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Review Article

Role of garlic (*Allium sativum* L.) in human and plant diseases

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A resurgence of interest in garlic due to recent revelations of its beneficial effects in the treatment of various human and plant diseases and also due to validation of claims made in traditional systems of medicine has resulted a plethora of publications on different aspects of garlic in recent years. Chemical constituents of garlic and their variations on the methods of isolation have been discussed in the present review. Effect of garlic and its constituents against various human and plant pathogenic and saprophytic microorganisms has also been reviewed.

Garlic (*Allium sativum* L.) is used worldwide as a food additive, spice and medicine. In Ayurveda[1], it has been described as a gastric stimulant. It aids digestion and is given in flatulence. It has special influence over the bronchial and pulmonary secretions and in promotion of flow of menses. Garlic is used as a tonic, carminative and stimulant. In large doses, it is an irritant and produces flatulence, headache, nausea and even diarrhoea. As a local stimulant and irritant, it causes redness of the skin and vesication. It is applied to the nose of hysterical patients when in a state of swooning. Given with common salt, it relieves colic pain and nervous headache. It is used as a vermifuge to expel roundworms. It causes diuresis and hence used in dropsy. In cold catarrh of children, bruised garlic is applied to the chest as a poultice or liniment. It is also used as a remedy to bites of venomous reptiles. The rubificent action of garlic is enhanced by addition of mustard powder. It is rubbed over ringworm for relief. Garlic juice boiled with salad oil gives relief from earache. It is beneficial in several forms of atonic dyspepsia and is effective in the treatment of tuberculosis.

Many of these effects have been substantiated by modern research and garlic has been proved to elicit antimicrobial[2-5], antihypertensive[6], hypolipidemic[7-9], hepatoprotective[10], antidiabetic[11-15], and insecticidal[16] properties. Immunomodulation and antitumor activities of garlic have also been reported[17]. Garlic extract has also been shown to reduce serum cholesterol levels[18,19] and increase blood coagulation time[18,20]. Antifungal activity of garlic bulbs[21-23] and aqueous extract of its leaves[24,25] are also on record. In view of the variety of bioactivities that garlic and its various preparations exhibit, it is considered appropriate to compile the various findings in a systematic manner and an attempt in this direction has been made in this brief review.

Chemical constituents of garlic - Garlic is a rich source of organosulphur compounds showing a variety of biological activities. The chemical constituents from garlic cloves (bulbs) vary with the isolation procedure and obviously many of the compounds reported from this source have been proved to be artifacts. Thus, while garlic oil obtained by steam distillation of garlic cloves was proved to be essentially diallyl disulphide (**1**) accompanied by lesser amounts of diallyl tri-and tetra-sulphides[26]. Extraction of garlic with ethyl alcohol at room temperature yields oxide of diallyl disulphide (**2**) called allicin[27]. Extraction of garlic with ethanol at subzero temperature yields[28] an odourless amino acid, (+)-S-allyl-L-cysteine sulfoxide (**3**) which is named alliin (Fig. 1). Odourless alliin has been proved to be the precursor of allicin, the main odorous principle of garlic and conversion of the former to latter takes place under the influence of an enzyme, allinase which comes in contact with alliin only when garlic bulbs are cut or crushed. It is for this reason that the bulbs are odourless unless cut or bruised.

Allinase, a pyridoxal-phosphate-dependent enzyme, which is responsible for conversion of alliin (**3**) to allicin (**2**) has been isolated in a fairly pure state from *Allium tuberosum*[29] and the mechanism of this transformation has been established[28,30]. Allinase breaks down alliin to 2-propenesulphenic acid (**4**),

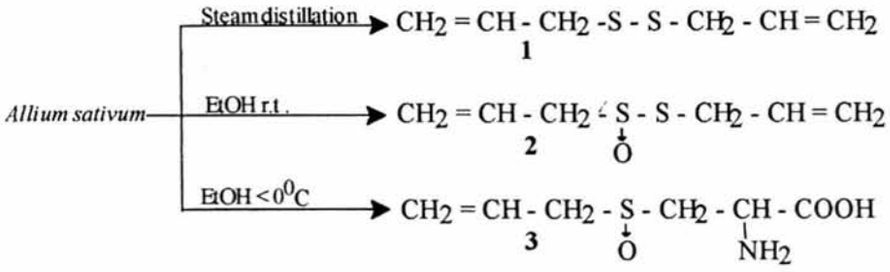
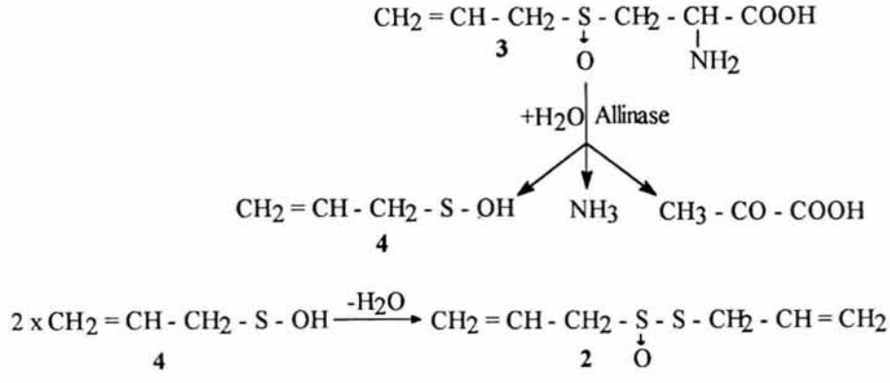


Fig. 1—Variation in chemical constituents of garlic due to change in isolation procedure

