

Antibacterial activity of Clove (*Syzygium aromaticum*) and Garlic (*Allium sativum*) on different pathogenic bacteria

Yashab Kumar, Sakshi Agarwal, Abhinav Srivastava, Satyaprakash Kumar, Garima Agarwal and
Mohammad Zeeshan Alam Khan*

Department of Microbiology & Fermentation Technology, Jacob School of Biotechnology & Bioengineering
Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed to be University)
Allahabad- 211007 (U.P) INDIA

*Corresponding Author E-mail: khan_zak47@live.com

ABSTRACT

Antimicrobial activity of Clove (*Syzygium aromaticum*) and Garlic (*Allium sativum*) was tested against two gram positive (*Bacillus cereus* and *Staphylococcus aureus*) and two gram negative (*Salmonella typhi* and *Escherichia coli*) pathogenic bacteria at different conc. (1000 ppm, 1500 ppm, 2000 ppm) of their extracts among which garlic was found to be more effective as compared to clove. *Bacillus cereus* was found to be most sensitive while *E.coli* was most resistant.

Keywords: Antibacterial activity, parts per million (ppm), *Syzygium aromaticum*, *Allium sativum*, etc.

INTRODUCTION

Spices have been used for many centuries by various cultures to enhance flavour and aroma of our foods as our ancestors have recognized the usage of spices in food preservation and in treatment of clinical ailments and there are several reports on development of antibiotic resistance in diverse bacterial pathogens⁴. This shift in susceptibility of pathogens to antibiotics greatly affects its ability to successfully treat patients empirically. Plant derived products have been used for medicinal purposes many centuries. At present it has been estimated that about 80% of the world population rely on botanical preparations as medicine to meet the needs as they are considered safe and provided to be effective against certain ailments^{5,6}.

Garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Allium vegetables, particularly garlic (*Allium sativum* L.) exhibit a broad antibiotic activity against both Gram-positive and Gram-negative bacteria. Therapeutic effect of garlic is possible because of its oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic. Thiosulfinates play an important role in the antibiotic activity of garlic³.

Cloves are used in Ayurveda, Chinese medicine and Western herbalism. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. It is also used in dentistry where the essential oil of clove is used as an adjuvant for dental emergencies. In addition, the cloves are antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic, antibacterial and anti-inflammatory⁷.

This study has been done to determine the antimicrobial activity of *Allium sativum* (garlic) and *Syzygium aromaticum* (clove) against certain pathogenic bacteria.

MATERIAL AND METHOD

Place of work

The present study entitled “**Antibacterial activity of clove and garlic on different pathogenic bacteria**” was carried out in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology & Sciences (Deemed-to-be-university), Allahabad.

Test microorganisms

The pathogenic organisms were obtained from the research laboratory of the department of Microbiology and Fermentation technology. The microbes used in this process were:-

Bacillus cereus (MCCB-008)

Staphylococcus aureus (MCCB-0065)

Escherichia coli (MCCB-0018)

Salmonella typhi (MCCB-0022)

Preparation of the culture broth

Nutrient broth was prepared and was inoculated with the test organism. A loop full of microorganism was taken and inoculated in the NB and was incubated at 37°C for 24 hrs to obtain a viscous growth.

Preparation of spices extracts

The fresh spices samples (clove and garlic) used in the present study were obtained from the local market of Allahabad. They were collected and surface sterilized with 0.1% HgCl₂. Spices were then crushed with distilled water using mortar and pestle. The sample was then poured in sterilized centrifuge tubes and centrifuged at 3000 rpm for 15 min. The supernatant was taken and different concentrations (1000, 1500, and 2000 ppm) were made using sterile distilled water.

Preparation of Nutrient agar plates

The freshly prepared and autoclaved NA media was poured in the Petri plates, after cooling it to 45°C, and was kept to solidify. Cotton swabs were dipped in the culture broth and were swabbed on the solidified media surface.

