manures in accelerating the metabolism reactions as well as stimulating enzymes. The quality and yield of essential oils have also been reported previously to be influenced by environmental factors (Malik *et al.*, 2011; Nadim *et al.*, 2011) including drying temperatures (Khangholi and Rezaeinodehi, 2008), fertilizer application (Sangwan *et al.*, 2001) and the pH of soils (Alvarez-Castellanos and Pascual-Villalobos, 2003). Fertilizers have been found to increase the yield of essential oil from other crops like *Matricaria recutita* (Upadhyay and Patra, 2011), *Rosmarinus officinalis* (Abdelaziz *et al.*, 2007) and *Valeriana officinalis* (Letchamo *et al.*, 2004).

Gas-chromatographic analyses of the composition of these essential oils revealed little variation of active constituents. In *A. annua*, nineteen components were characterized in the essential oil from untreated plants; twenty-two components were identified in the essential oil from SSB treated plants; twenty constituents were identified in the essential oil from plants treated with CPM and twenty components were identified in the essential oil from FYM treated plants. Camphor was the major component present in all the four samples. Artemisia ketone was found in considerable quantity in all samples. Artemisinin was not found among the chromatographic profiles of any of the essential oils. It has long been reported that artemisinin undergoes decomposition under the steam distillation conditions and that its decomposition products are not steam distillable (Tellez *et al.*, 1999). It is noteworthy to mention here that organic residues did not result in complete absence of sesquiterpenes in any of the oils as mentioned in our earlier report (Malik *et al.*, 2009). It is well established that the glandular trichomes possess the biosynthetic machinery to produce secondary metabolites including isoprenoids (Guo *et al.*, 1994; Clark *et al.*, 1997). The results presented here suggest that different organic amendments increased the glandular trichome density in *A. annua*, observed by the increase in essential oil content. However, anatomical study is required to establish that organic treatments indeed favour the induction of trichomes in the leaves of *A. annua*.

## CONCLUSION

Oil percentage and oil constituents were lower in plants cultivated in unamended soil compared to the SSB, FYM and CPM amended soil. The addition of organic matter rich in nutrients to the soil is thus a positive factor in essential oil production for *A. annua*. Therefore, greater attention needs to be paid for encouraging use of municipal wastes, organic industrial by-products and other locally available organic materials for improving the productivity of medicinal and aromatic plants whose secondary metabolites are directly influenced by the nutritional treatments. So, the agricultural recovery of municipal wastes and industrial organic by-products is a way to protect human health besides, solving serious environmental and plant nutritional problems.

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