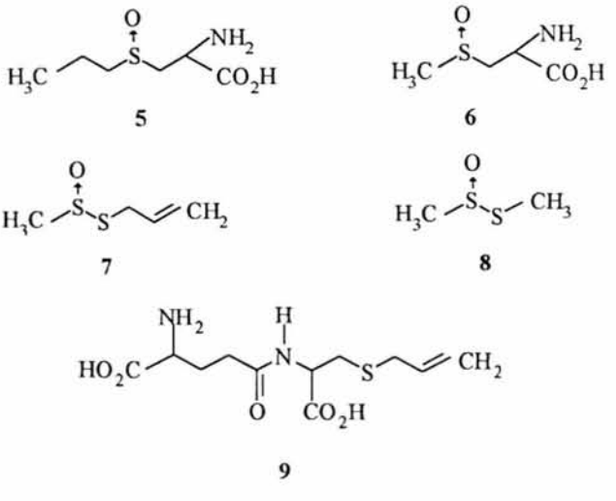


Scheme I

ammonia and pyruvic acid. Two molecules of sulphenic acid then react together to give allicin by loss of a molecule of water (Scheme I).

Subsequent research has revealed that cysteine sulfoxide fraction of garlic consists of 85% alliin (**3**) along with 2% S-propylcysteine sulfoxide (**5**) and 13% S-methyl cysteine sulfoxide (**6**). Action of allinase on this mixture affords, besides allicin, allylmethanethiosulfinate (**7**), methyl methanethiosulfinate (**8**) and other mixed or symmetrical thiosulfinates of the general structure, R-S(O)-S-R′ where R and R′ represent any one of methyl, propyl or allyl groups. Obviously all these thiosulfinates are found to be present in garlic extract[31]. Lawson & coworkers[32] have shown that in addition to endogenous alliin (**3**), a reserve for alliin exists in the precursor compound, γ-glutamyl-S-allyl cysteine (**9**) and is present in a significant quantity in freshly-picked garlic cloves.

Analysis of garlic extracts[33] using GC-MS revealed the presence of mixture (2.4 : 1) of two compounds (79%) which have been proved to be 3-vinyl-[4H]-1,2-dithiin (**10**) and 3-vinyl-[6H]-1,2-dithiin (**11**) (Scheme II), on the basis of comprehensive spectral analysis. Compounds (**10**) and (**11**) are regarded as dehydration products of allicin (**2**) and their formation is considered to take place during gas chromatography according to the equation:



$$C_6H_{10}S_2O \rightarrow C_6H_8S_2 + H_2O$$

Mechanistically speaking, allicin first breaks down into 2-propene sulfenic acid (**4**) and thioacrolein (**12**). While two molecules of sulfenic acid regenerate a molecule of allicin by loss of a molecule of water, two molecules of thioacrolein undergo Diels-Alder cyclisation to give cyclic 1,2 dithiins (Scheme II).

Thioacroleins may also cyclise in a different manner to give 2-vinyl-[4H]-1,3-dithiin (**13**; Scheme II) which is also found to be present in the fraction. Other volatile components of garlic extract detected by GC-MS analysis have been proved to be allyl alcohol (5.4%), methyl allyl disulphide (1.2%), diallyl

disulphide (5.7%), dimethyl trisulphide (2.4%), allyl methyl trisulphide (1.5%), diallyl trisulphide (1.0%) and sulfur dioxide[33]. Volatiles identified in garlic oils are suprisingly few and it has been considered that investigation of volatiles using modern techniques such as HPLC or capillary GC-MS would considerably extend the list of components. In fact, a temperature-programmed GC-MS analysis of garlic essential oil has revealed a group of previously unknown cyclic and acyclic organo-sulphur compounds of which the compounds **14** - **17** are particularly important because of their lipo-oxyginase inhibitory activity. Interestingly, most of these compounds are generated upon heating pure samples of diallyl disulphide[34].

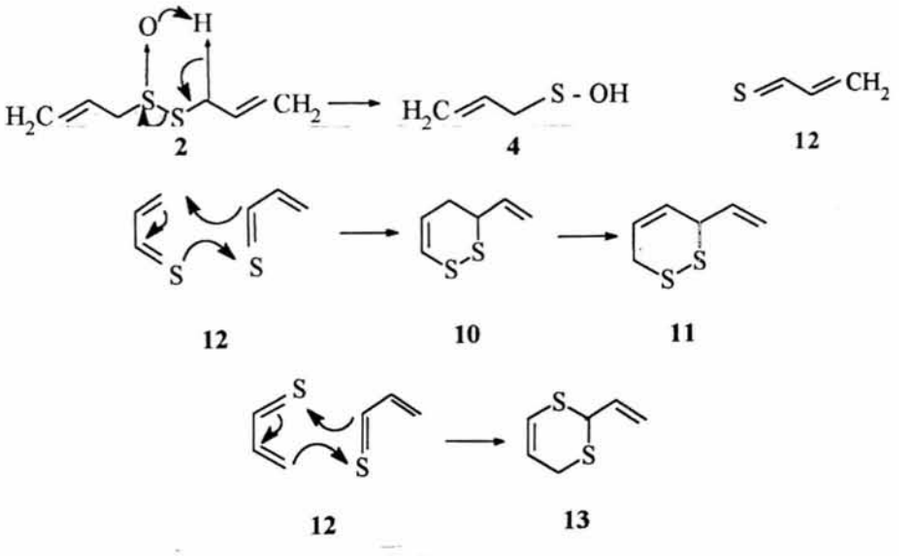
Allicin (**2**) undergoes self-decomposition even at room temperature and decomposition products may condense spontaneously to give a variety of compounds. One of these compounds of importance is ajoene (both *E* and *Z* varieties) which is well known for its antithrombotic properties. Taking a cue from the self-condensation of methyl methane thiosulfinate to 2,3,5-trithiahexane 5-oxide, the structure of ajoene is settled as 4,5,9-trithiadodeca-1,6,11-triene-9-oxide (**18**) and it is prepared by heating allicin (**2**) with a mixture of water and acetone. Three molecules of allicin give two molecules of ajoene (*E* and *Z*) and one molecule of water. The mechanism of formation of ajoenes from (**2**) has also been postulated[26] (Scheme III).

