ABSTRACT

Cymbopogon pendulus (Nees ex steudel) W. Watson, a native aromatic grass belonging to the tribe Andropogoneae of the family poaceae, has been observed to grow wildly in the plains of Darjeeling district, West Bengal, India. Due to rapid spread of urbanisation, the distribution of the species is becoming very much restricted to a certain area in the district. Under the present day environmental condition, the undisturbed fallow land of North Bengal University campus may be considered as the store house of such an endangered plant. Each year, the plants are being harvested by the local people and used as a material for thatching purpose without considering its commercial value in pharmaceutical industry.

The plant has been identified with the help of Central National Herbarium Section, Botanical Survey of India, Howrah, West Bengal. That the plant yield lemon grass oil has been confirmed by the presence of citral content in the crude extract of its essential oil.

Lemon grass oil is used as an ingredient of aerosol, deodorants, floor polishes, household detergents and a whole range of domestic and industrial products in which a pleasant fresh fragrance is desired. Besides, it is used as mosquito repellant. Moreover, it is commercially utilised for the preparation of vitamin A and E as well as medicine.

Lemon grass oil industry in India is passing through a critical period due to keen competition in the international market. Thus, it is necessary to explore the new source of lemon grass oil so far neglected in the region.

It is the objective of this part of work to investigate the wild plant like *C. pendulus* (Nees ex Steudel) W. Watson from plant physiological point of view for understanding various factors responsible for its optimum growth and development to show maximm herbage yield and production of essential oil in the ecological condition of Darjeeling district. The knowledge derived out of the work will be helpful for its commercial utilisation in pharmaceutical as well as in cottage indusrty in the region.

Up to date no attempt has been made in connection with chemical examination of different plant parts of *C.pendulus* (Nees ex Steudel) W. Watson available in Darjeeling district. Thus phytochemical investigation has been carried out to understand quality and quantity of different terpenoid constituents in essential oil extracted from the leaves of *C.pendulus* (Nees ex Steudel) W. Watson, immediately after their harvest.

Extraction of crude essential oil from the fresh leaves of the plant has been undertaken with the help of hydrodistillation method and analysis of terpenoid constituents in the crude essential oil obtained from the leaves of the plant has been made by Gas Liquid Chromatography. Essential oil has been observed to contain α and β-citral, terpine, and three other unknown volatile components. Essential oil in the leaves harvested in October from the plant at the flowering stage contains 82.92% of total citral content on fresh weight basis, out of which α and β-citral contents are 58.05% and 24.87% respectively. The high α-citral content is generally used commercially as flavouring agent and β-citral content is usually utilised for commercial preparation of vitamin A and E. The high content of these chemicals definitely support the commercial potentiality of the plant. Terpine content has been observed to yield only 9.76% while the yield of three unknown terpenoids i.e. no.1, 2 and 3 have been estimated to become 1.97%, 2.06% and 3.29% respectively.

In order to isolate nonterpenoid chemical constituents, dried leaves of the plant have been subjected to soxhlet extraction first with petroleum ether followed by methanol. For purification of chemical constituents, petroleum ether extract has directly been column chromatographed over alumina and eluted with different solvents and their mixture starting from nonpolar to polar solvent. Whereas methanolic extract of leaves has been separated into two fractions i.e. (a) ether soluble and (b) ether insoluble methanolic part. Ether soluble methanolic part again has been separated into four subfraction i.e. (a) phenolic acid, (b) acidic, (c) basic and (d) neutral subfractions. The petroleum extract shows the presence of a type of solid hydrocarbon in crystalline state and has been identified as n-alkane. Besides this, the fraction has been observed to contain two phytosterol i.e. sitosterol and lanosterol. Caffeic acid, a phenolic

acid, has been isolated and identified in the phenolic acid subfraction. Basic and acid subfractions have been examined not to contain any solid mass in appreciable quantity so that they are not in a position to be identified. Neutral subfraction also shows the presence of sitosterol. Apigenin-7-glucoside has been isolated from ether insoluble methanolic part. An anthocyanin pigment has been isolated from the red stem of the plant and identified as glucoside of cyanidin. Besides, coumarin, a lactone of hydroxy cinnamic acid, has been isolated and identified from the phenolic acid subfraction of dry husks of seeds of the plant. This chemical has been verified to be responsible for causing dormancy of seed of the plant under study.