Antimicrobial sensitivity test using filter paper method

Filter paper discs of 5 mm diameter were prepared and sterilized by dipping them in 95% ethanol using sterile forceps. These discs were dipped aseptically in respective spices extract of appropriate concentration and placed over NA plates seeded with respective pathogens, with the help of sterile glass spreader. The plates were incubated in an upright position at 37°C for 24 h. The diameter of inhibition zones formed was measured in mm and the results were recorded. Discs with 7 mm diameter were considered as having no antibacterial activity. Diameter between 7 and 12 were considered as moderately active and those with > 12 mm were considered as highly active.

RESULT AND DISSCUSSION

The Result of the present study entitled antibacterial activities of clove and garlic on different pathogenic bacteria were recorded according to the size of inhibition zone formed on the agar plates by disc diffusion method.

Effect of garlic Extract

Garlic showed excellent antibacterial activity at all concentrations, that is, (1000, 1500 and 2000 ppm). It was found effective against the entire organism tested. The maximum effect was observed at 2000 ppm. The antibacterial effect decreases with decreasing concentration at 1000 ppm as it showed the minimum effect (table no. 3.1).

The maximum effect of garlic extract was shown on *Bacillus cereus* with maximum zone of inhibition and the minimum effect was shown in *Escherichia coli*.

On *Bacillus cereus* the maximum zone of inhibition was 27mm at 2000 ppm concentration and Minimum zone of inhibition was 11mm at 1000 ppm concentration. On *Staphylococcus aureus* the maximum zone

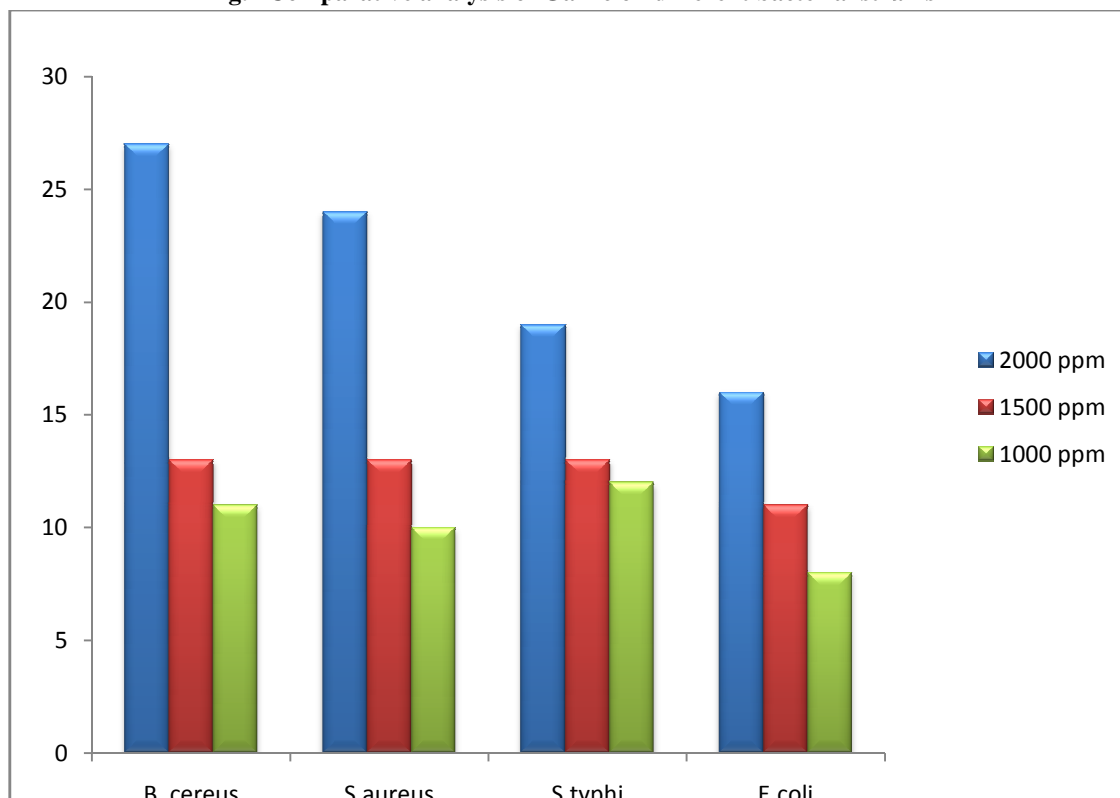
of inhibition was 24mm at 2000 ppm concentration and minimum zone of inhibition was 10mm at 1000 ppm concentration. On *Salmonella typhi* the maximum zone of inhibition was 19mm at 2000 ppm concentration and minimum zone of inhibition was 12mm at 1000 ppm concentration and on *Escherichia coli* the maximum zone of inhibition was 16mm at 2000 ppm concentration and minimum zone of inhibition was 8mm at 1000 ppm concentration.