Ajoene may also be prepared synthetically from diallyl disulfide. Peracetic acid oxidation of diallyl disulfide (**1**) gives allicin (**2**) which on refluxing with aqueous acetone for 4 hr gives a mixture containing ajoenes. Removal of nonpolar fraction by washing of this mixture with pentane and subsequent extraction with CH_2Cl_2 yields mixture (4:1) of *Z*-ajoene (**18a**) and *E*-ajoene (**18b**). The synthetic material is indistinguishable from ajoene obtained from garlic.

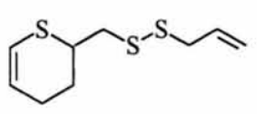
When an ether-soluble fraction of methanolic extract of garlic is stored in aqueous methanol for 4 days at 25°C and then successively extracted with hexane and methylene chloride, ajoene has been found to be present in the latter fraction. Hexane-soluble fraction contains several divalent sulfur compounds and these are identified as diallyl disulphide (**1**), diallyl trisulphide, diallyl tetrasulphide, allicin (**2**), and cyclic dithiin (**10**) and 2-vinyl-[4H]-1,3-dithiin (**13**). A compound isolated from oil-macerated garlic by silica gel column chromatography and preparative TLC, has been identified as Z-4,5,9-trithiadeca 1,6-diene-9-oxide (**19**) from NMR and MS analyses[5].

Essential oil of garlic, an item of commercial importance, is obtained mainly by steam distillation of garlic and this product differs from garlic extract prepared at room temperature. In place of allicin (**2**), ajoenes (**18**) and a variety of dialkyl thiosulphinates, known to be present in garlic extract, the essential oil of garlic has been reported to contain a mixture of diallyl and allylmethyl sulphides, disulphides and trisulphides and other minor components[35].

In addition to organosulphur compounds, peptides, steroidal saponins, flavones and other phytochemicals



Scheme II



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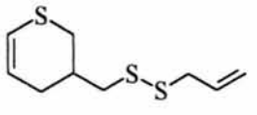

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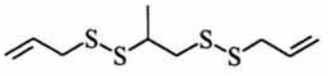

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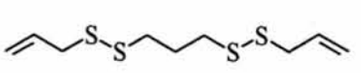

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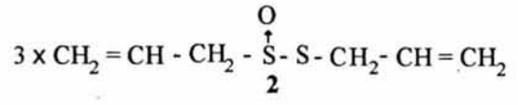

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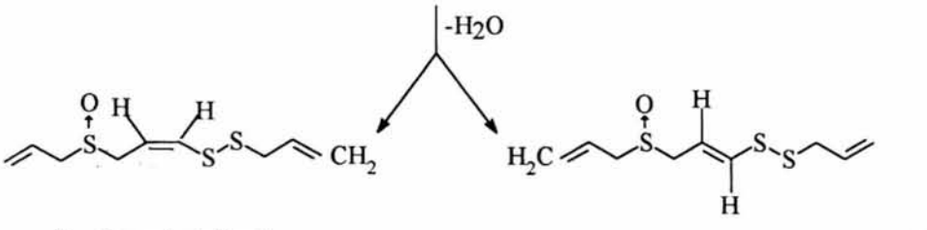


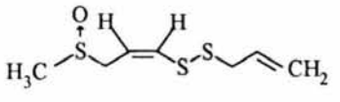
Z - ajoene (*cis* form)

a

18

E - ajoene (*trans* form)

b



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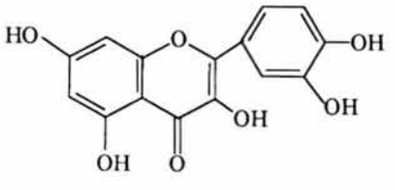

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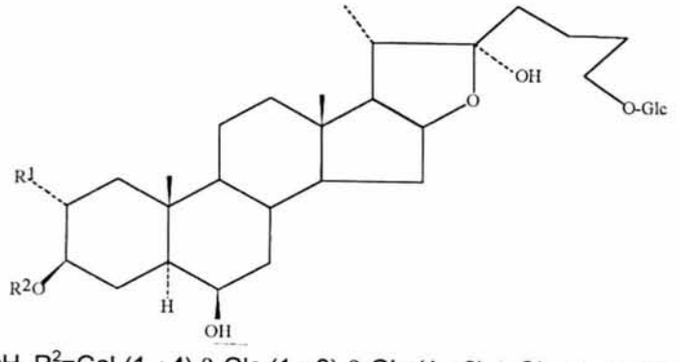

21 : R^1=H, R^2=Gal-(1→4) β-Glc-(1→3)-β-Glc-(1→3)-β-Glc-(1→2)-Glc

22 : R^1=OH, R^2 = β-Gal-(1→4)-β-Glc

Scheme III

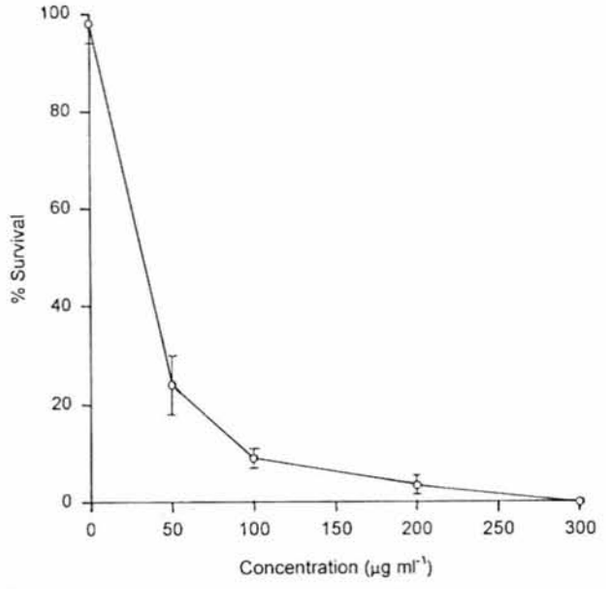

Fig. 2—Effect of ajoene on the survival of second stage juveniles of *Heterodera cajani*.