All these identified chemical constituents are now being claimed for the first time to be isolated from the leaves of *C. pendulus* (Nees ex Steudel) W. Watson and these have been identified on the basis of determination of melting point, chromatographic behaviour, chemical tests and IR spectrum of the isolated natural products and are similar in observation with those of authentic markers.

Investigation has been carried out in connection with productivity of essential oil and its terpenoid constituents in different plant parts of *C. pendulus* (Nees ex Steudel) W. Watson. In this respect leaf blade and leaf sheath have been considered separately. Mature leaf blade has been observed to yield 0.58% of crude essential oil on fresh weight basis as compared to only 0.30% in young leaf blade. Oil content is much less in leaf sheath in comparison to that of leaf blade at both the young and mature conditions of leaf. Young leaf sheath has shown lower percentage yield of oil (0.15) as compared to mature leaf sheath (0.40%). Maximum oil content of 0.72% on fresh weight basis has been recorded in flowering top of the plant. On the other hand, stem and root show only 0.20% and 0.10% of oil respectively.

So far as the terpenoid constituents are concerned, maximum content of total citral has been observed in mature leaf blade (82.92%) as against mature leaf sheath which shows only 65.20%. Root shows the lowest value (52.28%). Though young leaf blade, young leaf sheath and stem show total citral content of 67.83%, 63.72% and 60.80% respectively but the total citral content becomes

moderately high (71.28%) in flowering top. α-citral is maximum in mature leaf blade (58.05%) when β-citral content is only 24.87%. Root yield minimum of α-citral (33.59%) but its β-citral content (18.69%) has been observed to be slightly higher than 16.20% in young leaf sheath. Though essential oil content in flowring top has been recorded to become moderately high but its α and β-citral contents are 43.62% and 27.66% respectively.

It has been noted that terpine content becomes high (23.16%) in stem as compared to minimum value (9.76%) in mature leaf blade. Root has moderately high content (21.81%) of terpine along with maximum content (25.91%) of other unidentified terpenoid constituents which is observed to occur in minimum quantity (7.32%) in mature leaf blade.

Thus for commercial utilisation of citral the mature leaves of *C.pendulus* (Nees. ex Steudel) W. Watson, should be used while for good source of rest of the terpenoid constituents, stem and root of the plant may be considered and flowering top could be the best for maximum productivity of crude oil.

It has been observed that volatile oil enclosed in the plant tissue is affected by the drying of the plant materials after their harvest. After oven drying of harvested leaves at 60± 1°C the essential oil content increases to reach 0.80% after 24 hours of treatment and the content gradually declines when duration of treatment increases. But drying at room temperature (29± 1°C) the leaves have been observed to yield maximum of 1.10% after 96 hours of treatment and the value declines in subsequent increase in hours of treatment.

Thus induction of wilting of leaves of *C.pendulus* (Nees ex Steudel) W. Watson after keeping the harvested leaves at 29±1°C for 96 hours could have beneficial effect so far as the yield of oil in the leaves of the plant is concerned.

During storage of extracted oil for a considerable period of time upto 6 years, it has been noted that total citral, α-citral, β-citral and terpine contents have been observed to be decreased. On the other hand unidentified terpenoid content shows proportionate increase in accumulation. Total citral content (82.92%) in freshly harvested leaves in the first year of collection decreases to

70.72% during storage of oil for five years. Similarly α-citral (58.05%) and β-citral (24.87%) content in the leaves of 1st year collection decreases to 48.29% and 22.43% respectively in the oil stored for five years. Terpine content also has been observed to become lowered from 9.76% during the some period of storage of oil.

