PLANT BIOCHEMISTRY

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BIOLOGICALLY ACTIVE SUBSTANCES OF AQUEOUS-ETHANOLIC EXTRACT OF *TAGETES SIGNATA* BARTL. № 13152-8 'VETVISTY' SPECIMEN OF NIKITA BOTANICAL GARDENS COLLECTION

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Introduction

Tagetes L. genus includes more than 30 cultivars growing *in vivo*, mainly in Central America. *Tagetes patula* L. *Tagetes erecta* L., *Tagetes signata* Bartl., syn. *Tagetes tenifolia* Cav. are considered the most popular within crop [5, 18, 27].

Different *Tagetes* cultivars are used to treat liver or kidneys disorder, for pain control – as a diuretic and pathogenic remedy. *Tagetes* L. preparations permit to control nervous tension and improve spirit. The plant is popular in dermatology as well, because it cures blackheads and possesses tonic and regenerative properties, so important for skin [1, 10, 13, 16, 17, 19, 21, 26, 30].

Essential oils (EO) of different *Tagetes* L. cultivars are known as matters with antibacterial, antimicrobial, larvicidal, repellent and fungicidal effect [7, 10, 11, 22-24, 26, 28, 29].

Tagetes flowers are possible to use as a spice "Imeretian Saffron" [30]. Besides some species of *Tagetes* L. are source of carotenoids (for example, in poultry farming – for intensifying egg color) [2, 4, 9], pigments and lutein [3, 20].

Allowing for variety of useful *Tagetes* L. properties, introduction of new cultivars is of great importance now, particularly on South Coast of the Crimea, as well as breeding new sorts with valuable characteriristics.

Nikita Botanical Gardens – National Scientific Centre bred promising (by ornamentality, disease- and pest-resistance, crop capacity, essential oil content) specimenTagetes signata L. № 13152-8 "Vetvisty". It was found out that essential oil composition of this specimen included trans-tagetenon, dihydro-tageton, trans-tageton, cis-tagetenon, limonene, sabinene, trans-ocymen, eugenol and a number of minor components [15].

In this way EO extracting out of raw material is accompanied with oxidation process if to apply method of steam distillation; getting native substances demands their transition into water-ethanol extracts.

It`s a well-known fact, that flower extracts of T.*signata* is a result not only of volatile substances transition, but phenol substances, which present antioxidant properties: hydroxycoric acids (caffeic, rosemary, chlorogenic, p-Coumaric acid), flavonoids and their glycosides (apigenin, hyperoside, isoquercetin, quercetin, rutin) [12, 15]. At the same time there is dependence of chemical composition in raw material from cultivar and sort belonging, soil and climatic conditions of habit.

Allowing for data mentioned above, investigation of chemical composition of T.*signata* specimen water-ethanol extract, № 13152-8 "Vetvisty", cultivated on South Coast of the Crimea, is a vital question.

The study purpose is to research qualitative and quantitative composition of biologically active substances (volatile terpenes, phenol compounds, vitamins) of T.*signata* № 13152-8 "Vetvisty" under conditions of South Coast of the Crimea, to get an opportunity for use it in medioprophylactic production.

Objects and methods of the research

Study research is water and ethanol extract out of specimen T.*signata* №13152-8 "Vetvisty" flowers, yielded during mass blooming.

Specimen was bred in NBG-NSC applying individual selection from seed population. Plant of 50-60 sm high. Stem is straight, branchy from the bottom, well-leafed. Leaves are sectile. Inflorescence is a basket of 19-24 mm across diameter, 5 semiflosculous flowers, corolla is golden-yellow. Blooming period is the whole summer, mass blooming happens from July the 10-20th. Specimen is disease- and pest-resistant. Crop capacity made 84,7 center/ha, essential oil content – 0,46% of the raw material, EO yield made 40,8 kg per hectare.

Concentration of biologically active substances was determined in water-ethanol extract, made of air-dry plant mass, yielded during mass blooming. Extraction was carried out applying 50-% ethanol having ratio of raw material and leach – 1:10 what was prepared for 10 days at room temperature.

