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Volatile Compounds of the Microalga *Chlorella vulgaris* and Their Phytotoxic Effect

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Abstract: The volatile metabolites of green microalga, *Chlorella vulgaris,* grown under outdoor conditions were isolated by steam distillation and fractionated on a silica gel column using different organic solvent mixtures. The volatile metabolites and the individual fractions were analyzed using GLC and GC/MS. The volatile compounds were a mixture of 105 compounds of which 30 were identified. These components consisted of hydrocarbons, acids, alcohols, esters, aldehydes and ketones, having 33.67, 23.93, 15.62, 8.02, 3.24 and 2.71% of the total volatile components, respectively. The total volatile metabolites had strong inhibitory action on α-amylase activity and growth of the coleoptiles as well as the germination rate of barley grains. The acid fraction had a significantly higher phytotoxic effect in comparison with the other fractions and its action was mainly due to the presence of linoleic acid in this fraction. Hydrocarbon and polar fractions did not show any significant activity as phytotoxic agents.

Key words: *Chlorella vulgaris,* plant growth inhibition , volatile compounds and acid fraction

Introduction

Secondary metabolites, such as volatile compounds of microalgae grown in fresh water may impart characteristic odors to the water (Juttner, 1983, 1992; Rzama *et al.,* 1995; Borowitzka, 1997 and Ogbonna *et al.,* 1998) but these volatile compounds have received little investigation due to their low content in comparison with higher plants (Kajiwara *et al.,* 1993). However, these secondary metabolites have become of increasing interest in recent years because they have proved to be a rich source of different compounds with diverse structural features and interesting biological activities (Brown and Miller, 1992; Anggadiredja *et al.,* 1997). These biologically-active compounds have been identified as carbonyls, alkenes, saturated and unsaturated aliphatic alcohols, aldehydes, ketones, esters, thioesters, sulfides terpenes, fatty acids, isoprenylated and brominated hydroquinones, and phycotene, which have served as potential compounds in the pharmaceutical areas including: antibacterial, antifungal, antiviral and anticancer agents (Kajiwara *et al.,* 1993; Mathew *et al.,* 1995; Morinoto *et al.,* 1995; Borowitzka, 1997 and Aboul-Enein *et al.,* 2001). However , most of these studies are concerned with the chemical composition of volatile metabolites, but not their bio-activities.

In present study, the steam-volatile metabolites of the microalga *Chlorella vulgaris,* grown in fresh water culture were isolated and analyzed using GC and GC mass spectrometry to identify their components. The bio-activity of the volatile metabolites was evaluated (as phytotoxic) on α-amylase activity, the growth of coleoptiles and germination rate of barley grains.

Materials and Methods

The algae: A pure culture of *Chlorella vulgaris* , isolated and identified by Shaheen *et al*. (1974), was mass-cultured in a medium in 200 L outdoor glass tanks. The nutrient solution, NS1 was used for the cultivation of *Chlorella* , as described by Payer and Trultzch (1972). Carbon dioxide was used as a carbon source and the culture was aerated with 5 % by volume CO_2 in air mixture. The pH of the medium was maintained between 6 and 7 during the growth period by adjusting the flow rate of CO_2 in the culture. The other details of cultivation conditions and processing were described by El-Fouly *et al*. (1985). At the end of the long phase of growth, the culture was harvested by centrifugation at 6000 r.p.m. for 10 min. and kept at -20°C until used.

Isolation of volatile compounds: Suspension of *Chlorella vulgaris* cells (100 g.) was placed in a flask (4 L) containing 2 L of double distilled water. The suspension was distilled slowly until about 250 ml of its volume was collected as a distillate in a receiver cooled by NaCl and ice. The final distillate was extracted with methylene chloride, dried over anhydrous Na_2SO_4, and then reduced to 2 ml by evaporation using a Kuderna Danish concentrator (Tsuchiya and Matsumoto, 1988).

Fractionation of volatile compounds : A portion of the volatile components was placed on a column (12×500 mm^2) of silica gel (Merck 60, 230-400 mesh). The hydrocarbon fraction was eluted with pentane (150 ml). The polar fraction was then eluted with distilled diethyl ether (polar fraction I) and with an organic solvent mixture composed of diethyl ether and methanol (9:1) (polar fraction II, contained highly polar and oxygenated compounds). Finally, the acidic fraction was eluted with an organic solvent mixture composed of diethyl ether, methanol and acetic acid (45:4:1, v / v / v), as reported by Tava *et al*. (1991) and Rzama *et al*. (1995).

