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# Extraction and biological activity of allicin from In Vitro cultured garlic (Allium sativum L.)

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## Abstract

The shoots of garlic (*Allium sativum* L.) were initiated on MS (Murashige and Skoog) medium supplemented with Benzyl adenine (BA), 4mg/L which is significantly the best among other treatments (1,2,3,5mg/L); the shoots also initiated from shoot tip in the presence of combination (NAA, 3mg/L and kinetin, 1mg/L); callus was also initiated from basal disc and shoot tip in the presence of combination (NAA, 5mg/L and kinetin 0.5mg/L) and (2,4-D, 1.5mg/L and kinetin 0.5mg/L).$R_{fs}$ values of extracted allicin from bulbs, shoots, and callus were 0.97, 0.94, 0.95 respectively and the amount percentages were 1.3%, 1.8%, 2.2% respectively; identification of allicin by IR spectrum showed the similarity between the groups of extracted allicin from shoots and those of extracted allicin from bulbs (Standard); identification of allicin by UV showed one band at wave length 338nm for extracted allicin from shoot and 296nm for extracted allicin from bulbs (Standard).The results also showed the difference in the biological activity of extracted allicin from callus, shoots and bulbs of garlic toward *Staphyllococcus aureus* and *Escherichia coli*.

**Key words:** In Vitro , garlic, allicin , biological activity.

Cited from: M.Sc. Thesis

# الاستخلاص واالفعاليه البايولوجيه للاليسين من نبات الثوم Allium sativum L. المزروع خارج الجسم الحي

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## الخلاصه

نشأت فروع الثوم ( *Allium sativum* L. ) على وسط MS ( مورا شيجي وسكوك) المزود بالبنزيل ادينين BA، ( ٤ ملغم / لتر) الذي كان الافضل معنويا" بين التراكيز ( ٥،٣،٢،١) ملغم / لتر، ونشأت الفروع ايضا" من طرف الفرع Shoot tip بوجود التوليفه ( NAA ٣ ملغم/لتر والكاينتين ١ ملغم/لتر) ، ونشأ الكالس من القرص القاعدي لطرف الفرع بوجود التوليفه ( NAA ٥ ملغم/لتر والكاينتين ٥و٠ ملغم/لتر) او ( D– ٤و٢ ٥و١ ملغم /لتر والكاينتين ٥و٠ ملغم /لتر.كانت قيم السريان النسبي (Rf) للاليسين المستخلص من البصلات والفروع والكالس ( ٩٧و٠ ، ٩٤و٠ ، ٩٥و٠) على التوالي والنسب المئويه للكميات ٣و١ % ، ٨و١ % و ٢و٢ % على التوالي.اظهر تشخيص الاليسين بواسطة طيف الاشعه تحت الحمراء IR التشابه بين مجاميع الاليسين المستخلص من الفروع وتلك المستخلصه من البصلات ( القياسي standard) اظهر تشخيص الاليسين بواسطة الاشعه فوق البنفسجيه حزمة واحدة بطول موجي ٣٣٨ نانومتر للاليسين المستخلص من الفروع و ٢٩٦ نانومتر للاليسين المستخلص من البصلات ( القياسي).اظهرت النتائج كذلك الفرق بين الفعاليه البايولوجيه للاليسين المستخلص من الكالس و الفروع والبصلات للثوم تجاه البكتريا Staphyllococcus aureus و Escherichia coli.