have also been isolated from garlic. Seven dipeptides isolated from the concentrated aqueous extract of garlic have been identified as Ser-Tyr, Gly-Tyr, Phe-Tyr, Asn-Tyr, Ser-Phe, Gly-Phe and Asn-Phe from sequence analysis, FAB mass spectral analysis as well as by synthesis[6]. In addition, several other tri- and di-peptides have also been isolated[36]. The presence of flavonol, quercetin (**20**) has also been detected in garlic[37]. Reports of isolation of bioactive steroidal saponins from garlic are there but the structures of all of these saponins could not be retrieved from the rather obscure journals[20,38,39]. Two of the saponins reported earlier[38], have been identified as (**21**) and (**22**).

Effect of garlic on fungi - Antifungal activity of garlic started with the testing of garlic bulb extract against some fungi. Appleton and Tansey[21] first used garlic extract against some zoopathogenic fungi with significant growth inhibition. Later, Barone and Tansey[40] have reported the isolation, purification, identification, synthesis and kinetics of activity of the inhibitory principle of *A. sativum* on some *Candida* species and postulated a hypothesis for its mode of action. Effect of aqueous garlic extract on fungi causing mycotic diseases in human beings has also been investigated[22]. In a preliminary experiment, fungicidal effect of garlic leaf extract has been reported by Mishra and Dixit[24]. Effect of aqueous extract of garlic leaf on chickpea seeds and wilt and stem rot of chickpea caused by *Fusarium oxysporum*

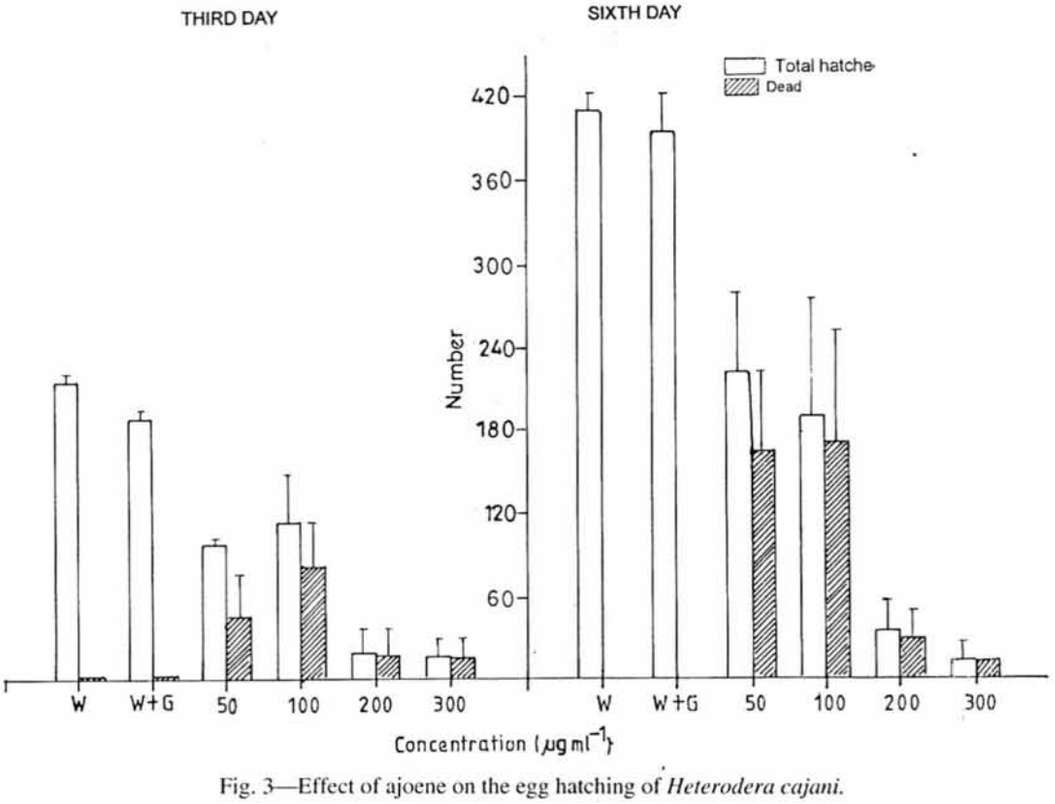

Fig. 3—Effect of ajoene on the egg hatching of *Heterodera cajani*.

f. sp. *ciceri* and *Sclerotinia sclerotiorum* has been seen. It has been observed that germination of treated chickpea seeds is delayed and seedlings arising from them do not show wilt symptoms[25]. Possible use of garlic leaf extract in controlling plant diseases under field conditions has been suggested.

Chicken infected with *Candida albicans* have been successfully treated by garlic[41]. Antifungal activity of garlic has also been reported against seven species of *Candida* and two species of *Cryptococcus* present in human serum[42]. Intravenous administration of garlic extract inhibits the growth of *Cryptococcus neoformans* present in plasma and cerebral fluid[43].