Identification of volatile compounds: The total volatile components and their different fractions (four fractions) obtained by CC were analyzed using a Pye Unicam PU 4550 gas chromatography system equipped with a flame ionization detector (220°C). A coiled glass column (1.5 m × 4 mm) packed with Diatomite C (100-120 mesh) and coated with 10% PEGA was used with nitrogen as a carrier gas. The oven temperature was programmed at 4°C/ min from 60 to 180°C and was held at 180°C for 15 min. The injector temperature was 300°C. Gas flow rates for N_2, H_2 and air were 30, 33 and 330 ml/min, respectively. Identification of peaks were performed by comparing the relative retention times of each peak with those of known compounds. Also, volatile compounds were mixed with reference compounds and injected into the GLC to verify the peaks identity (co-chromatogram) as mentioned by El-Baroty *et al*. (1997).

Gas chromatography-Mass spectrometry (GC/MS) of volatile compounds: The volatile components and their different fractions were analyzed by GC/MS using a Hewlet Packard capillary GC- quadruple MS system (Model 5970) fitted with

a 50 m × 0.32 mm, i.d., fused silica column coated with carbowax 20 m (0.32 mm thickness) programmed as follows: 60-180°C (4°C min^{-1}) GS-MS analysis was made in splitless mode with helium as the carrier gas at a flow rate of I ml min^{-1}. The mass spectrometer was operated at 70 eV and the identification of the compound was based on a comparison of retention time and mass spectra with those of authentic samples and with literature data. Fragmentation patterns were used to determine molecular structure when reference spectra were unavailable.

Phytotoxicity bioassay : The physiological effect of volatile components of *C . vulgaris* and their fractions as well as the basic compounds occurring in these fractions were evaluated on grains of barley using the Edney and Rizvi (1996) method. Barley grains (5 g) were soaked in 25 ml of either total volatile sample at different concentration levels (50, 100, 250, 500 and 1000 ppm) or their different fractions as well as their individual components [tested at similar concentration levels present in net volatile oil (hexadecene (100 ppm); octadec-9,10-dieneoic (100 ppm)] and hexadecanol (150 ppm)) for 4 h at 28°C ± 1 . The grains were then transferred to Petri dishes containing two disks of filter paper moistened with 20 ml of respective solution and incubated at 28 ± 1°C for 48h. Grains with prominent radical growth were considered to have germinated. The germination rate (%) was calculated. Then, the grains were incubated for 5 days in a dark chamber at 28 ± 1°C. At the end of the experiment the coleopitle lengths were measured to calculate the percentage of inhibition compared with those of the control.

The α-amylase activity of imbibed grains was determined according to Marambe *et al.* (1992) method. The α-amylase activity of tested grains (crude extracts) was determined by a spectrophotometric method using starch as a substrate and I_2/KI solution as reagent. The developed colour was measured at 620 nm. Standards of starch were analyzed simultaneously and the α-amylase activity of the extract of grains was expressed as μg of starch degraded by 1ml of extract in 30 min at 30°C. In all cases, replicate samples were analyzed and any sample that showed 20% reduction or greater at 100-1000 ppm levels in comparison with control were considered significantly active (Steven and Merrill, 1980).

Statistical analysis: The standard analysis of variance procedure of factorial experiment in completely randomized design was applied for all biological data according to the method outlined by Snedecor and Citron (1973).

Results

The volatile components of *Chlorella vulgaris* cultivated under outdoor conditions were obtained by steam distillation in a yield of 0.08% (v/w) with a fishy odour. The total volatile components and their four fractions obtained by column chromatography were analyzed by GC and GC-MS. The total volatile compounds of *Chlorella* were a complex mixture of about 105 compounds (detected by FID-GC), of which 30 were identified (by GC-MS) and listed with their relative peak areas in Table 1. The 30 identified and 75 unidentified compounds represented 87.28% and 12.72% of the total volatile components, respectively. However, the unidentified compounds were in very low abundance (trace < 0.1%) and are not listed. The identified compounds were grouped under chemical class and their percentages are given in Table 1. The *C. vulgaris* volatile compounds were composed of hydrocarbons, acids, alcohols, esters, and aldehydes as well as ketones, which presented 33.67, 23.93, 15.62, 8.02, 3.24 and 2.71% of the total volatile substances, respectively.