Table 3. 1 Comparative analysis of garlic extract on different strains of bacteria

Organisms	Zone of inhibition of Garlic extract at different concentration (mm) – including 5 mm disc diameter.		
	2000 ppm	1500 ppm	1000 ppm
<i>Bacillus cereus</i>	27	13	11
<i>Staphylococcus aureus</i>	24	13	10
<i>Salmonella typhi</i>	19	13	12
<i>Escherichia coli</i>	16	11	8

The antibacterial activity of garlic is widely attributed to allicin. Allicin interferes with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA. If amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure. Also, as lipid synthesis is affected, other parts of the cell are interfered with. The main effect being that the phospholipid bilayer of the cell wall cannot form correctly in both Gram positive and Gram negative bacteria. All these things contribute to the bacteria cannot grow in the presence of allicin¹, observed a significant bactericidal effect of garlic extract against *Staphylococcus epidermidis*, *Salmonella typhi* and various yeasts. Even bacteria resistant to antibiotic agents were sensitive to extracts of garlic³.

Fig.1 Comparative analysis of Garlic on different bacterial strains



Effect of clove Extract

Clove showed comparatively lower effect on all the pathogenic strains of bacteria. Clove showed maximum effect on *Salmonella typhi* and minimum effect on *E. coli* (table 3.2).