Effects of ajoene and other fractions of garlic against *Aspergillus niger* and *Candida albicans* have been studied by Yoshida *et al.*[2]. Out of six fractions, ajoene has shown strongest activity against the growth of *A. niger* and *C. albicans* at a concentration of <20 µg/mL. Ajoene which is highly inhibitory to *C. glabrata, C. tropicalis, Trichophyton mentagrophytes, Tricosporon beigelii* and *Saccharomyces cerevisiae* has been found to be superior to allicin. Scanning electron microscopic study has revealed severe damage of cell wall of ajoene-treated hyphae and conidia of *A. niger* and it has been postulated that like other antifungal agents, ajoene also acts on the cell wall of fungi[2].

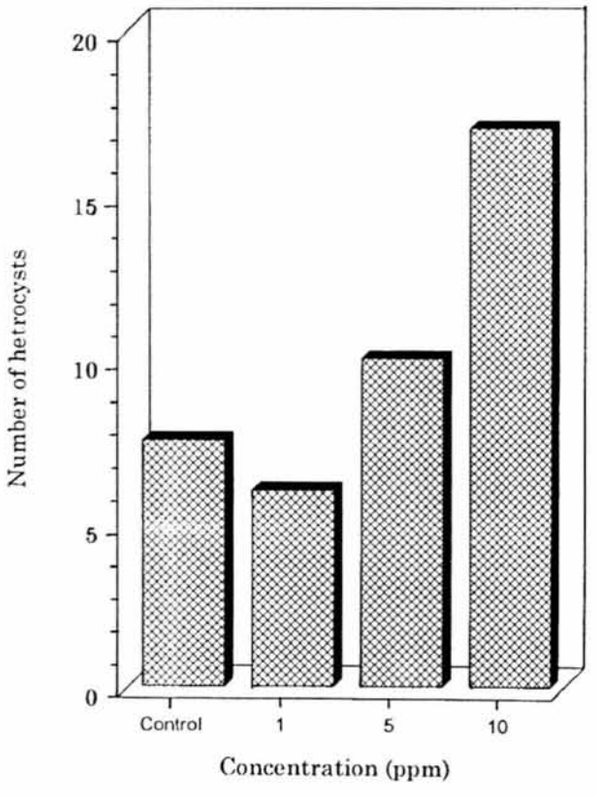

Fig. 4—Effect of ajoene on heterocyst formation in Nostoc calcicola.

Effect of ajoene on spore germination of some plant pathogenic fungi, viz., *Alternaria solani, A. tenuissima, A. triticina, Alternaria* species, *Colletotrichum* species, *Curvularia* species, *Fusarium lini, F. oxysporum, F. semitectum* and *F. udum* has been noticed *in vitro* by Singh *et al.*[3] and it has been observed that at 100 µg/mL concentration, spore germination of some fungi is completely inhibited. While working on the effect of ajoene on *Phytophthora drechsleri* f.sp. *cajani*, Singh *et al.*[44] have noticed differential response of this chemical on different developmental stages of the fungus. For instance, while mycelial growth is completely inhibited at 25 µg/mL, maximum effect is observed on sporangium formation and its germination at a very low concentration (2.5 and 3.5 µg/mL). Zoospore germination is affected at slightly higher concentration (20 µg/mL). Treated zoospores produce 2-4 germ tubes and is not dose-dependent.

Fromthing and Bulmer[23] have also noticed inhibitory effect of aqueous extract of garlic on *Cryptococcus neoformans*. Effect of garlic, its constituents and commercial garlic supplement products has also been studied[45] on several fungi, *viz., Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. schoenleinii, Microsporum audouinii, M. canis, Aspergillus fumigatus, Candida albicans* and *C. stellatoidea* and their efficacy is compared with ketoconazole. Allicin is generally less effective than ketoconazole, but is superior to latter against *Aspergillus fumigatus* and *Candida* spp. Ajoenes (*E&Z*) are effective against *C. stellatoides*, but less effective against *C. albicans*. In general, the antifungal effects of garlic compounds have been correlated with their allicin content.

Effect of garlic extract as well as of ajoene in the field and glasshouse has been observed by some workers[46-48]. Reimers *et al.*[46] noticed the effect of ajoene on 10 phytopathogenic fungi including three powdery mildews, viz., *Oidium lycopersicum, Sphaerotheca fuliginea* and *S. pinnosa* var. *roseae* and two yeasts. The results of *in vitro* tests indicate that minimum inhibitory dose for growth depends on organism, method and nutritional medium used and it varies from 2-200 ppm. *Cladosporium fulvum, Verticillium dahliae* and *Erwinia amylovora* are the

most sensitive species. Singh *et al.*[47] have studied the effect of ajoene on conidial germination of *Erysiphe pisi* as well as on powdery mildew development in growth chamber. According to them only higher doses of ajoene affect conidial germination and production of multiple germ tubes of *E. pisi.* The post-inoculation treatment of pea leaves with ajoene is as effective as commercially available fungicides against the development of powdery mildew of pea. The observations made by Reimers *et al.*[46] and Singh *et al.*[47] indicate that ajoene acts not only on germination of spores, but also on other stages of fungal development or growth. Bitsch[49] has reported strong reduction in mildew (*Sphaerotheca*) on cucumbers upon repeated spraying of garlic tea (aqueous infusion of garlic).

Effect of garlic on bacteria-Several workers studied the antibacterial activity of garlic extracts and attributed this activity to diallyl sulphide, unstable sulphur in alkyl polysulphides, a bacteriophage and acrolein or some similar unsaturated aldehydes. Phytoncides, a chemically undefined group of substances, have been reported to be antibacterial[27,50].

Allicin, isolated from garlic by steam distillation of crushed cloves has been found to be effective against many gram-positive and gram-negative bacteria belonging to *Staphylococcus, Streptococcus, Bacillus* and *Vibrio*[27]. Mechanism of action of allicin has been reported to be due to its ability to inhibit sulfhydryl containing enzymes[51] and RNA synthesis in bacteria[52]. Effect of a bacteriostatic concentration of allicin (0.2 to 0.5 m*M*) on growth of *Salmonella typhimurium* shows a pattern characterised by (i) a lag of approximately 15 min between addition of allicin and onset of inhibition; (ii) a transitory inhibition phase whose duration is proportional to allicin concentration and inversely proportional to culture density; (iii) a resumed growth phase which shows a lower rate of growth than in uninhibited controls; and (iv) an entry into a stationary phase at a lower culture density[52].

