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INTERPLANT QUANTIFICATION OF ALLIIN CONTENT IN DIFFERENT VARIETIES OF GARLICS (*ALLIUM SATIVUM L.*)

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ABSTRACT

There are several herbs growing in India, some are cultivated and others are growing wild, among these herbs is well known name Garlic (*Allium sativum L.*) Garlic (*Allium sativum*) cultivated and almost globally consumed as spice or pickle Garlic (*Allium sativum L.*) is an erect herb of *Alliaceae* family, 30 to 60 cm tall bulb is on disc like stem, consisting of multiple cloves covered with common dry membrane. Each clove consists of a protective cylindrical sheath and small central bud. Leaf blade is linear, flat, and solid, 1 to 2.5 cm wide and 30 to 60 cm long, having an acute apex. Leaf sheaths form a pseudo stem, having smooth scup, round solid coiled at first, subtended by membranous, long beaked spathe splitting on one side and remaining attached to umbel. Flower mostly variable in number and sometime absent: they seldom open and may wither in bud. Seeds are seldom – if ever produced. Garlic use as spice/pickles and it contains most important medicinally active compounds like Alliin/Allicin, Di-allyl disulfide, 2-vinyl-4H-1, 3-dithiin and Ajoene. Alliin (Allicin) is cholesterol lowering and antihypertensive (Ref. see Sukhdev, 1997, Sculz et al, 1998 and Hung, 1999). Alliin is an amino acid, in presence of enzyme Alliinase, Alliin converted into Allicin and it has produce characteristic smell of garlic.

KEYWORD: Garlic (*Allium sativum*), Alliin, Allicin, Diallyl cysteine etc.

INDEX TERMS: Garlic (*Allium sativum L.*) Alliin, Allicin.

1. INTRODUCTION

The Garlic (*Allium sativum L.*) is a species in the onion genus belongs to Alliaceae family.

Traditionally it is used as spice /Pickles over 7,000 years.

Allium sativum is a bulb. It grows up to 0.5 m (2ft) in height.

Within the species, *Allium sativum*, there are also two main subspecies or varieties.
Allium sativum var. ophioscorodon (Link) Döll, called ophioscorodon, or hard necked garlic, includes porcelain garlics, rocambole garlic, and purple stripe garlics.

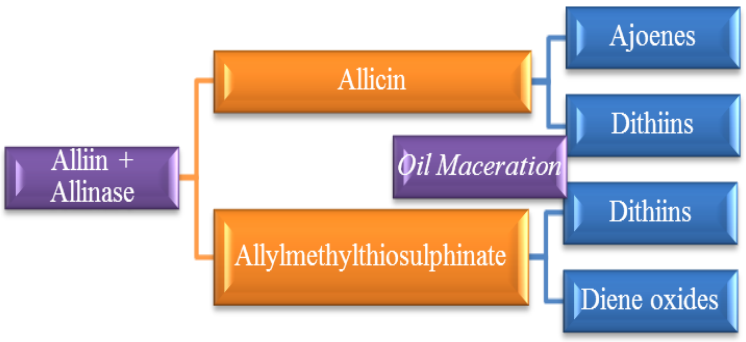
Allium sativum var. sativum, or soft-necked garlic, includes artichoke garlic, silver skin garlic and creole garlic.

Alliin/Allicin is a major secondary metabolite of Garlic apart from Alliin following are the other secondary metabolites-
Alliinase, S-allycystein, Diallylsulphide, Allylmethyltrisulfide, Ajoene, Diallyltrisulfide (DATS).

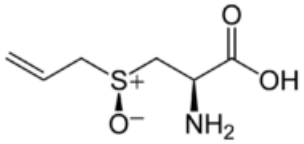
Alliin (S-allyl-l-cysteine sulfoxide), is an amino acid, which converted into Allicin (Diallylsulphide) in presence of enzyme Alliinase and Allicin produce characteristic smell of Garlic (*Allium sativum L*) whiles it is injured or crushed.

It is Organo-Sulphur compound that contributes to its therapeutic value and pharmacological importance.

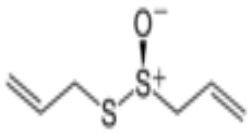
Metabolism of secondary metabolites i.e. Alliin



Chemical Structure of Alliin & Allicin



Alliin



Allicin

Allicin features the thiosulfate functional group, R-S-(O)-S-R. The compound is not present in garlic unless tissue damage occurs and is formed by the action of the enzyme Alliinase on Amino Acid which is found in Garlic known as Alliin.

Allicin is chiral but occurs naturally only as a racemate.

The racemic form can also be generated by oxidation of diallyl disulphide.

Medicinal use and health benefits

Reduces Serum Cholesterol, Blood Pressure, & Platelet Aggregation.