It is very interesting to note that contents of total citral, α-citral, β-citral, terpine and other terpenoids remain more or less stable for two years of storage of oil and the duration of which should be taken into consideration while utilising stored oil for commercial purpose.

C. pendulus (Nees ex Steudel) W. Watson growing in the district has the capacity to produce viable seeds alike majority of the species of *Cymbopogon.* Thus the study of seed germination and seedling growth is very important for its utilisation in agronomic trial and also in connection with the imporvement of the plant by mutation breeding utilising the seed.

From germination behaviour of seeds it appears that 22± 1°C is the optimum temperature showing maximum (90%) seed germination. In connection with the effect of different quality of light, red has been noted to accelerate seed germination as compared to blue and white (control) light.

It has been observed that husked seeds immediately after harvest in the winter month of December show maximum percentage of germination (95) but gradually become deteriorated in course of time and understanding proper chemical treatment to show stimulation of seed germination, investigation has been carried out to study the effect of various chemicals such as growth hormones, growth regulators, vitamins, sugars, micronutrients and heavy metals.

Maximum stimulation of seed germination over control has been observed due to treatment of IAA, ABA, D-mannose, Nicotinic acid, Pyridoxine-hydrochloride, $MnCl_2$, $CuCl_2$, and $HgCl_2$. Moderate type of stimulation has been achieved by the treatment of GA, Phenoxy acetic acid, P-chlorophenoxy acetic acid, 2,4,5-trichlorophenoxyacetic acid, 3,4-Dichlorophenoxy acetic acid, 2, 4 - Dichloro phenoxy acetic acid, Pantothenic acid, Thiamine, Riboflavin, Xylose,

Glucose, Fructose, $NiCl_2$, $CoCl_2$, $CdCl_2$, $PbCl_2$ and $BoCl_3$. The treatment of sucrose, biotin and ascorbic acid shows very little stimulation of seed germination over control. Neither stimulation nor inhibition of seed germination has been recorded in connection with the effect of 2,4, 6-trichloro phenoxy acetic acid and kinetin. Inhibition of seed germination has been expressed during the treatment of galactose, mannose and Napthalin acetic acid.

Seeds of *C. pendulus* (Nees ex Steudel) W. Watson when covered by papery husk show low percentage of germination, maximum of 28%, as against 100% of germination in husked seeds. It is expected that the husk covering the matured seeds has some effect to cause dormancy during seed germination. With a view to understanding the nature of chemical inhibitor concerned, chemical investigation has been carried out specially with the husks of the seed subjected to soxhlet extraction with different solvents. After a thorough analysis it is observed that $NaHCO_3$ subfraction of ether soluble methanolic part shows the presence of appreciable quantity of solid substance having a characteristic flavour identified as coumarin after comparing melting point, chemical tests, chromatographic behaviour and IR spectrum of the isolated product with those of authentic coumarin. In presence of isolated coumarin the husked seeds show germination maximum of 30%.

In order to observe the effect of N,P and K on the seed germination and seedling growth in *C. pendulus* (Nees ex Steudel) W. Watson, sand culture experiment has been conducted in presence of nine sets of nutrient solution such as complete N.P.K. nutrient solution, complete -N, -P, -K, -NP, -NK, -PK, -NPK and distilled water as control. -P culture shows maximum percentage (53.33%) of seed germination as compared to complete culture which yields 40%. Low percentage of seed germination has been noticed in -NK culture. Germination is strikingly minimum (18.88%) in -NPK culture. Maximum height (76 cm) of the plant has been recorded in complete solution as compared to control (63cm) when the plantation period becomes 150 days. Complete culture also shows the highest number (6) of tiller per plant as compared to control (3). Similarly, maximum number of leaves per plant (9) has been observed in complete solution as compared to control (6).