Kinetics of decline of populations of *Salmonella typhimurium* and *Escherichia coli* in the presence of freshly reconstituted dehydrated garlic powders has been studied by Johnson and Vaughn[53]. According to them, there is a significant bactericidal property of this powder on the above mentioned bacteria. Though the use of garlic for the treatment of tuberculosis with remarkable cure rate had been reported from Dublin Hospital and New York city around the turn of twentieth century, the concentration of the garlic extract necessary for inhibition of *Mycobacterium tuberculosis* and other strains of mycobacteria of 17 different species has been quantitatively assessed by Delaha and Garaguci[54] rather recently and it has been observed that minimum inhibitory concentration (MIC) of garlic extract ranged from 1.34 to 3.35 mg/mL. Inhibitory activity of garlic extract against some food pathogens, viz.. *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Listeria monocytogenes* has been measured by turbidity method and all the bacterial strains tested have been inhibited by garlic[4]. MIC of the essential oil of garlic against *L. monocytogenes* is 1: 1280 (v/v units)[55]. Like ajoenes, *Z*-10-devinyl ajoene (**19**) also exhibits a broad spectrum of antibacterial activity and its activity is comparable to that of *Z*-ajoene (**18a**); but is superior to that of *E*-ajoene (**18b**)[5]. This shows that the replacement of allyl group by methyl group does not alter the activity.

Effect of garlic on mites - Effect of garlic or garlic products on mites has not been reported earlier. Only recently acaricidal effect of ajoene has been studied using spotted mite (*Tetranychus urticae* Koch) and complete mortality (100%) has been observed with 0.075% solution (w/v) after 14 hr of treatment which is comparable with conventionally used acaricides. At lower concentration (0.05%), ajoene affects facundity and juvenile survival (the survival rate being 37.56% at this concentration without affecting the male:female ratio). The results suggest that ajoene, besides having direct acaricidal effect, can also check resurgence of the pest. Use of ajoene for the control of *T. urticae* under field conditions is quite possible[16].

Effect of garlic on nematodes - Effect of ajoene (**18**) on survival of second stage juveniles of *Heterodera cajani* which causes root knot disease in pigeonpea (*Cajanus cajan* (L.) Millsp.) has been tested in our laboratory and it has been noticed that survival percentage is reduced to zero at 300 µg/mL of ajoene (Figs. 2,3).

Effect of garlic on blue-green algae - In order to explore the possibility of use of garlic or its products for control of plant pathogens in the field conditions, it is of paramount importance to know their effect on non-target organisms, particularly, blue-green algae. With this objective in view, the effect of ajoene on the growth of *Nostoc calcicola* has been studied in our university (unpublished work) and it has been observed that at the concentration of 6-20 ppm, the growth of alga decreases up to 4th day of incubation

and thereafter increases up to 14th day while lower doses do not have any effect.

In another experiment, it has been observed that the heterocyst formation is affected by ajoene. At 1 ppm, the number of heterocysts slightly decreases as compared to control but at 5 and 10 ppm the number increases significantly in comparison to control (Fig. 4). The use of ajoene against plant pathogens may, therefore, indirectly help in nitrogen fixation.

Effect of garlic on rats - With the objective of finding out whether ajoene, a constituent of garlic, has any effect on rats, it was injected intraperitonially to female and male wister rats (unpublished work). In female rats it was injected before and after fertilization of the ovum at the rate of 10, 20 and 40 mg/kg body weight in single and multiple doses. In all the experimental groups, the average number of live fetuses was higher than those of corresponding controls and the weight of ovaries at full term was comparatively increased. In pregnant female rats, at full term, when the fetuses were removed, the weight of viscera, like liver, spleen and kidney, was found to be nearly similar to those of controls, whereas in nonpregnant female rats, the weight of these viscera was comparatively reduced. In male rats the weight of liver, spleen, kidney and testes was reduced in treated groups as compared to control male rats.

Antioxidant activity of garlic - Garlic oil and some of its components have been reported to inhibit lipooxygenase (LO), one of the enzymes involved in arachidonic acid metabolism which may be related to its biological properties[34]. Of the different components of garlic oil that shows significant LO inhibitory activity, 2-(2',3'-dithia-5'-hexenyl)-3,4-dihydro-2H-thiopyran (**14**), 3-(2',3'-dithia-5'-hexenyl)-3,4-dihydro-2H-thiopyran (**15**), 6-methyl-4,5,8,9-tetrathiadodeca-1,11-diene (**16**) and 4,5,9,10-tetrathiatrideca-1,12-diene (**17**) display IC_{50} values toward soybean 15-lipooxygenase enzyme within a range of 8-37 μM, in comparison to 90 μM shown by *E*- and Zajoenes (**18**). A structure - activity relationship study made by these authors using garlic oil components and structurally related synthetic model compounds reveals that LO inhibitory activity increases with molecular weight up to a certain point and that changes made in the molecules by addition or removal of unsaturation have only minor influence. While LO inhibitory activity of garlic oil components and homologues may be attributed primarily to lipophilic interactions, the ability of these organosulphur compounds to bind to LO iron merits further study. Diallyl disulphide (**1**) has been reported to inhibit linear microsomal lipid peroxidation induced by doxorubicin, a well known anticancer drug. As the oxidative injury caused by doxorubicin restricts its clinical use, the clinical efficacy of this drug can be improved, if used in combination with diallyl disulphide[56]. It is interesting to note that most of the garlic oil components could be obtained simply by pyrolysis of diallyl disulfide (**1**) and, therefore, their presence is regarded as a consequence of heat (during steam distillation) on allicin (**2**) and diallyl disulfide (**1**). Obviously, any culinary procedure that exposes garlic or garlic spiced food to heat generates compounds with possible health benefits associated with antioxidant or LO inhibitory activity.