Garlic has been shown to reduce blood clotting and to reduce blood pressure, therefore making it an important part of the treatment for cardiovascular disease (Mabey, et al., 1988). Allicin is the most potent antiplatelet constituents of garlic because of its in vitro effects (Agarwal, 1996).

Dithiins and Ajoenes possess antithrombic properties (Passwater, 1997). Ajoene is currently being developed as a drug for the treatment of thromboembolic disorders (Agarwal, 1996).

Dithiin and Ajoenes decrease clotting time because they are anticoagulants and blood thinner. This activity indirectly reduces the risk of stroke and cardiovascular disease. Garlic improves cardiovascular function because it provides protection against hypercholesterolemia, arthrosclerosis, ischemia-reperfusion-induced, arrhythmias and infarctions. Potent enzymes that inhibit the activities of adenosine deaminase and cyclic AMP phosphodiesterase have been identified from garlic extracts. The presence of such enzyme inhibitors in garlic may perhaps explain its effect as an antithrombotic, vasodilatory and anticancer combatant (Agarwal, 1996).

It is anticancer (intestinal tract), antimicrobial with antioxidant.
Allium sativum has shown significant effects on cancers that affect the stomach and intestine. Persons who regularly ingest garlic have lower incidence of stomach cancer (Anon., 1994b). The Chinese Academy of Medical Sciences reports that epidemiological studies show that dietary intake of garlic is inversely related to gastric cancers (Howe, 1997).

Di-allyltrisulfide (DATS) is a compound in garlic that lowers the spread of human lung cancer cells. DATS is extremely effective in reducing growth of human lung carcinoma cells in culture. Also, two other compounds in garlic have anti-carcinogenic properties: S-allyl cysteine (SAC) and diallyldisulfide (DADS) (Anon., 1997c).

Garlic stimulates the immune system. The garlic stimulates the activity of macrophages and bulbs increase the activity of helper T cells. It is also effective in treating upper respiratory viral infections and protects cell membranes from DNA damage (Holladay, 1997).

The Objective of this study was "To identify the high Alliin/Allicin (medicinally active compound) containing Biomass of Garlic Plant (*Allium sativum*) and varieties of Garlic (*Alliums'* species*)* which are cultivated in similar agro-climatic condition.

This study also confirm about the effecting factors like agro-climatic (Eco-factors) condition (Soil and temperature) on its large scale production.

Earlier it was estimated medicinally active content i.e. Alliin/Allicin in various samples (bulb/cloves) of Garlic (*Alliums*' species) which was cultivated in different regions of Indian States [Ref. Comparative study of Alliin containing different Varieties of Garlics (*Allium sativum L.*) - published at: "International Journal of Scientific and Research Publications (IJSRP), Volume 3, Issue 12, December 2013 Edition"].

And medicinally active compound content i.e. Alliin/Allicin estimated from biomasses Sample leaves of various species (variety) of Garlic (Allium sativum), which are cultivated in similar agro-climatic condition. [Ref. Rajesh Kumar Singh, Dr. S.N. Hiremath - Scaling Studies of Effecting Factors* on Large Scale Production of Medicinally Active Compound in Garlic (*Allium sativum L*) - published at: "International Journal of Scientific and Research Publications (IJSRP), Volume 5, Issue 3, March 2015 Edition"].

The purpose for continuation of the study was to identify high medicinally active compound (Alliin/Allicin) containing Garlic (*Allium sativum L.*) Biomass - Leaves, bulb/clove and flower of Garlic (*Allium sativum*) and its variety of Garlic (*Allium sativum L.*) & affecting factors (Ecotype or Genotypes) are responsible for Alliin/Allicin content variations of Biomass samples within the Garlic (*Allium sativum L*) Plant as well as in different varieties of Garlic (*Allium sativum L.*) plant biomass sample which has been cultivated in similar agro-climatic condition.

Production of Alliin/Allicin - in different varieties of Garlic (*Allium sativum L*) Biomass (plant organs like bulb/clove and flowers), which was grown under similar conditions.

Selected varieties were cultivated in a single pot in multiple set of blocks.

Alliin estimated from various biomass samples of Garlic plant part of selected different varieties of Garlic which has been cultivated in similar agro-climatic condition.

2. MATERIALS AND METHODS

A. Soils preparations for Garlic cultivation.
B. Pots selection for Garlic cultivation.
C. Collection of Garlic Seeds samples for cultivation.
D. Cultivation.
E. Monitoring of Agro-climatic conditions.
F. Analytical Method - Estimation and Quantization of Medicinally active compound i.e. Alliin/Allicin from Biomass of Garlic (*Allium sativum L*).
G. Sample collections.
H. Testing of collected samples.
I. Analytical Results of Bio mass.