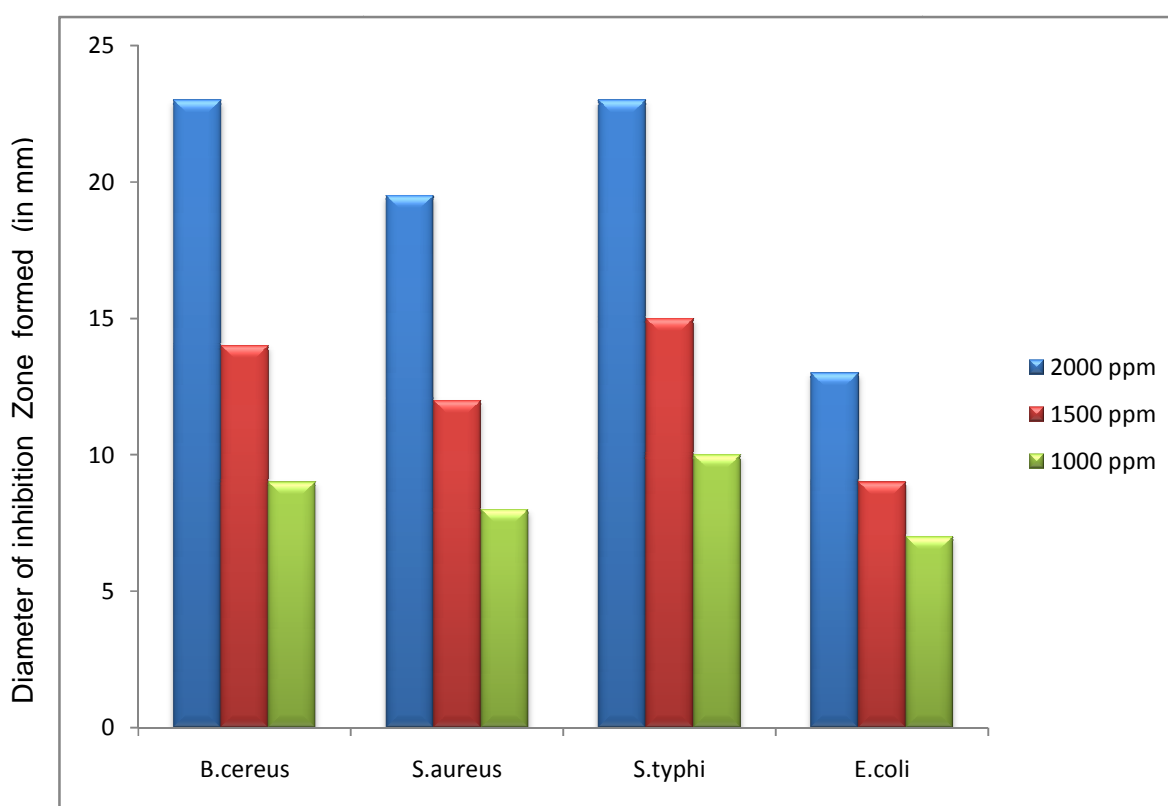
On *Bacillus cereus* the maximum zone of inhibition was 23mm at 2000 ppm concentration and Minimum zone of inhibition was 9mm at 1000 ppm concentration. On *Staphylococcus aureus* the maximum zone of

inhibition was 19.5mm at 2000 ppm concentration and minimum zone of inhibition was 8mm at 1000 ppm concentration. On *Salmonella typhi* the maximum zone of inhibition was 23mm at 2000 ppm concentration and minimum zone of inhibition was 10mm at 1000 ppm concentration and on *Escherichia coli* the maximum zone of inhibition was 13mm at 2000 ppm concentration and minimum zone of inhibition was 7mm at 1000 ppm concentration.

Table 3. 2 Comparative analysis of clove extract on different strains of bacteria

Organisms	Zone of inhibition of Garlic extract at different concentration (mm) – including 5 mm disc diameter.		
	2000 ppm	1500 ppm	1000 ppm
<i>Bacillus cereus</i>	23	14	9
<i>Staphylococcus aureus</i>	19.5	12	8
<i>Salmonella typhi</i>	23	15	10
<i>Escherichia coli</i>	13	9	7

Fig.2 Comparative analysis of Clove on different bacterial strains



Cloves were used in Ayurveda, Chinese medicine and Western herbalism and also as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. All the dilutions of clove showed good inhibitory activity against all four bacteria. The best effect was shown on *Salmonella typhi* and the least on *Escherichia coli*. The results of the present study are in harmony to those reported by Burst & Reinders² that clove oil was found effective against non-toxicogenic strains of *E. coli* O157:H7. Similarly, in another study clove oil was found active against foodborne Gram positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *P. aeruginosa*)⁸.

Natural products of plant origin have played significant role in the search of new drugs such as quinone from cinchona^{5, 6}. The results of the present study are quite encouraging as both the spices exhibited antimicrobial activity against the pathogens, but the antimicrobial activity varies widely, depending on the type of spices, test medium and microorganism. This study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics.