As shown in Table 1, the hydrocarbons represented a large part of the total volatile components obtained from *C. vulgaris*. These compounds included saturated and unsaturated hydrocarbons, some of which contain one or two double bonds and a cyclic ring. However, hexadecene was the most abundant (15.30%) in *C. vulgaris*, while the β-pinene (7.63%) was the second most important compound. Other hydrocarbons such as dodecane (1.76%), heptadecene (2.11%) and octadecane (3.21%) were identified as the minor compounds (< 10.0%) while heptadecane (0.74%), tetracosane (0.35 %) and heptacosane (0.55 %) ,were detected as trace constituents (< 1.0%).

The acidic fraction obtained from the total volatile components of *Chlorella* by column chromatography was converted into methyl esters which were composed of saturated and unsaturated monocarboxylic acids with chain lengths ranging from C_{12}-C_{18}. Four saturated fatty acids: $C_{12:0}$ (0.2%), $C_{14:0}$ (0.9%), $C_{16:0}$ (8.73%), $C_{18:0}$ (2.2%) and three unsaturated acids: $C_{18:1}$ (0.73%), $C_{18:2}$ (10.2%) and $C_{18:3}$ (0.97%) were detected in the acid fraction. Most of the acids occurred in very low abundance (< 1.0%). However, $C_{16.0}$ and $C_{18.2}$ were the most important ones.

The alcoholic group makes the third major part of the volatile components of *C. valgars* (15.62 %, total oil). The hexadecanol (10.8%) was present as the most abundant constituent of this group. Other minor compounds such as nondecanol (0.34 %) and octadecanol (0.52 %) were also identified.

The ester group was characterized by esterification of saturated, mono and di-unsaturated fatty acids. The methyl esters of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:.2}$ were present as minor components. $C_{18:0}$ was the predominant compound in this fraction.

Ketone and aldehyde groups were present in relatively small amounts and among these compounds, hexadecanone (1.7 %) and hexadecanal (2.2 %) were found to be the minor compounds (< 10% - > 1%). Also, nonadecanal ,hexanal , decanone and α and β- ionone were identified as trace compounds.

Phytotoxic effect of *Chlorella vulgaris* steam volatile compounds and their different fractions : The influence of total steam volatile compounds of *Chlorella vulgaris* and their fractions obtained by CC as well as the identified compounds on α-amylase activity, coleoptile growth and germination (%) of barley grains are shown in Table (2). The (%) germination rate of barley was significantly (LSD value at P = 0.01 was 7.84) reduced with exposure to the total volatile components and this effect was increased by increasing the level of volatile component concentrations. The total volatile compounds of *Chlorella* at low concentrations of 100 and 250 ppm reduced the germination (%) of barely grains to 82.7 % and 72.3%, respectively. At 1000 ppm the germination (%) of barley grains was reduced to 45.9% in comparison with the control. Also, the (%) germination rate of barley was significantly reduced with exposure to the solution of the acid fraction (240 ppm) and its individual identified compounds (linoleic acid: octadec-9, 10-dieneoic, 100 ppm). The rates of reduction in germination were 50.1% and 52.4% as compared with the control. In addition, the rate of the reduction of acid fraction was 50.1% and it is higher than octadec- 9,10-dieneoic (52.4%) .The hydrocarbon fraction did not show a significant reduction in the germination rate of barley. The polar fractions I and II , hexadecanol and hexadecene showed significant reductions in the germination rate, but these reductions were less than 20 % and were therefore, not

Table 1: Composition (%) of the green microalga *Chlorella vulgaris* volatile metabolites