## Introduction

Garlic (*Allium sativum* L.) which is belonging to Liliaceae is useful as medicine and food (Novak, 1990); the demand of micropropagation of garlic is for two reasons; the first is sexual sterility of the plant and the second is the capability of the production of secondary metabolites; Chen and Huang (1991) used the shoot tip in tissue culture of garlic and they emphasized on the activity of induction of adventitious shoots from basal disc tissues on (MS) medium supplemented with NAA (naphthalene acetic acid), 0.6mg/L, BA (Benzyl adenine), 2mg/L. Ayabe and Sumi (2001) proved that the basal disc of garlic has atotipotency of micropropagation; Masuda *et al.* (1994) reported that the rings of basal disc of bulbs produce a multiplied shoots on MS medium containing NAA and BA; however, BA was the most effective stimulator for shoot formation and increased the percentage of growth (Choi *et al.*, 1993; Kudou *et al.*, 1995); Changhua *et al.* (1995) found that MS medium supplemented with BA, 1-2mg/L, NAA, 0.1mg/L causes an increase in the regeneration of garlic buds. Nagasawa and Finer (1988) obtained callus from leaf primordial and meristematic tissues of garlic by using 2,4-5T (2,4-5 trichlorophenoxy acetic acid), dichamba and picloram; Khan et al. (2004) stated that the high percentage of embryagenic callus initiation was at combination 2,4-D, 1.5mg/L and kinetin 5mg/L., the callus itself produces a high number of shoots on MS medium supplemented with BA (10mg/L); Cavalito et al. (1944) isolated allicin (volatile oil ) and studied its chemical and physical properties; inactive allicin was converted to active allicin in garlic extract at 37°c for an hour (Tynecka and Gos, 1973); Jerzy and Lipo (1990) found that fresh garlic extract is more active than commercial type during to the high content of allicin; garlic is rich with organic sulfur compounds of medical uses in curing some diseases such as bronchitis (Chakravarty, 1976 ; Block, 1985); Saleem (1978) found that the yeast Sacharomyces cereviceae more sensitive than bacteria toward the garlic extract; garlic has biological activity as antibiotic to bacteria Vibrio spp. , Streptococcus fecalis, Proteus vulgaris, Salmonella typhi, Pseudomonas aerreginosa and Bacillus cereus (Chen et al. 1985; Takagi, 1990; Koch, 1993; Pai and Platt, 1995); Barros and Maia (1995) stated a close inverse relationship between the diameters of fungal colonies and the concentration of garlic water extract; Lowson (1998) stated that the allicin derivatives DATS (Diallyl trisulfide) and Ajoene have antimicrobial activity in human body less than allicin itself; our current study has been aimed to recognize if there is an effect of tissue culture treatment on the induction of allicin production and its biological activity.

## Materials and Methods

### Tissue culture:

MS medium was prepared from MS basal salts (Murashige and Skoog, 1962) supplemented with following materials (mg/L):Sucrose (30000), NaH2PO4 (170), meso-lnositol (100) thiamine-HCl (0.5), Agar (8000), activated charcoal (3000) whereas auxins and cytokinins were added in the following combinations and purposes: BA at levels (0, 1, 2, 3, 4, 5)mg/L used for culturing shoot tip with basal disc; NAA (3mg/L) and kinetin (1mg/L) used for culturing shoot tip; BA (1mg/L) used for culturing shoot tip with basal disc; NAA (5mg/L) and kinetin (0.5mg/L) used for culturing basal disc; 2,4-D (1.5mg/L) and kinetin (0.5mg/L) used for culturing basal disc; pH was adjusted at (5.8) than agar and activated charcoal were added, the medium was heated, cooled and dispensed at 25ml liquotes in tubes (15x180 mm) or in 50ml liquotes in flasks, the containers were autoclaved at 121°c for 15min.

### Extraction of allicin:

Allicin was extracted from bulbs and In vitro propagated shoots of garlic; Procedure of Cavalito et al. (1944) was applied with modification that normal hexanol was substituted by normal butanol in separation and filteration (Al-Wafi, 1998); allicin was identified by TLC (Thin Layer Chromatography Technique) In which ethanol (95%) was used as solvent .according to (Khanna and Rathore, 1977); Relative flow coeffecients (Rfs) were determined, IR (infra red) spectrum of isolated allicin from alcoholic extract was measured at frequency 500-4000cm-1 according to (Shriner, 1980); UV (altra violet) spectrum of isolated allicin from alcoholic extract was measured at frequency 200-800nm by spectrophotometer according to Harborne, (1984).