Component composition of volatile substances was determined applying chromatograph Agilent Technology 6890N with mass-spectrum detector 5973N. Chromatographic column – capillary HP-1 (30 m long); inside diameter – 0,25 mm. Thermostat temperature was programmed in a range 50-250 °C with velocity of 4°C/min. Injector temperature - 250°C. Carrier gas is helium, stream velocity – 1,0 sm^3/min. Transfer from gas chromatograph to mass spectrometric detector was heated till 230°C. Source temperature was kept up at 200°C. Electronic ionization was carried out at 70 eV with mass range m/z from 29 till 450. Identification of essential oil components was guided by comparison results obtained in terms of chemical substances mass spectra with publishing data of mass spectra NIST05-WILEY2007 (about 500000 of mass spectra).

Component composition of phenol substances was determined applying chromatograph "Agilent Technologies" (model 1100), completed with running vacuum degasifier G1379A, 4-channeled pump of low pressure gradient G 13111A, automotive injector G 1313A, thermostat of heaters G13116A, diode-matrix detector G1316A, fluorescent detector G1315B. Chromatograph heater 2,1mm x 150 mm, filled with octadecilsilil sorbent "ZORBAX" SB-C18 and 3,5 mkm graining was applied during analysis. Gradient regime of chromatography that forsees changes of component A (0,1% orthophosphoric acid, 0,3%-tetrahydrofuran, 0,018%- triethylamine) and B (methanol) components in eluting mixture. Feed rate of mobile phase made 0,25 sm^3/min; working pressure of eluent – 240-300 kPa; sample volume – 2 mkl; scanning time 2 sec, scale of measurements – 1,0. Identification of phenol substances was carried out according to time of standard keeping and spectrum characteristics (wave length – 313 (for phenol acids and their derivatives), 350 (for glycosides of flavonoids), 371 nm (for flavonoids)).

Concentration of carotinoids was determined applying photometric method [14], ascorbic acid – by titration of potassium iodate [6].

Results and discussion

It was found out that concentration of volatile substances in specimen extract (T.*signata* № 3152-8 "Vetvisty") made 32,96 mg/100 g of raw material.
7 components were identified (table 1, fig.1).

Table 1

Volatile substances of water and ethanol extract *Tagetes signata* Bartl. № 13152-8 "Vetvisty"

№	Output time, min	Component	Concentration, %
1	6.38	cyclohexanone	8,49
2	7.25	3,5-dimethylcyclohexe-2-en-1-on	10,2
3	8.27	propylvaleriate	2,28
4	8.36	hexe-1,2,6-triol	2,76
5	14.12	tagetenone	17,3
6	15.11	4-vinylphenyl acetate	8,23
7	16.63	P-vinylguaiacol	50.8

The principal volatile components of this extract are P-vinylguaiacol (50,8%) and typical for T.*signata* № 13152-8 "Vetvisty" tagetenone (17,3%). Dominant group of compounds in the extract is aroma (59%; P-vinylguaiacol and 4-vinylphenyl acetate). The biggest number of components (3,5-dimethylcyclohexe-2-en-1-on, cyclohexanone, hexe-1,2,6-triol, propylvaleriate) belongs to aliphatic compounds, what is 23,7% in amount. Typical for T.signata component – tagetenone is a monoterpenic compound.