Class	Compounds	%	Mode of I. D*
Hydrocarbons	α-pinene	2.11	a,b,c
	β-pinene	7.63	a,b,c
	Dodecane	1.76	c
	Hexadecene	15.30	a,b,c
	Heptadecane	0.74	b,c
	Heptadecene	2.11	b,c
	Octadecane	3.21	b,c
	Tetracosane	0.35	b,c
	Heptacosane	0.55	b,c
Acids	Odecanoic	0.2	b,c
	Tetradecanoic	0.9	b,c
	Hexadecanoic	8.73	a,b,c
	Octadecanoic	2.2	a,b,c
	Octadec-9-enoic	0.73	b,c
	Octadec-9,12-dienoic	10.20	a,b,c
	Octadec-9,12,15-trienoic	0.97	c
Alcohols	Hexadecanol	10.80	a,b,c
	Octadecanol	2.30	a,b,c
	Nonadecanol	2.01	c
	Phytol	0.51	c
Esters	Methyl-hexadecanoate	2.20	a,b,c
	Methyl-octadecanoate	3.52	a,b,c
	Methyl-octadec-9-enoate	0.98	a,b,c
	Methyl-octadec-9,12-dienoate	1.32	a,b,c
Aldehydes	Hexanal	0.14	c
	Nonadecanal	0.90	c
	Hexadecanal	2.20	c
Ketones	Decanone	0.7	c
	Hexadecanone	1.70	c
	β-ionone	0.13	c
	α-ionone	0.18	c
Unidentified	----------	12.72	----------
Total		100	

*(a) Identified by co-chromatography with authentic sample, *(b) Identified by comparison of retention time and GC/MS data with those of authentic sample and *(c) Identified by comparison of GC/MS data with literature data

Table 2: Effect of steam volatile compounds and their fractions of *Chlorella vulgaris* on germination rate (%), coleoptile growth (%) and α-amylase activity (%) of barley grains

Treatments	Concentrations (ppm)	Relative germination	Cleoptile Growth	α-amylase activity
Control	-----	100a	100a	100a
Total volatge	100	82.7b	86.6b	82.7c
compounds	250	72.3d*	79.5c*	74.8c*
	500	63.9e*	70.8d*	60.0e*
	1000	45.9f*	53.4e*	52.8f*
Acid fraction	150	50.1f*	58.7e*	55.5f*
Hydrocarbon fraction	330	95.5a	98.3a	95.9a
Polar fraction (PI)	250	90.6b	89.7b	92.5b
Polar fraction (PII)	250	86.1b	88.9b	86.9b
Linoleic acid	100	52.4f*	58.0e*	54.6f*
Hexadecanol	100	87.3b	85.7b	89.3b
Hexadecene	150	90.8b	92.5b	90.2b

Numbers in each column followed by same letters are not significantly different at P=0.01 (LSD = 7.84, 8.29 and 6.50 for germination rate, coleoptile growth and α-amylase activity, respectively).
*The treatment showed 20% reduction or greater from those of control (100%) were considered significant bio-active (Steven and Merrill, 1980).

considered to be significantly bio-active as reported by Steven and Merrill (1980).

Table 2 shows the influence of total volatile components of *Chlorella* and their fractions on the development of coleoptile growth (lengths of coleoptile were used as an indicator of growth) of barley grains. The results demonstrated that the coleoptile growth of barley was gradually decreased by increasing the concentration of total volatile compounds. The coleoptile lengths of barley were reduced by 86.6%, 79.5%, 70.8 % and 53.4 % from the control (untreated barley grains 100 %) when exposed to total volatile compounds of *Chlorella* at concentration levels of 100, 250, 500 and 1000 ppm, respectively. In addition, the coleoptile of barley grains exposed to the volatile components of *Chlorella* were more

thick and quite distinct from the thin control. The coleoptile growth of barley was significantly reduced with exposure to the solution of the acid fraction and its individual components (linoleic acid: octadec- 9,10-dieneoic, 100 ppm). The rates of reduction in coleoptile growth were 58.7 and 58.0%, as compared with control . The acid fraction had a significantly higher effect on coleoptile growth of barley than octadec-9,10-dieneoic. The other fractions (hydrocarbon, polar fractions I and II) and their individual components did not show any significant bio-activity for reduction in coleoptile growth rate of barley.