### Biological activity:

Staphyllococcus aureus ATCC25923 and Escherichia coli ATCC25922 were prepared on slant nutrient broth medium and incubated at 37°c for 24hrs; dilutions were made up to 0.1 (optimum density) by spectrophotometer; Muller-Hinton agar medium was prepared in petri dishes according to Kirby and Bauer test (Bauer et al. 1966); after autoclaving the medium was incubated at 37°c for 24 hrs. for the following tests: 400mg of allicin were dissolved in 10ml of ether in test tube, small discs of filter papers were imbibed with

allicin for 3hrs in petri dishes up to saturation level 40mg by evaporation of ether from the petri dishes ; 0.1ml of nutrient broth which contains activated bacteria was cultured homogenously on Muller-Hinton medium for 15-30min., then inoculated with discs of allicin and control in a close contact between the discs and the surface of culture media, the cultured petri dishes were incubated at 37°c for 24hrs; diameters of inhibition zones were measured. SPSS program is used for statistical analysis including L.S.D. test at P<0.01, 0.05 and complete randomized design were applied.

## Results and Discussion

### Tissue culture:

The shoots were initiated from shoot tip with basal disc in the presence of BA (4mg/L) or (1mg/L) (fig.1), thus the treatment (4mg/L) showed a significant differences among other treatments (1, 2, 3, 5mg/L) (Table 1) and the shoots were also initiated from shoot tip in the presence of combination (NAA, 3mg/L and kinetin, 1mg/L) (fig. 2).

Table (1): The effect of BA on the shoots initiation

| (mg/L) | 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Fresh weight (mg) | 550 *b | 562 *b | 524 b | 776 b | 1064 a | 784 b |

*different letters indicate significant differences at P≤0.01.

These results corroborated with the finding of Kodou et al. (1995) and Choi et al. (1993) who reported that BA was most effective stimulator for shoot formation and increasing the percentage of shoot regeneration, however, Hassan et al. (2007) used MS medium containing BA (0.01mg/L) in micropropagation of garlic.Shoot induction in the presence of combination (NAA, 3mg/L and kinetin, 1mg/L) was corroborated with the finding of Roksana et al. (2002) who observed the shoot induction on semisolid medium in the treatment NAA, 2mg/L and Kinetin, 2mg/L, however Roksana *et al.* (2002) reported that the combination of cytokinin and auxin is better for early establishment of shoot apex than cytakinin alone; the callus was initiated from basal disc and from shoot tip in the presence of combination (NAA, 5mg/L and kinetin 0.5mg/L) (fig. 3, 4) or from shoot tip at combination (2,4-D, 1.5mg/L and kinetin 0.5mg/L) (fig. 5); these results corroborated with the finding Khan et al. (2004) who obtained the higher percentage of callus formation at combination 2,4-D, (1.5mg/L) and kinetin (5mg/L); Robledo et al. (2000) who reported that the good callus induction was observed by using root tip in the presence of combination 2,4-D and kinetin; Generally. the importance of basal disc in the initiation of both shoots and callus may be due to the meristematic activity, however, Masuda et al. (1994) reported the importance of basal disc in the micropagation of garlic.

### Extraction:

Rfs values of extracted allicin were: 0.97, 0.94, 0.95 from bulbs, shoots and callus respectively (fig. 6); these results were in approach with the finding of Alwafi (2001) who reported that the Rf value of extracted allicin was 0.96.Table (2) showed the amounts and the percentages of extracted allicin from three of garlic plant parts:

Table 2: The amounts and percentages of extracted allicin from bulbs, shoots and callus of garlic

| Percentage | Amount (mg) | Plant part |
|---|---|---|
| 1.3% | 325 | Bulbs |
| 1.8% | 450 | Shoots |
| 2.2% | 560 | Callus |

These results indicate the importance of tissue culture in increasing the amount of extracted allicin; the important groups of IR spectrum of extracted allicin were indicated in table (3) and fig. (7) whereas those of extracted allicin from bulbs were showed in fig. (8).