A. Soil Preparation for Garlic cultivation

About 50 square feet Soil was collected from agro-field of a farmer from Manpura Village, Tehsil Nalagarh Dist. Solan, and Himachal Pradesh, India. The earlier crop of this farming field was Maize.

It was blended with about 15 square feet rotted cow dung and one kg chemical fertilizer i.e. DAP (ammonium phosphate).

After thoroughly mixing, it was moisturized with five liter of potable water.

Soil Preparation snap shot A-1.

Soil Preparation Rotted Dung mixing (snap shot A-2).

B. Pot selection for Garlic Cultivation

Cylindrical Cone shaped Pots having Height -12" inch, top diameter-15" inch, bottom diameter-4.5" inch and Rectangular pots having size (Outer) 36" X 14" X 9" inch and (inner) 32" X 10" X 7" inch were purchased from local market.

Mixed soil with rotted cow dung and chemical fertilizer i.e. DAP (di ammonium phosphate) (Step A) were loaded into each pot up to similar level.

C. Selected Garlic Seeds Samples for Cultivation

Following selected varieties of Garlic were collected for cultivation in similar agro-climatic condition.

Table -1.

S. No.	Variety
1.	Agrifood– I (Seed Shop Pinjore
2.	Sabji Mandi Kalka
3.	Kumaoni Garlic(Nainital)
4.	Elephant Garlic(Pahadi Lahsun)

D. Cultivation

Multiple Blocks of Garlic were cultivated in similar agro Climatic conditions. (Refer Image B).

Image B.

E. Monitoring of Agro-climatic Conditions.

Table-2.

Date	Temperature ^{0}C		Date	Temperature ^{0}C	
	Minimum	Maximum		Minimum	Maximum
3rd week of Oct	10.0 – 14 .0	25.0 – 30.0	2nd week of Feb	6.5 – 10.0	22.0 – 24.0
4th week of Oct	8.0 – 13.0	24.0 – 28.0	3rd week of Feb	7.0 – 11.0	22.5 – 25.0
1st week of Nov	8.0 – 11.0	23.0 – 27.0	4th week of Feb	7.5 – 11.5	23.0 – 25.5
2nd week of Nov	8.0 – 11.0	24.0 – 27.0	1st week of Mar	9.1 – 12.0	23.2 – 27.8
3rd week of Nov	8.0 – 10.0	24.0 – 26.0	2nd week of Mar	9.5 – 13.0	24.0 – 28.0
4th week of Nov	8.0 – 10.0	21.0 – 25.0	3rd week of Mar	10.0 – 13.8	24.0 – 30.0
1st week of Dec	8.0 – 11.0	23.0 – 27.0	4th week of Mar	11.0 – 15.0	24.2 – 31.0
2ndweek of Dec	8.0 – 11.0	24.0 – 27.0	1st week of Apr	18.0 – 20.0	27.0 – 30.0
3rd week of Dec	8.0 – 10.0	24.0 – 26.0	2nd week of Apr	15.0 – 19.0	22.0 – 35.0
4th week of Dec	8.0 – 10.0	21.0 – 25.0	3rd week of Apr	18.0 – 21.0	30.0 – 37.0
1st week of Jan	2.0 – 7.0	8.5 – 13.5	4th week of Apr	20.0 – 23.0	33.0 – 40.0
2nd week of Jan	2.1– 6.0	6.1 – 19.6	1st week of May	20.0 – 26.0	36.0 – 42.0
3rd week of Jan	7.6 – 11.5	12.8 – 24.1	2nd week of May	21.0 – 28.0	30.0 – 41.0
4th week of Jan	5.6 – 7.0	18.0 – 20.0	3rd week of May	24.0 – 27.0	39.0 – 42.0
1st week of Feb	6.0 – 9.5	21.0 – 23.0	4th week of May	25.0 – 28.0	37.0 – 43.0

F. Analytical Method: Estimation and quantization of Alliin from Biomass of Garlic(*Allium sativum L*)

The Medicinally active compound of Garlic (*Allium sativum L*) i.e. Alliin/Allicin was estimated and Quantified chemically by using nitrite titration method. Reference: USP 29 <451> Nitrite Titration and analytical results summarized in Table -3.

a) Required Reagents and Glass ware for Estimation of Alliin from Biomass of Garlic (*Allium sativum L*)

Reagents

- 0.01M Sodium Nitrite Solution (Standardized).
- Sulphanilamide (RS) (USP reference standard)
- Starch Iodide Paper.
- Concentrate Hydrochloric acid.
- Potassium Bromide.
- Ice cubes.
- Distilled Water

Glass ware

- Burette 50 ml.
- Graduated pipets 10 ml.