Plate no. 3.1 Antibacterial activity of garlic and clove on *Bacillus cereus*

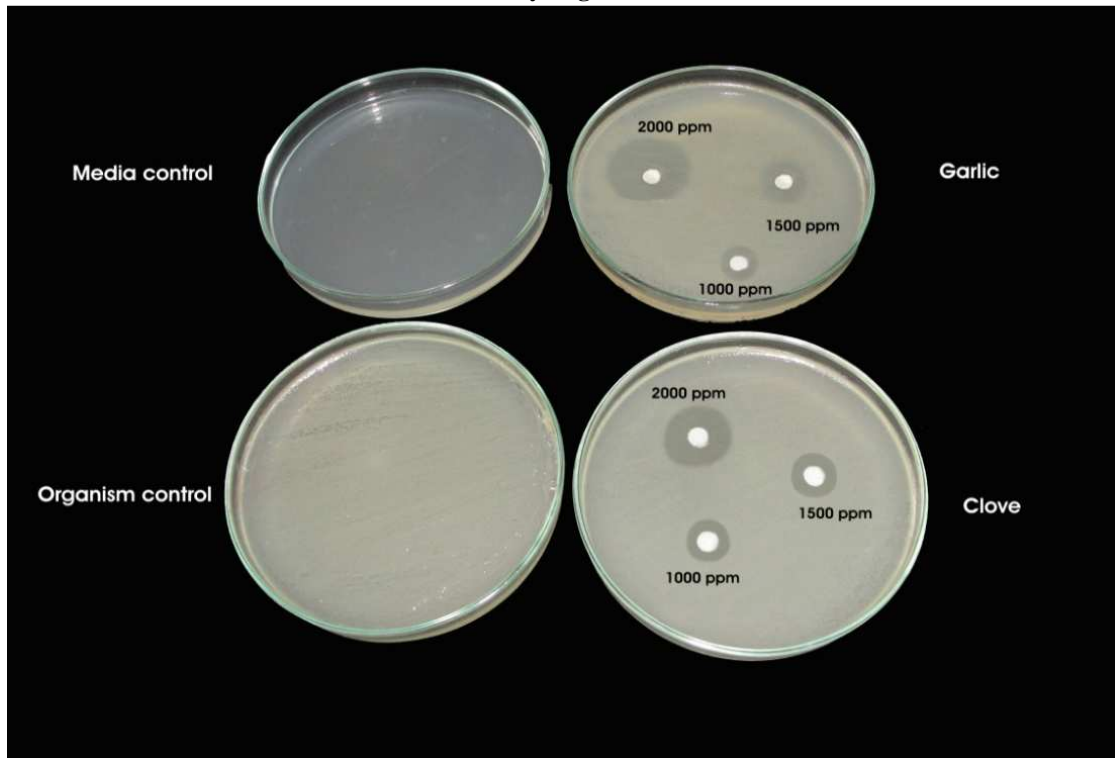


Plate no. 3.2 Antibacterial activity of garlic and clove on *Staphylococcus aureus*