Table (3): Absorption bands and related active groups in IR spectrum of allicin

| Band frequency $cm^{-1}$ | Band | Active group |
|---|---|---|
| 3062-3026 | $= CH_2$ | $= CH_2$ |
| 2921 | $- CH_2 -$ | - CH in aliphatic compound |
| 2850 | $- CH_2-$ | - $CH_2$ in aliphatic compound. |
| 1598 | -C = C- | Doublet due to rotational isomerism |
| 1026 | S = O | S = O in alkyl sulfoxides |
| 696 | C – S | C – S in sulfonyl compound |

As a comparison between important groups of extracted allicin from shoots and these from bulbs (standard) were observed nearly similar in their values which were 696, 1026, 1598, 2062, 3026, 2921, 2850cm-1 indicating the presence of the groups, (C-S), (S=O), C=C, =CH2, =CH2, -CH2-, -CH2 respectively for allicin from shoots of garlic whereas: 695, 1050, 1550, 3020, 3055, 3000, 2940, 2890, 2820cm-1 indicating the presence the groups, (C-S), (S=O), C=C, =CH2, =CH2, -CH2-, -CH2-, -CH2, -CH2 respectively for allicin from bulbs of garlic (fig. 8), however. Brace (2002) reported that etheral extraction of garlic bulbs

contain allicin related compounds such as cystein and cystein sulfoxides gamma; the results of UV spectrum showed one band at wave length 338nm of isolated extracted allicin from shoots (fig.9) and 296nm of standard allicin from bulbs (fig.10), those results due to the transition n→π as indicator of the presence of atoms with free lone pair of electrons such as N``, S``, O`` in the chemical structure of organic compounds, however. Staba (2001) pointed out that steam distillation of organic produces allyl sulfides compounds such as DAS diallyl sulfides, DADS (Diallyl disulfides) and DATS (Diallyl trisulfides); Lowson (1998) stated that fresh garlic contains Gamma-glutamyl S-allyl cystein) and alliin; the results also showed in (fig. 11) and (fig. 12) that the biological activity of extracted allicin from callus and shoots is higher than that of extracted allicin from bulbs, this may be due to an increase in the temperature and storage period length in the case of extracted allicin from bulbs cause the chemical and physical changes in the different compounds of garlic, especially, allicin, allinase.Borukh et al. (1975) found that antibacterial activity of garlic diminished during storage at 16°c more than at 0°c.The variance in biological activity of allicin between callus the induction of callus and bulbs may be also due to the induction of callus by hormone treatments, however, Zenk (1978) reported that there are many compounds of economic importance such as antibiotics could be produced from callus better than from organs cultures.

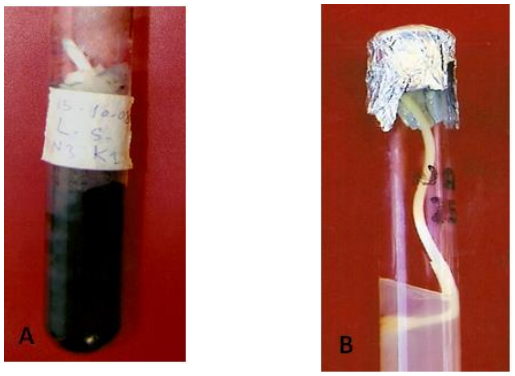


Fig. 1: Initiation of shoots on MS medium A-from shoot tip using (NAA, 3mg/L and kinetin 1mg/L)