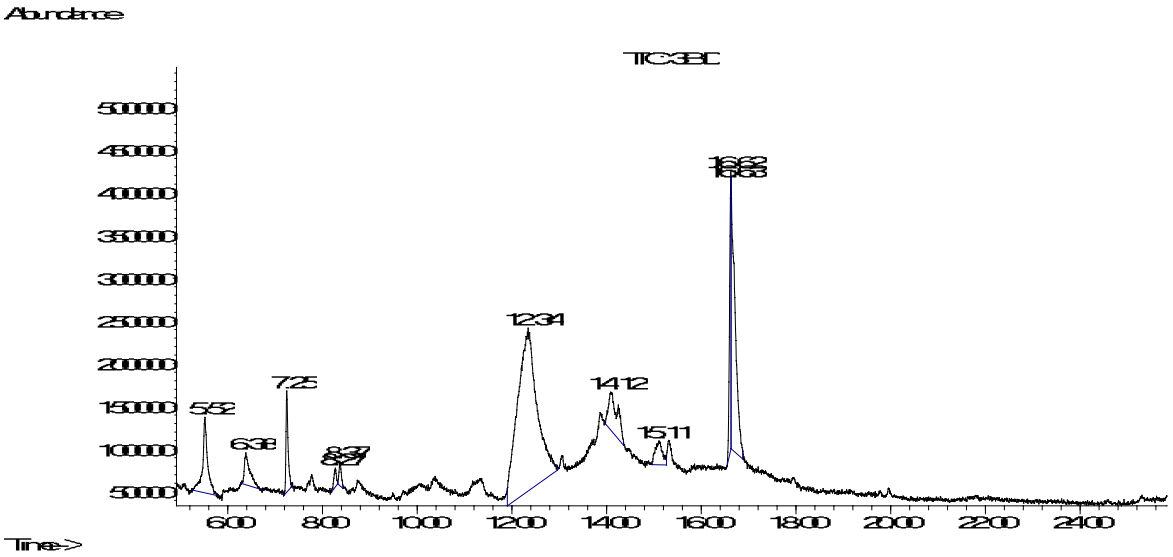

Fig. 1 Chromatogram of volatile substances in water-ethanol extract of *Tagetes signata* Bartl. № 13152-8 "Vetvisty"

It was found out that content of phenolic substances in extract of T.*signata* № 13152-8 "Vetvisty" made 1821 mg/100 g of plant material (table 2, fig.2). 11 components were revealed, 6 compounds – identified.

Table 2

Phenolic substances of water and ethanol extract Tagetes signata Bartl.№ 13152-8 "Vetvisty"

№	Output, min	Component	Concentration mg/100 g
1	15.35	Caffeic acid derivatives	142
2	17.92	Ferulic acid	152
3	18.73	Unidentified flavonoid	132
4	18.96	Unidentified flavonoid	79
5	19.34	Quercetin -3-O-galactoside	301
6	19.92	Rutin	331
7	20.28	Unidentified flavonoid	65
8	20.76	Unidentified flavonoid	332
9	21.40	Quercetin -3-O-glucoside	217
10	23.70	Unidentified flavonoid	38
11	24.39	Lutheoline	32

Among identified substances in study extract the following dominate: glicosides of quercetin – rutin (331), quercetine-3-O-galactoside (301) and quercetine-3-O-glucoside (217 mg/100 g). Hydroxycoric acids are presented by ferulic acid and caffeic acid derivatives, which make 294 mg/100 g in total.

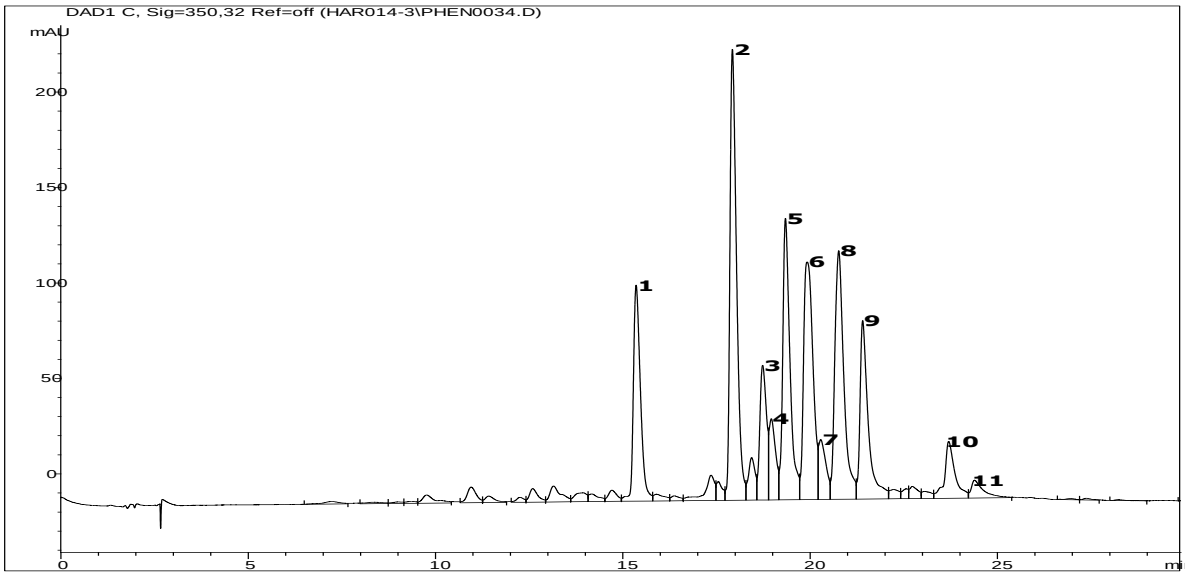

Fig.2 Chromatogram of phenolic substances in water-ethanol extract of *Tagetes signata* Bartl. № 13152-8 "Vetvisty"