Table 2 shows the α-amylase activity of barley grains exposed to the total volatile compounds of *Chlorella* and their fractions as well as the isolated compounds. The total volatiles at concentration levels of 100, 250, 500 and 1000 ppm reduced the α-amylase activity of barley grains to 82.7%, 74.8 %, 60.0% and 52.8 % relative to the control. Therefore, the total volatile oil of *Chlorella* significantly decreased α-amylase activity of barley grains and this decrease was increased with concentration level of oil. The other fractions (hydrocarbon, polar fractions II and I) and their individual components did not show any significant activity for decreasing α-amylase activity. However, the acid fraction and its major individual components (linoleic acid) possessed highly significant phytotoxic activity compared with other fractions, but its activity was lower than that of the total volatile oil. This means that the acid fraction and linoleic acid had a similar pattern on α-amylase activity as the total volatile compounds. In general , the phytotoxicity of *Chlorella* volatile compounds and their fractions as well as linoleic acid could be arranged in following order : total volatile oil ≥ acid fraction > linoleic acid > polar fraction (PII) > polar fraction (PI) > hydrocarbon fraction

Discussion

The volatil ecompounds of C*hlorella vulgaris* were characterized by containing high quantities of hydrocarbons (33.67 %) and acidic (23.93%) compounds. These compounds are found in many microalgae (e. g., *Scenedsmus sp*. *Chlorella sp*.) as major chemical classes and have been reported as originating from decarboxylation of fatty acids, e.g., palmitic and stearic acids (Gelpi *et al*., 1970; Juttner, 1992; Rzama *et al*., 1995). Also, the terpenoid compounds identified in *C. vulgaris* volatile oil are probably intermediates in the formation of higher terpene compounds (Zolotovish and Velev, 1973).

Ketones (2.1 %. and aldehyde (3.4%) were found as minor components in *Chlorella* oil and these may originate from the degradation of unsaturated fatty acids and carotenoids (Kodama, 1986). Also, Kajiwara *et al*. (1993) suggested that the aldehyde and ketone compounds are formed in algae as derivatives of carotenoids by oxidative cleavage of double bonds in various positions. On the other hand, the trace amount of α, β- ionone components could be formed during degradation of non-carotenoid compounds.

The alcoholic fraction was characterized by containing a predominance of saturated aliphatic alcohols ; alcohols are probably formed as intermediates during the formation of higher terpene compounds, e.g., carotenoids and others (Rzama *et al*., 1995).

Several investigators have examined the volatile compounds of *Chlorella* species grown in natural environmental conditions, e.g., Gelpi *et al*. (1970), Juttner (1983) and Rzama *et al*. (1995). There were both qualitative and quantitative differences between the obtained results in present work and that of the aforementioned authors and also between them. The differences may be due to environmental conditions (e.g. the light intensity, nutrient medium, temperature, etc.), the extraction methods of the oil (steam distillation and stripping), the degree of freshness of the algal material and the age of the algal cells (Juttner, 1983; Henatsch and Juttner, 1983; Tsuchiya and Matsumoto, 1988).

The total steam volatile compounds of C. *vulgaris* and their fractions as well as the characterized individual compounds, significantly reduced the α-amylase activity, coleoptile growth and germination rate (%) of barley and their effects gradually increased with increasing concentrations. Also, the acidic fraction and its major component {octadec- 9,10-dieneoic (linoleic acid)} produced the similar results. However, the phytotoxic effect of the total volatile compound was lower than that produced by the acid fraction and linoleic acid (when tested at concentrations similar to that present in the net volatile oil). Consequently, the activity of *C. vulgaris* volatile can be explained by reference mainly to its acidic fraction. These results were supported by those of Buller *et al*. (1976), and Edney and Rizvi (1996), who reported that the germination of sorghum seeds was significantly reduced with the exposure to fatty acids and their effect was increased with the increase in length of carbon chain of the fatty acids. Also, Buta (1983) reported that linoleic acid acts as a plant growth inhibitor against several plant species. On the other hand, the microalgae are releasing some volatiles into the aquatic ecosystem as natural growth inhibitors for submerged macrophytes and other microorganisms (Stevens and Merril, 1980; Henatssch and Juttner, 1983). Also, Ueda *et al*. (1991) identified methyl jasmonate and jasmonic acid in *Chlorella* and *Spirulina* which was found to be plant growth inhibitor.