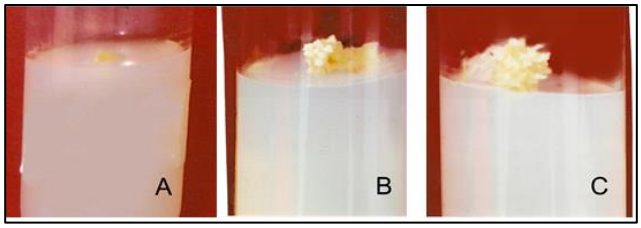


Plate 1: Initiation of callus from basal disc using (NAA, 5mg/L and Kinetin 0.5mg/L). A- after 2 weeks B- after 2 months C- after 3 months

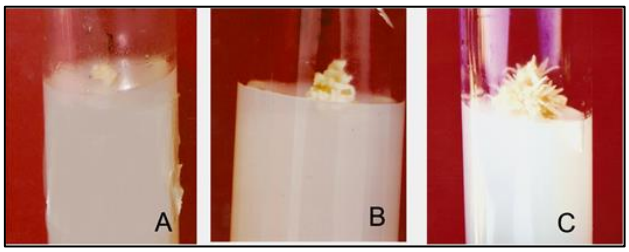


Plate 2: Initiation of callus from shoot tip using (NAA, 5mg/L and Kinetin 0.5mg/L). A- after 2 weeks

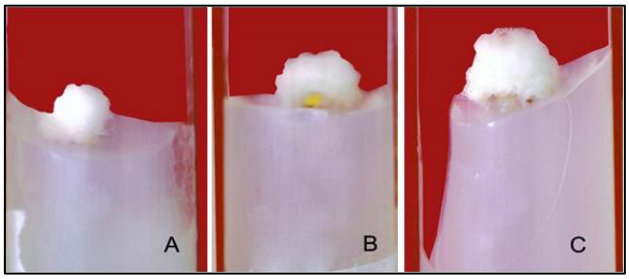


Plate 3: Initiation of callus from basal disc using (2,4-D, 1.5mg/L and Kinetin 0.5mg/L). A- after 10 days B- after 15 days C- after 30 days