Hypolipidemic, hypocholesterolaemic, antithrombotic and antidiabetic properties of garlic - Garlic preparations have been shown to lower the levels of serum cholesterol and triglyceride through inhibition of their biosynthesis in liver and of oxidation of low density lipoproteins[7-9]. The garlic constituent, alliin (**3**) has hypolipidemic[8] activity and both alliin and S-methyl cysteine sufloxide are endowed with antidiabetic actions[12,13]. A combination of garlic and ginger has been claimed to be superior to garlic alone in reducing serum lipids as well as blood glucose[14]. A recent study reveals garlic protein to be similar in composition to soya protein and it exhibits hypolipidemic action[57]. Effects of aspirin and garlic powder supplementation on some clotting parameters in rabbits show that garlic powder supplementation to cholesterol rich diet is more advantageous than aspirin alone because it has the beneficial effects on both blood clotting and plasma cholesterol level[58]. Some steroidal saponins isolated from fresh garlic bulbs also exhibited inhibitory effect on blood coagulability[20].

Beneficial effects of garlic and its preparation against atherosclerosis and coronary thrombosis are considered to be due to their ability to lower serum cholesterol[19] and inhibit aggregation of blood platelets[59]. Administration of aged garlic extract over an extended period significantly reduces epinephrine and collagen-induced platelet aggregation. While early work reports allicin (**2**), allyl methyl trisulfide and diallyl trisulfide to be the active constituents, 2-vinyl-[4H]-1,3-dithiin (**13**) and ajoene (**18**) have been proved to be the potent antithrombotic agents. Unlike several other inhibitors of platelet aggregation, these compounds are found to inhibit aggregation induced by all known inductors[60,61]. In an effort to understand the structure activity relationship, a comparative study

of antithrombotic activity of (*E* and *Z*)-ajoene and synthetic homologues has been made and it has been found that among the components of garlic oil, *Z*-ajoene (**18b**) is most active. The central carbon-carbon double bond and disulfide groups of ajoene have been proved to be essential for activity. On the basis of this study it has been postulated that platelet membrane sulphydryl groups participate in a disulphide exchange reaction with ajoene and the resultant alteration in the membrane prevents aggregation. Apitz-Castro *et al.*[62] have established the molecular basis of the antiplatelet action of ajoene and it has been shown that it directly interacts with fibrinogen receptor. One of the remarkable features of ajoene is that its antiaggregatory effect is synergistic with that of prostacyclin, aspirin and indomethacin[63].

Dipeptides isolated from concentrated aqueous extract of garlic show angiotensin 1-converting enzyme inhibitory activity. These are considered to be responsible, at least in part, for antihypertensive activity of garlic in animals and humans[6]. Efficacy of garlic powder, extract or oil in treatment of patients with hyperlipidemia has, however, been questioned[64,65]. Efficacy of garlic principles on hyperlipidemic patients may, however, differ according to etiology of the disease and this aspect needs further investigation.

Effect of garlic on AIDS (HIV) viruses and malarial parasites - Apitz-Castro and coworkers[62] have observed that ajoene inhibits platelet aggregation by allosterically inactivating the platelet integrin GP IIb/III, and this prompted Tatarintaev *et al.*[66] to propose that ajoene may also inhibit both adhesive interactions and fusion of leucocytes and may antagonize integrin-dependent processes in HIV-infected T-lymphoblasts. Study has shown that ajoene (**18**) inhibits platelet aggregation, HIV-mediated syncytia formation, replication of HIV-1 (RF) in H9 cells and replication of HIV-I (LAV-BRU). Based on their results it has been suggested that administration of ajoene in combination with conventional anti-HIV drugs may be a promising approach for treatment of AIDS.

Perez *et al.*[67] have investigated anti *Plasmodium berghei* activity of ajoene on experimental mice. They observed inhibition of malarial parasite following treatment with ajoene. However, when ajoene was used as a mixture with a noncurative dose of chloroquine there was a complete prevention of the development of parasitemia in mice. It is believed that ajoene synergistically enhances antimalarial activity of chloroquine against plasmodial parasite. The actual mechanism of action is not yet known.

Hepatoprotective activity and detoxification effects of garlic – Garlic preparations and organosulphur compounds present in garlic have been proved to be responsible for protection of liver against acetaminophen and bromobenzene toxicity[10,38]. Acetaminophen-induced acute cataract and other occular tissue damage in mice could be prevented by treatment with diallyl disulfide (**1**), preferably in combination with N-acetyl L-cystine. The plausible mechanism for this detoxification effect has been postulated[68]. Khanum and co-workers[69] have observed that long term feeding of fresh garlic, or its oil, reduces the toxic effects of azoxymethane, a carcinogen. Iron nitrilotriacetate-mediated nephrotoxicity is diminished by one week of oral pretreatment of male albino wister rats with garlic oil[70]. Most of the toxins damage tissues by free radical formation and garlic principles counter them by their detoxifying actions[71].