The phytotoxic effect of the volatile constituents of *Chlorella* and its acid fraction may be due to inhibition of enzymes of germinated grains that are involved in production of free sugar (e.g.,_α- amylase) and / or amino acids (proteases), where the germination is a process of reactivation of the metabolic machinary of the seed and the emergence of the radicals (Marambe *et al*., 1992, Edney and Rizvi 1996 and El-Baroty and Abddel-Lattif, 1997).

The use of natural volatile components has been welcomed in recent years in preference to synthetic herbicides from both environmental and health consideration (Bagchi *et al*., 1997). Most synthetic chemicals are more hazardous due to their long persistence, non-target toxicity, carcinogenic and mutagenic activities (Duke *et al*., 1988; El-Baroty, 1997). These volatile oils are characterized by fat solubility, volatility, ephemeral nature, easily biodegradable and recognized as safe (Mishra and Dubey, 1994; El-Baroty and Abddel-Lattif, 1997).

Finally, the potency of *Chlorella* volatile compounds, which have phytotoxic activity similar to an allelopathic action, could make these volatile metabolites a promising agent for emerging integrated pest management (IPM) strategies for weed control.

References

Aboul-Enein, A. M., R. K. El-Baz, G. S. El-Baroty, A. M. Youssef, H. H. Abdel-Baky , 2001. Antioxidant activity of algal extracts on lipid peroxidation. J. Appl. Phycol., (In press).

Anggadiredja J., R., Andyani and H. Muawanah, 1997. Antioxidant activity of *Sargassum polycystum* (Phaeophyta) and *Laurencia obtusa* (Rhodophyta) from Seribu islands. J. appl. Phycol., 9: 477-479.

Bagchi, G.D., D.C. Jain and S. Kamar, 1997. Arteether: A potent plant growth inhibitor from *Artemisia annua*. Phytochemistry, 45:1131-1133.

Borowitzka, M.A., 1986. Microalgae as sources of fine chemicals. Microbiol. Sci., 3: 372-375.

Borowitzka, M.A., 1997. Microalgae for aquaculture: Opportunities and constraints. J. appl. Phycol., 9: 393-401.

Brown, M.R. and K.A. Miller, 1992. The ascorbic acid content of eleven species of microalgae used in mariculture. J. appl. Phycol., 4: 205-215.

Buta, J.G., 1983. Lino;ieic acid as a plant growth inhibitor from seeds of *Sesbania punicea* . J. Nat. Prod., 46:755.

Duke S. O. R. N., S. M. Pasal, Lee, 1988. Terpenoids from the genus *Artemisia* as potential pesticides. ACS Sump. Ser., 335: 318-334

Edney N.A. and M. Rizvi, 1996 . Phytotoxicity of fatty acids present in dairy and hog manure. J. Environ. Sci. Health, 31: 269-281.

El-Baroty, G.S., 1997. Pesticidal activity of essential oil of *Schiuns* species. J. Agric. Sci. Mans. Univ., 22: 1277-1285.

El-Baroty, G. S. and M. S. Abdel-lattif, 1997. Essential oils of *Artemisia judaica* and *Artemisia cina* and their biological activities. J. Agric. Sci. Mans. Univ., 22: 4715-7424.

El-Fouly M., A.M. Yossef, F. K. El-Baz, F. E. Abdalla, M.M. Abdel Hafeez, 1985. Studies on fat accumulation in *Chlorella* and *Scenedesmus*. Arch. Hydrobiol-Alglogical. Suties, 40: 439-448.

Fytianos, K., E. Evgenidon and G. Zachariadis, 1999. Use of microalgae as biological indicators of heavy metal pollution in Thermaiks Gulf, Greece. Bull. Env. Contam. Toxicol., 62: 630-637.

Gelpi, E., H. Schneider, J. Mann and J. Oro, 1970. Hydrocarbons of geochemical significance in microscopic algae. Phytochemistry, 9: 603-617.

Henatsch J. J. and F. Juttner, 1983. Volatile odorous excretion products of different strains of *Synechococcus* (cyanobacteria). Wat. Sci. Technol., 15:259-266.

Jennings, W. and T. Shibamoto, 1980. Quantitative analysis of flavours and fragrant volatiles by Glass capillary gas chromatography. Academic Press, New York.