Vitamin concentration was determined in the study extract as well. Concentration of ascorbic acid made 14,54 mg/100 g, carotinoids – 5,19 mg/100 g.

In this sway water-ethanol extract of T.signata specimen №13152-8 "Vetvisty" contains not so much of volatile terpenes, but could be considered as a source of P-vinylguaiacol, glicosides of quercetine, ascorbic acid and carotinoids.

Conclusions

In terms of the research qualitative and quantitative composition of biologically active substances (volatile terpenes, phenolic substances and vitamins) in water-ethanol extract of Tagetes signata Bartl. specimen, № 13152-8 "Vetvisty", cultivated on South Coast of the Crimea.

It was found out that among volatile compounds of the specimen, P-vinylguaiacol and tagetenone dominate.

At the same time among phenolic substances glicosides of quercetine (rutin, quercetine-3-O-halactoside, quercetine-3-O-glucoside) and flavonoids prevail. Hydroxycoric acids are presented by ferulic acid and derivatives of coffeic acid.

In extract of T.*signata* № 13152-8 "Vetvisty" we determined vitamin concentration – ascorbic acid and carotinoids.

In general specimen of T.*signata* Bartl. № 13152-8 "Vetvisty" could be considered as a source of biologically active substances, especially of P-vinylguaiacol, glicosides of quercetine, ascorbic acid and carotinoids.

References

1. *Bender K.I., Gomenyuk G.A., Freidman S.L.* Ukazatel po primeneniyu lekarstvennyh rasteny v nauchnoy i narodnoy meditsine. – Saratov: SGU, 1988. – 112 s.

2. *Berdey T.S., Marchishyn S.M.* Doslidzhennya lipofilnoy fraktsii roslyn rodu chornobryvtsi (Tagetes L.) // Farmatsevtychny chasopys. – 2011. - №1. – S. 10-14.

3. Deineka V.I., Sorokopudov V.N., Deineka L.A., Tretyakov M.Yu. Issledovaniye tsvetkov *Tagetes* sp. kak istochnika lyuteina // Khimiko-farmatsevticheskiy zhurnak. – 2007. – T. 41, № 10. – S. 30-32.

4. Deineka V.I., Tretyakov M.Yu., Deineka L.A., Sorokopudov V.N. Nekotoriye osobennosti nakopleniya pigmentov v tsvetkah *Tagetes* sp. // Nauchniye vedomostu Belgorodskogo gosudarstvennogo universiteta. Seriya: Yestestvenniye nauki. – 2007. – T. 5, vyp.5. – S. 123-129.

5. Klassifikator roda *Tagetes* L. (Barkhatsy). – L.: VYR, 1088. – 15 s.

6. Kriventsov V.I. Metodicheskiye rekomendatsii po analizu plodov na biochimicheskiy sostav. – Yalta, 1982. – 22 s.

7. *Libus O.K., Rabotyagov V.D., Kutko S.L., Khlypenko L.A.* Efirnomaslichniye i pryano-aromaticheskiye rasteniya: Nauchno-populyarnoye izdaniye. – Kherson: Ailant, 2004. – 272 s.

8. Maksimenko N.V., Prokhorov V.N. Otsenka razlichnyh vidov *Tagetes* L. po osnovnym khozyaistvenno-tsennym priznakam // Vestnik Belorusskoy gosudarstvennoy selskokhozyaistvennoy akademii.- 2014. – №4. – S. 112-114.

9. Maksimenko N.V., Prokhorov V.N. Primeneniye razlichnyh genotipov rasteny roda *Tagetes* L. Kak perspektivnyh istochnikov karotinoidov dlya biodobavok v ptitsevodstve // Selskoye khozyaistvo – problemi i perspektivy: sb.nauchn.tr. – 2014. – T. 26. Zootekhniya. – S. 168-174.