- Conical flask 250/500 ml.
- Beakers 250/500 ml.
- Glass rod.
- Mortar & Pestle.
- Measuring cylinders 25 ml, 50 ml, 100 ml and 500 ml.
- Thermometer /Temperature probe (0.5 to 100 degree Celsius).

b) Analytical procedure

1. Take 5.0 g of **Sample*** in mortar and pestle add 5.0 ml Hydrochloric acid (concentrated) and Triturate to make paste, add another 5.0 ml Hydrochloric acid (concentrated) + 20 ml Distilled Water, triturate well and transfer into 250 ml beaker.
2. Add 3.0 g Potassium Bromide add 10 ml Hydrochloric acid (concentrated) + 30 ml water and mix, add about 50.0g Ice cube and check the temperature (should be 12 – 15^0C).
3. Titrate with 0.01 M Sodium Nitrite solution using starch iodide paper as external indicator. Each ml of 0.01ml Sodium Nitrite solution equivalent to 0.001722g of Alliin or 0.0016228 g of Allicin.

- Also perform Blank (without sample) and titrate with 0.01 M $NaNO_2$, 1 drop blue color observed.

[Sample* - Collected biomass of Garlic (*Allium sativum L*) i.e. Cloves or Roots or leaves or shoot (spathe) or flowers.]

G. Sample collection

Different Sample were collected and kept in air tight containers and labeled**. (Refer image C-1, C-2, C-3 and C-4)**

C-1.

C-2.

Ripe Crop

C-3

Freshly Collected Roots with Clove.

C-4

Freshly Collected Flower & Stem (leave spathe).

H. Testing of collected samples

Samples collected from the pots grown in similar Agro Climatic Conditions were tested in the lab for the Alliin Content by the aforementioned Analytical method ***(Refer Image D-1, D-2, and D-3).***

Image D-1

Image D-2

Image D-3.

3. Analytical Results of Bio mass

4. Table 3: Content of Alliin in different varieties of Garlic.

Variety	Content of Alliin (%) in Bulb	Content of Alliin (%) in Flower	Content of Alliin (%) in leave & Stem (leaves spathe)	Content of Alliin (%) in Root
Agrifood– I (Seed Shop Pinjore	0.35	*	0.004	ND
Sabji Mandi Kalka	0.43	*	0.005	ND
Kumaoni Garlic(Nainital)	0.34	*	0.11	0.07
Elephant Garlic(Pahadi Lahsun)	0.47	2.14	0.35	0.18

*Till the time of sample collection only Elephant Garlic was Green and Flowered other verities were dried and didn't flowered up to maturity.

ND Not Detected

Note: All other verities of Garlic were dried/dead at the time of sample collection, only Elephant Garlic was green growing well and Flowered.

4. DISCUSSION

Flower was produced only in Elephant Garlic while all others varieties were grown in similar agro-climatic condition. And highest Alliin content was found in flower as compare to other biomass of same plant.

Content of Alliin in leaves/stem (leaves spathe), cloves, flower and roots of different varieties of Garlic was found different, though grown in similar agro climatic conditions.

Highest Alliin content was found whole biomass of Elephant Garlic as compare to various biomasses of other varieties of Garlic. (In totality as well as individually).

5. CONCLUSION

Observed Analytical data reveal that Elephant Garlic have the highest Alliin content as compared to that of other verities, though all the verities were grown in the similar Agro Climatic conditions.

It can be concluded that Alliin content differs due to genotypic factors and Ecotype factors have none or very lesser effect.

Whole Green Plant has medicinally active compound i.e. Alliin, while in the dried plant Alliin is present only in the bulbs (Clove).

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REFERENCES

1. Biotechnology of the micro propagation of medicinal and aromatic Plants In: Bajaj Y.P.S. (ed.) Biotechnology in Agriculture and Forestry 4: medicinal and Aromatic plants I. Springer-verlag Co. New Yark. PP. 60-103.
2. U.S.P-24, NF- 19 PP. 2445 Garlic (official monograph) Estimation of Alliin by HPLC method.
3. USP 29 <451> Nitrite Titration.
4. International pharmacopoeia.
5. Abo. Ei. Nil MM (1977) organogenesis and embryogenesis in callus culture of Garlic (Allium sativum L.) Plant Science Lett., 9: 259: 224.
6. Ali A. Metwally EE91992), Somatic embryogenesis and Plant Regeneration as a tool for Garlic Improvement. Egypt j Appl., 7: 727. 73.
7. Rajesh Kumar Singh and Dr. S. N. Hiremath - Comparative study of Alliin containing different Varieties of Garlics (Allium sativum L.) - published at: "International Journal of Scientific and Research Publications (IJSRP), Volume 3, Issue 12, December 2013 Edition"].
8. Rajesh Kumar Singh, Dr. S.N Hiremath - Scaling Studies of Effecting Factors* on Large Scale Production of Medicinally Active Compound in Garlic (Allium sativum L) - published at: "International Journal of Scientific and Research Publications (IJSRP), Volume 5, Issue 3, March 2015 Edition"].