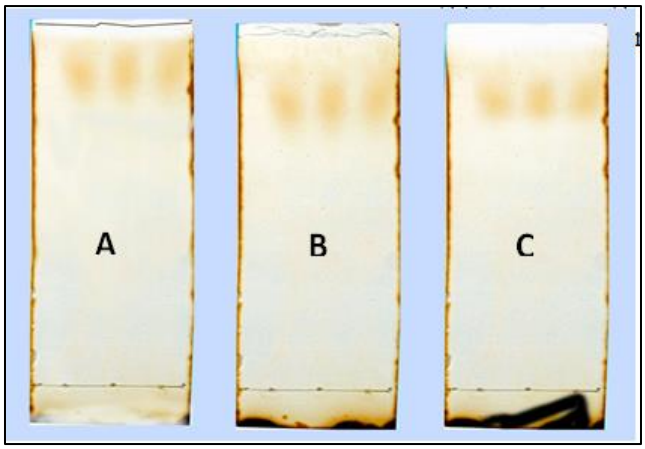


Plate 4: TLC of isolated allicin from: A- Bulbs B- Shoots C- Callus

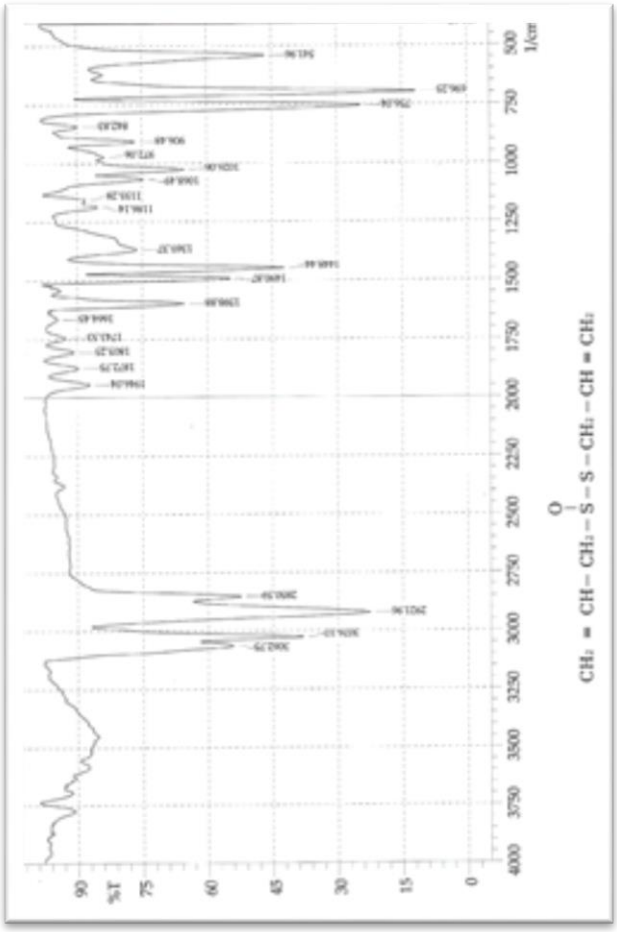


Fig. 7: I.R spectrum of isolated allicin from shoots of garlic

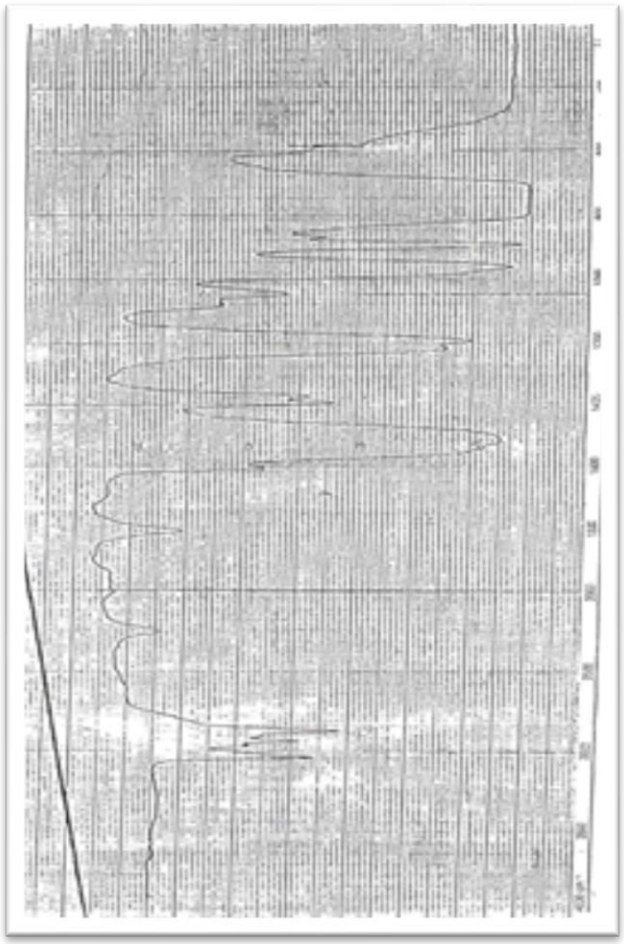

Fig. 8: I.R spectrum of isolated allicin from bulbs of garlic

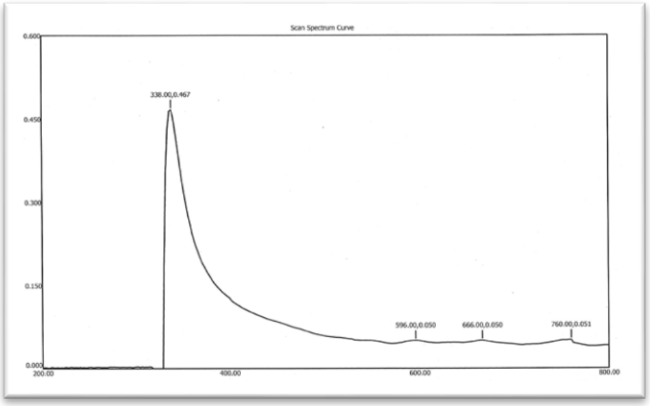


Fig. 9: U.V spectrum of isolated allicin from shoots of garlic