10. Malakhov V.A., Zavgorodnyaya A.N., Chernishova T.I. Encyklopediya naturoterapii. – M.: Eksmo, 2008. – S. 75.

11. Malyugina O.O., Mazulin O.V., Smoilovska G.P., Mazulin G.V., Yerenko O.K. Komponentny sklad ta protymikrobna diya efirnoyi olii sutsvit chornobryvtsiv

pryamostoyachyh (*Tagetes erecta* L.) // Farmatsevtychny zhurnal. – 2014. - № 1(14). – S. 86-92.

12. *Marchishyn S.M., Berdey T.S., Kozachok S.S., Demydyak O.L.* Opredeleniye flavonoidov i hydroksikorichnyh kislot v trave *Tagetes erecta* L., *Tagetes patula* L., *Tagetes tenuifolia* Cav. metodom VEZhKh // Meditsina i obrazovaniye v Sibiri: setevoye nauchnoye izdaniye. – 2013. - № 6. – Rezhym dostupa: http://ngmu.ru/cozo/mos/article/text_full.php?id=1259.

13. *Mashkovska S.P., Grigoryuk I.P.* Chornobryvtsi – dzherelo efektyvnyh likiv // Phytoterapiya. Chasopys. – 2003. - № 4. – S. 41-47.

14. Pleshkov B.P. Praktikum po biochimii rasteny. – M.: Kolos, 1969. – 183 s.

15. Rabotyagov V.D., Khlypenko L.A., Bakova N.N., Mashanov V.I. Annotirovanny katalog vidov i sortov efiromaslichnyh, pryano-aromaticheskih, i pishchevyh rasteny kollektsii Nikitskogo botanicheskogo sada. – Yalta: Nikitsky botanichesky sad, 2007. – 48 s.

16. *Oganesyan E.T., Andreyeva O.A., Terekhov A.Yu., Podgornaya Zh.V.* Biologicheski aktivniye veshchestva lipofilnoy fraktsii iz tsveykov *Tagetes patula* i izucheniye protivoozhogovoy aktivnosti na ih osnove // Sovremenniye naukoyomkiye tekhnologii. – 2004. - № 6. – S. 109-110.

17. *Papayani O.I., Dukhanina I.V., Sergeyeva Ye.O.* Izucheniye khimicheskogo sostava i antimikrobnoy aktivnosti sukhogo ekstrakta iz tsvetov barkhatsev rasprostyortyh (*Tagetes patula* L.) // Izvestiya Samarskogo nauchnogo tsentra Rossiyskoy akademii nauk. – 2012. – T. 14, № 5-3. – S. 745-747.

18. *Yurchak L.D.* Kultura chornobryvtsiv v umovah Lisostepu Ukrainy // Introduktsiya Roslyn. – 1999. - № 1. – S. 49-54.

19. *Basher S., Gilani A.H.* Studies on the antioxidant and analgesic activities of Aztec marigold (*Tagetes erecta*) flowers // Phytotherapy research. – 2008. – Vol. 22(12). – P. 1692 – 1694.

20. *Bhattacharyya S., Datta S., Mallick B., Dhar P., Ghosh S.* Lutein content and *in vitro* antioxidant activity of different cultivars of Indian Marigold flower (*Tagetes patula* L.) extracts // J. Agric. Food Chem. – 2010. – Vol. 58(14). – P. 8259 – 8264.

21. *Céspedes C.L., Avila J.G., Martinez A., Serrato B., Calderón-Mugica J.C., Salgado-Garciglia R.* Antifungal and antibacterial activities of Mexican Tarragon (*Tagetes lucida*) // J. Agric. Food Chem. – 2006. – Vol. 54(10). – P. 3521 – 3527.

22. *Chamorro E.R., Ballerini G., Sequeira A.F., Velasco G.A., Zalazar M.F.* Chemical composition of essential oil from *Tagetes minuta* L. leaves and flowers // Journal of the Argentine Chemical Society. – 2008. – Vol. 96(1-2). – P. 80 – 86.

23. *Dharmagadda V.S.S., Naik S.N., Mittal P.K., Vasudevan P.* Larvicidal activity of *Tagetes patula* essential oil against three mosquito species // Bioresource Technology. – 2005. – Vol. 96(11). – P. 1235 – 1240.