Juttner, F., 1983. Volatile odorous excretion products of algae and their occurrence in the natural aquatic environment. Water Sci. Tech., 15:247-257.

Juttner, F., 1992. Flavour compounds in weakly polluted rivers as a means to differentiate pollution sources. Water Sci. Tech., 25:155-164.

Juttner F. and B. Hoflacher, 1985. Evidence of β-carotene 7,8 oxygenase (β-cyclocitrol, crocetindial generation) in Microcystis. Arch. Microbiol., 141: 337-343.

Kajiwara T., K. Kodama, K. Halanaka and K. Matsui, 1993. In: Bioactivity volatile compounds from plant, (Teranishi R, Buttery R, Sugisawa H (eds)), ACS Symposium Series, 525 pp: 103-120.

Kodama, H., 1986. In Modern Methods of Plant Analysis ,New Series 5.Liskens H, Jackson JF eds. Springer, Perlin, p: 277.

Likensm, S.T. and G.B. Nickerson, 1966. Isolation and identification of volatiles. J. Chromatog, 21:1-8.

Marambe, B., T. Ando and K. Kouno, 1992. Alpha-amylase and protease activity and water relation in germination sorghum (Sorghum biocolor Moench) seeds as affected by animal waste composts. Soil. Sci. Plant Nutr., 38: 123-131.

Mathew, B., R., P. Sankaranarayanan, C. Nair, T. Varghese, B. Smanathon, N.S. Amma, N.S. Amma and M. Nair, 1995. Evaluation of chemoprevention of oral cancer with *Spirulina fusiformis*. Nutr. Cancer, 24: 197-202.

Mishra, A. K. and N.K. Dubey, 1994. Evaluation of some essential oil for their toxicity against fungi causing deterioration food commodities. Appl. Envron. Microbiol., 60: 1101-1105.

Morinoto, T., A. Nagatu and N. Murakami, 1995. Anti-tumor promoting glyceroglycolipid from green alga *Chlorella vulgaris*. Phytochem., 40: 1433-1437.

Mrechie, G., P. Lavens, P. H. Dhert, M. Dehasque, H. Nelis, A. Deleenheer and P. Sorgeloas, 1995. Variation of ascorbic acid content in different live food organisms. Aquaculture, 134: 325-337.

Ogbonna J., T. Shota and H. Tanaka, 1998. Heterotrophic cultivation of *Euglena gracilis* for efficient production of α-tocopherol. J. Appl. Phycol., 10: 67-74.

Payer, H. D. U. and Trultzch, 1972. Meeting the trace element requirements of dense culture of microalgae under special conditions of manganes and vandium. Arch. Mikrobiol., 84: 43 – 53.

Rzama, A., A. Benharref, B. Arreguy, J. Dufourc, 1995. Volatile compounds of green microalgae grown on reused waste water. Phytochem., 38: 1375-1379.

Shaheen, A. B., M. M. El-Fouly, E.Y. El-Ayouty, A.M. Saleh, 1974. Growth and protein content of *Chlorella* during indoor and outdoor cultivation. Al-Azhar Agric. Res. Bull., 2:

Snedecor, G. and W.C. Citron, 1973. Statistical Methods, 6 th ed., Iowa State Univ. Press Amas, Iowa, USA.

Steven, K.L. and G.B. Merrill, 1980. Growth inhibitors from spikeruch. J. Agric. Food Chem., 28: 644-646.

Tava, A., N. Berado and M. Odordt, 1991. Composition of essential oil of tall fescue. Phytochem., 30: 1455-1458.

Tsuchiya, T. and A. Malsumoto, 1988. Identification of volatile metabolites produced by blue-green algae. Wat. Sci. Tech., 20: 149-155.

Ueda, J. and J. Kato, 1982. Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiol. Pl., 54: 249-252.

Ueda, J., K. Miyamoto, T. Sato, Y. Momotani, 1991. Identification of jasmonic acid from *Euglena gracilis* Z as a plant growth regulator. Agric. Biol. Chem., 55: 275-276.

Zolotovich, G.D. and T.K. Velev, 1973. Content of alcohols in concrete from *Scenedesmus acutus*. Compt. Rend. Acad. Bulg. Sci., 26: 795-798.