Investigation has been conducted to grow seedlings of *C.pendulus* (Nees ex Steudel) W. Watson at three trials of spacings i.e. 75cm x 75cm, 60cm x 60cm and 50cm x 50cm. The height of the plants, number of tillers per plant and number of leaves per tillers have been observed to show comparatively good growth at all the three stages of development i.e. vegetative, pre-reproductive and reproductive stages due to spacing of 50cm x 50cm as compared to all other types of treatment.

N.P.K. fertilizer trial on *C. pendulus* (Nees ex Steudel) W. Watson has been conducted taking three doses, 0, 50 and 100 kg of each of N,P and K per hectare in 27 different combinatins including control ($N_0P_0K_0$) arranged in randomised block design. Entire dose of P, K and 50% of total N have been applied basally i.e. at the time of transplanting of seedling of 30 days old in each plot of 1m x 2.5m size and the other 50% applied after 30 days of initial application. Altogether 10 plants have been raised in each plot with a spacing of 50cm x 50 cm. Transplantation has been done in May. Cultural operations like, weeding, aerating the soil and watering were regularly done at 10 days interval.

The height of the plant has been observed to be maximum due to treatment of $N_{100}P_{100}K_{50}$ combination showing 140.60cm, 173.50 cm and 200.26 cm at the vegetative, pre-reproductive and reproductive stages of the plant respectively as compared to control values of 96.43cm, 120.50 cm and 137.30cm at the three stages of development of plant respectively. Maximum number of 39, 46 and 51 tillers per clump have been observed at vegetative, pre-reproductive and reproductive stages respectively during the treatment of $N_{50}P_{100}K_{50}$ combination. Maximum number of leaves / tiller at all the three stages of development of the plant has also been observed in the same combination compared to control.

The total yield of herbage and that of oil per plot under consideration have been estimated and expressed in terms of tonnes / ha/ year and kg / ha / year respectively. This is for understanding the efficiency in productivity of *C. pendulus* (Nees ex Steudel) W. Watson, growing in Darjeeling condition as compared to that of other species of *Cymbopogon* utilised commercially in India.

Maximum herbage yield of 75.88 tonnes/ ha/ year as compared to control (28.50 tonnes/ ha/ year) due to treatment of $N_{100}P_{50}K_{100}$ and which is the total of 17.80, 34.00 and 24.08 tonnes/ ha/ year at the 1st, 2nd and 3rd harvest respectively.

So far as the annual oil yield is concerned, maximum of 328.55/ kg/ ha/ year has been observed due to treatment of $N_{100}P_{50}K_{100}$ combination as compared to control which shows total annual oil yield of 91.19 kg/ ha. As regards percentage of oil yield, 0.49% has been observed due to treatment of $N_{50}P_{100}K_{50}$ as compared to control (0.32%). Percentage of citral has been observed to become 88.00 due to treatment of $N_{100}P_{100}K_{50}$ with the corresponding α and β-citral of 54.56% and 33.44% respectively against control showing 86% of total citral out of which 57.77% and 28.23% of α and β-citral respectively.

From fertilizer trial it appears that higher doses of nitrogen and phosphorus have positive effect on the yield of vegetative growth of *C.pendulus* (Nees ex Steudel) W. Watson, in Darjeeling plains of West Bengal while potassium has a beneficial effect on the yield of oil in the leaves of the plant.

Thus on the basis of overall performances of the plant it may be suggested that for commercial utilisation of the plant $N_{100}P_{50}K_{100}$ combination may be utilised during agronomic trial at the present day environmental condition of Darjeeling plains.

There is no doubt that though various other species and strains of lemon grass oil yielding plants are being utilised commercially now-a-days in our country, the wild plant like *C.pendulus.* (Nees ex Steudel) W. Watson, available in the plains of Darjeeling district, West Bengal, India, should not be underestimated so far as its commercial potentiality is concerned and more investigation is needed for its conservation and imporvement so that the plant may be utilised for economic development in the region.