24. *Gillij Y.G., Gleiser R.M., Zygadlo J.A.* Mosquito repellent activity of essential oils of aromatic plants growing in Argentina // Bioresource Technology. – 2008. – Vol. 99(7). – P. 2507 – 2515.

25. *Gong Y., Liu X., He W.-H., Xu H.-G., Gao Y.-X.* Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) residue // Fitoterapia. – 2012. – Vol. 83(3). – P. 481 – 489.

26. *Lima B., Agüero M.B., Zygadlo J., Tapia A., Solis C., Rojas de Arias A., Yaluff G., Zacchino S., Feresin G.E., Schmeda-Hirschmann G.* Antimicrobial activity of extracts, essential oil and metabolites obtained from *Tagetes mendocina* // J. Chil. Chem. Soc. – 2009. – Vol. 54(1). – P. 68 – 72.

27. *Lim T.K. Tagetes tenifolia* // Edible medicinal and non-medicinal plants. – 2014. – P. 469 – 472.

28. *Romagnoli C., Bruni R., Andreotti E., Rai M.K., Vicentini C.B., Mares D.* Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. // Protoplasma. – 2005. – Vol. 225(1-2). – P. 57 – 65.

29. *Rondon M., Velasco J., Hernández J., Pecheneda M., Rojas J., Morales A., Carmonia J., Diaz T.* Chemical composition and antibacterial activity of the essential oil of *Tagetes patula* L. (Asteraceae) collected from Venezuela Andes // Rev. Latinoamer. Quin. – 2006. – Vol. 34(1-3). – P. 32 – 36.

30. *Vasudevan P., Kashyap S., Sharma S.* Tagetes: A multipurpose plant // Bioresource Technology. – 1997. – Vol. 62(1-2). – P. 29 – 35.

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Kornilyev G.V., Paliy A.Ye., Rabotyagov V.D., Feskov S.A. Biologically active substances of aqueous-ethanolic extract of *Tagetes signata* Bartl. № 13152-8 'Vetvisty' specimen of Nikita Botanical Gardens collection // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 39-45.

The qualitative and quantitative composition of *Tagetes signata* Bartl. № 13152-8 'Vetvisty' specimen was investigated in terms of the research. It was established that among volatile substances there were aromatic (59%; p- vinylguaiacol prevails), aliphatic (23,7 %) and monoterpenoide (tagetenon content is 17,3%) compounds. Phenolic compounds were presented by flavonoids and their glycosides (rutin, quercetin-3-O-galactoside, quercetin-3-O-glycoside), and hydroxycinnamic acids (caffeic, ferulic). The ascorbic acid (14,54) and carotenoids (5,19 mg/100 g) were identified as well. On the whole, the specimen *T. signata* № 13152-8 "Vetvisty" can be considered as a sources of biologically active substances such as p- vinylguaiacol, flavonoid glycosides, ascorbic acid and carotenoids.

Key words: *Tagetes signata* Bartl.*; specimens; aqueous-ethanolic extract; volatile substances; phenolic substances; vitamins.*

PHYTOMONITORING

UDK 635.055:504.753:712.253

LOWER LAYER ORNAMENTAL PLANTS OF ARBORETUM IN NIKITA BOTANICAL GARDENS AND THEIR GROWTH AND STATUS DEPENDING ON ILLUMINATION CONDITIONS

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Introduction

Investigation of shade tolerance of ornamental plants, peculiarities of their light conditions plays a big role for matching of cultivars and crop combinations projecting ladscape compositions. Shade-tolerant plants have wide ecological amplitude; concerning light, good illumination favors their better growth, but at the same time they adapt to weak light easily. As a rule they are divided into more and less shadow-tolerant. Those trees and bushes that grow on open areas and don`t stand continuous shade can be classified as less shadow tolerant (light-requiring) arboreal crops. Photosynthesis gets the highest point if good sun illumination. This group includes: birch, willow, larch, aspen, walnut, locust, pine, ash. More shadow tolerant arboreal plants are presented by trees and bushes standing some shadow, but intensively growing under conditions of good illumination: box tree (*Buxus),* arrowwood *(Viburnum), Euonymus Japonicus,* cherry laurel, Japan laurel (*Aucuba japonica*),