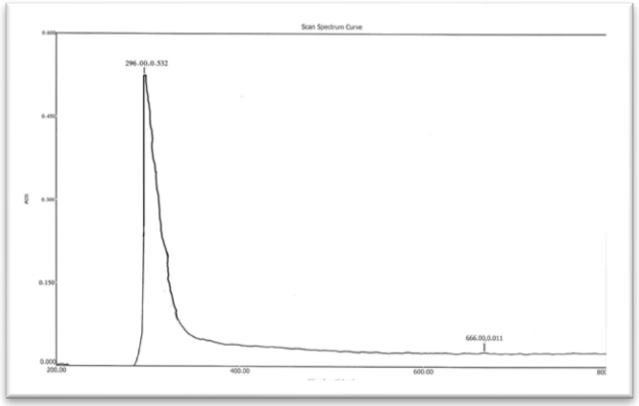


Fig. 10: U.V spectrum of isolated allicin from bulbs of garlic

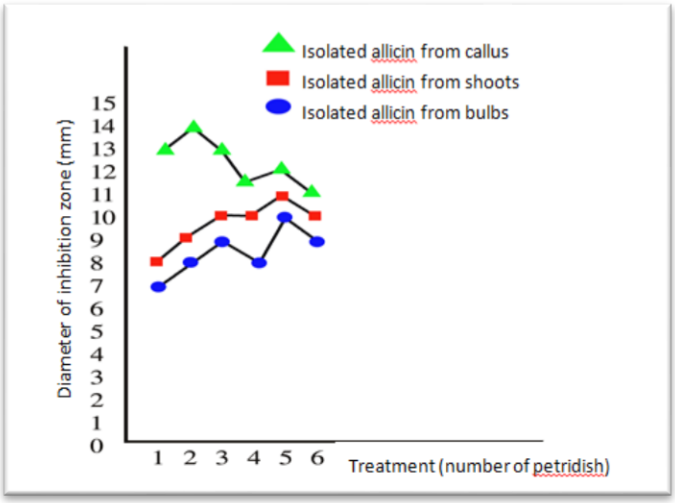


Fig. 11: Sensitivity test of Staphyllococcus aureus toward isolated allicin

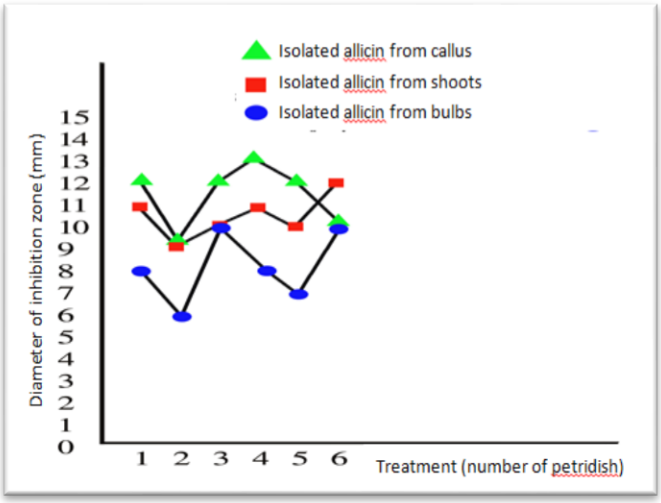


Fig. 11: Sensitivity test of *Escherichia coli* toward isolated allicin

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