Anticancer and immunomodulatory activity of garlic - Several ingredients of our dietary system are known to have the potential against occurrence of cancer in human beings. A variety of phytochemicals present in edible fruits and vegetables has been proved to inhibit carcinogenesis by appropriate experimental studies but evaluation of anticancer activity of garlic, its extract and of its constituents has been done recently despite the reference of garlic as an effective remedy for tumors in Codex Ebers, an Egyptian Medical Papyrus dating back to around 1550 BC (Ref. 33,72). Kyo *et al.*[17] have studied the antitumor activities of aged garlic extract and observed that it stimulates the proliferation of mouse spleen cells and the release of cytokines, increases NK activities and enhances phagocytosis of peritoneal macrophages. It also stimulates the reactivity of lymphocytes in response to cytokines and mitogens. Aged garlic extract (a rich source of ajoene) significantly inhibits the growth of sarcoma-180 (allogenic) and LL/2 lung carcinoma (syngenic) cells transplanted into mice. Increased NK and killer activities of spleen cells have been observed in sarcoma-180 bearing mice treated with aged garlic extract. Aged garlic extract has thus been proved to be a potent biological response modifier in NK cells and T-lymphocytes as it inhibits the growth of transplanted tumors. A protein fraction from aged garlic extract has also been reported to enhance

cytotoxicity and proliferation of human lymphocytes[73].

Direct cytotoxic effect of garlic and its extract has been studied. Dietary (5% in diet) and subcutaneous administration of garlic extract have been reported to inhibit the growth of Morris hepatoma 3924A in rats[74]. Inhibition of growth of murine transitional cell carcinoma (MBT-2) in mice by intraperitoneal injection of garlic extract has been reported[75]. Belman[76] has shown inhibition of 7, 12-dimethylbenzanthracene-induced phorbol-myristate-acetate promoted skin papillomas in mice by topical application of garlic oil.

Diallyl sulphide and diallyl disulphide have been used to determine inhibition of aryl amine N-acetyltransferase (NAT) activity in a human colon tumor (adenocarcinoma) cell line and it has been observed that NAT activity is inhibited by these organosulphur compounds in a dose-dependent manner[77]. Modulation of hepatic drug-metabolizing enzymes in rats treated with dialkyl sulphides and disulphides has been studied. The results suggest a possible protective effect of diallyl disulfide on the first step of carcinogenesis *via* modulation of enzymes involved in carcinogen metabolism[78].

Garlic has also been found to modulate immunological response against cancer. Mice injected with aqueous garlic extract-treated Ehrlich carcinoma cells acquire resistance against a challenge with untreated tumor cells.[79] In a human study, administration of raw garlic (0.5 g per kg body wt/-. day) for three weeks induced the increase of natural killer cell activity by 140% (Ref. 80). Diallyl sulphide, a constituent of garlic has been shown to inhibit carcinogen induced nuclear damage in mouse colon epithelial cells and esophageal cancer in rats[81,82]. The cancer preventive role of organosulphur compounds of garlic has been reviewed by Sumiyoshi and Wargovich[83].

Essential oil of garlic known to inhibit lipooxygenase and cyclooxygenase enzymes has also been found to inhibit mouse skin tumor promotion. Of the different components of garlic oil tested, di-(1-propenyl) sulphide and ajoene have been found to inhibit both lipooxygenase and tumor promotion[84]. Scharfenberg *et al.*[85] have tested the cytotoxic effect of ajoene using human primary fibroblasts (FS 4), a permanent, non-tumorgenic cell line derived from baby hamster kidney cells (BHK 21) and a tumorogenic lymphoid cell line derived from Burkitt lymphoma (BJA-B) and observed the cytotoxic action in the range 2-50 μg/mL depending on the cell density. Although the exact mechanism of cytotoxic action of ajoene is not known, it is assumed that it reacts with cellular SH functions to exhibit its cytotoxic activity. *Z*-ajoene (**18a**) has also been shown to induce cell death by apoptosis in human HL-60 promyelocytic leukemia cells, MGc-803 gastric mucoid adenocarcinoma cells and Molt-4T lymphocyte leukemia cells[86].

There are several firms now in India and abroad which prepare garlic capsules that are recommended for the diseases of lungs, atherosclerosis, high blood pressure, gout, rheumatism, asthma, chronic bronchial catarrh, intestinal complaints and loss of appetite. These capsules are also claimed to renew the blood, clean it of all impurities, regulate digestion, remove all parasites from the intestines and cure bacterial infection. Garlic fried in mustard or coconut oil is an excellent antiseptic suitable for application in scabies and wounds. Its juice mixed with salt is applied to bruises and is claimed to relieve neuralgia and earache. The essential oil of garlic diluted with edible oil is administered to dogs and other pets to expel parasites from the digestive tract as well as from other parts of the body. It has also been pointed out that feeding of dogs with a teaspoonful of fresh garlic juice twice a week keeps them free from parasites.

In spite of beneficial effects of garlic against a variety of diseases, it suffers from the disadvantage of producing bad breath. Incidentally, the constitutents in human breath after ingestion of garlic have been analysed using proton-transfer-reaction mass spectrometry by Taucher *et al.*[87] and the components have been identified as allylmethyl sulfide, allylmethyl disulfide, diallyl disulfide, diallyl trisulfide, dimethyl sulfide and acetone. Various preparations and formulations of garlic are available in the market but all of them may not be equally useful for all kinds of diseases. It was observed by Eric Block[30] that the antithrombotic principles, ajoene (**18**) and 2-vinyl-[4H]-1,3-dithiin (**13**) are virtually absent in dehydrated garlic powder, pills, oils, extracts and other proprietary garlic preparations. It is presumably due to this reason that in many of these preparations, essential oil of garlic obtained by steam distillations is used. Again many of the volatile compounds known to have lipooxygenase inhibitory or antioxidant activity are present only in essential oils and in products prepared by application of heat.

Thus, while essential oil of garlic lacks most of the antibacterial and antithrombotic activity of garlic extract prepared at room temperature, it appears to possess equally interesting antitumor and antioxidant properties among other types of biological activities[34]. Appropriate formulations of garlic for the cure of different diseases are, therefore, a necessity. The research on garlic may go a long way in finding out its newer therapeutic values, in validating the claims made in different systems of medicine and also in assessing its potential in plant disease control.

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