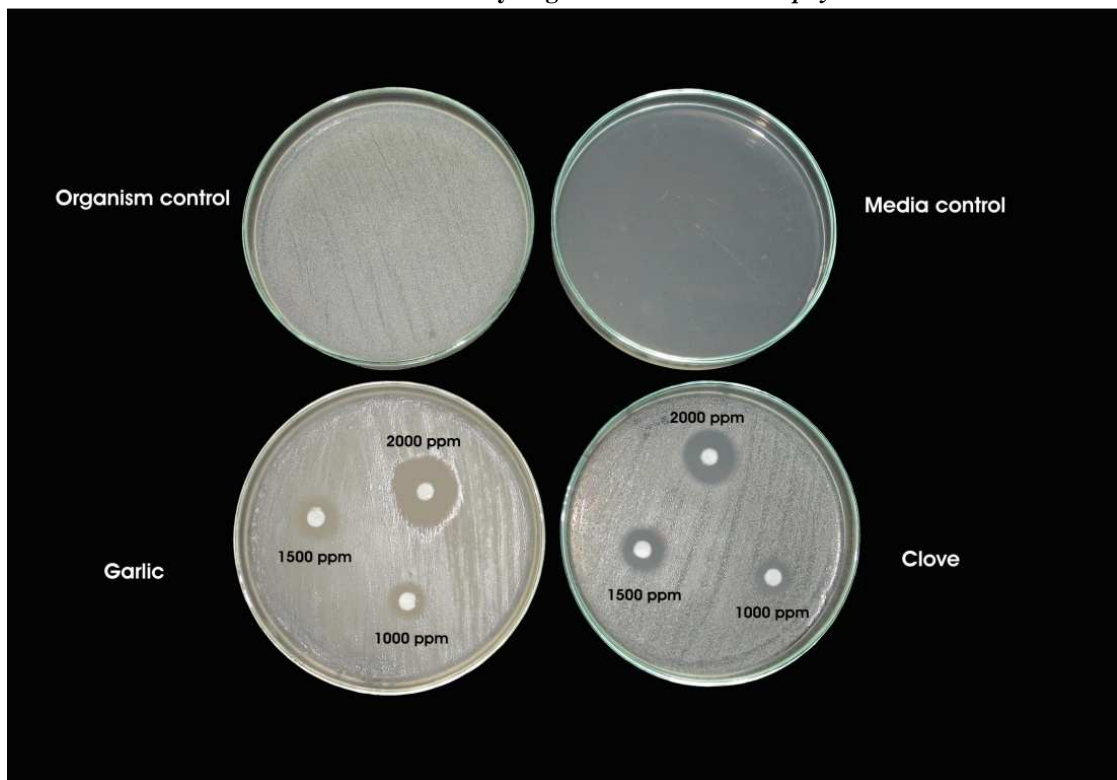


Plate no. 3.3 Antibacterial activity of garlic and clove on *Salmonella typhi*

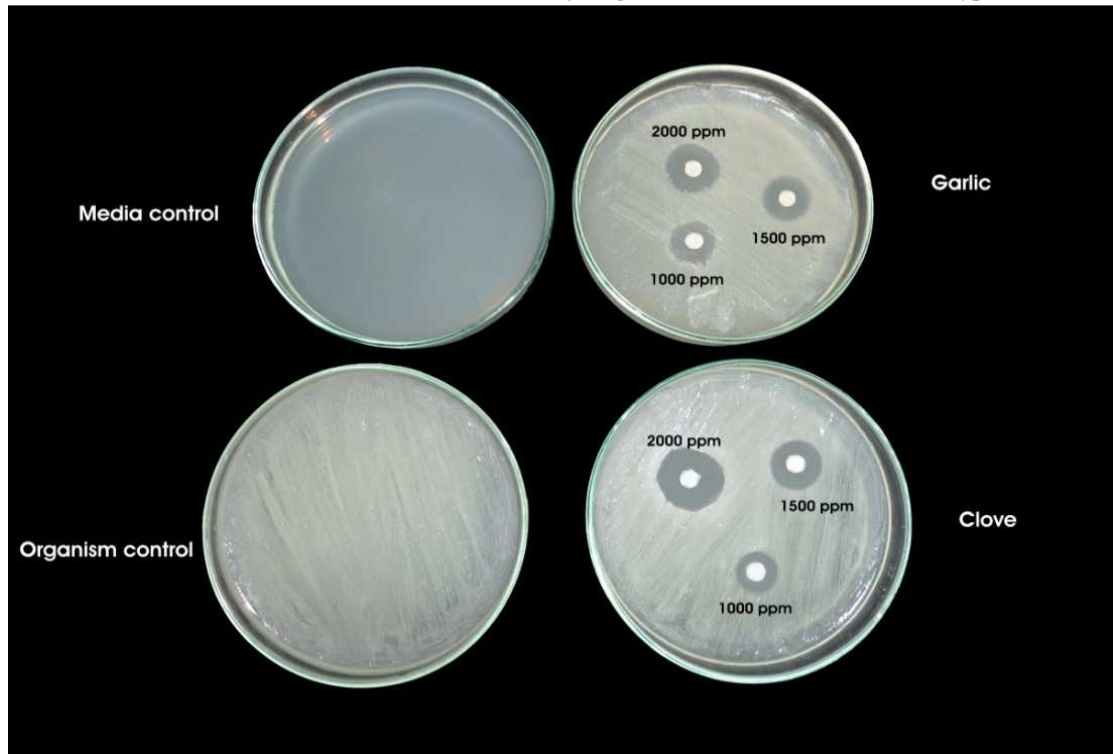
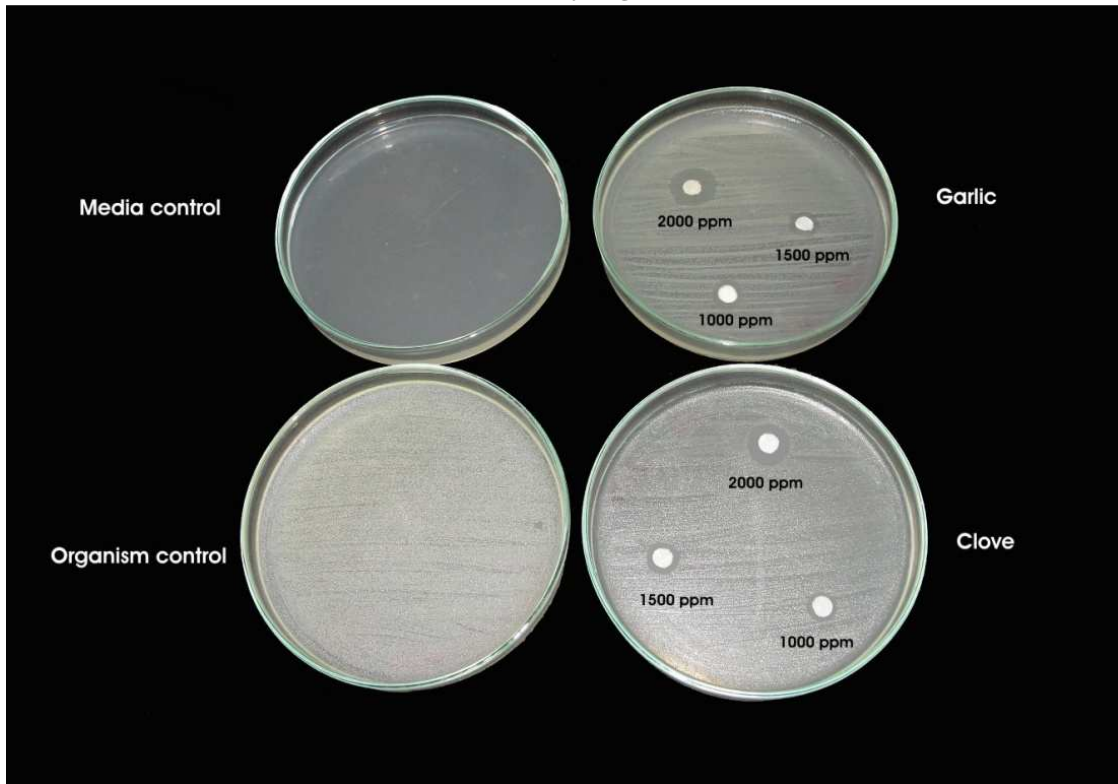


Plate no 3.4 Antibacterial activity of garlic and clove on *Escherichia coli*



SUMMARY AND CONCLUSION

The present study was carried out on the, “Antibacterial activity of clove and garlic on different pathogenic bacteria” in the Department of Microbiology And Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences. The study included the preparation of aqueous extract of garlic and clove. This was followed determination of antibacterial activity by agar well diffusion method. The result obtained has been summarized as follows:

Garlic showed more antibacterial effect on all the pathogenic strains of bacteria as compared to clove. Maximum zone of inhibition 27mm at 2000 ppm was shown by garlic on *Bacillus cereus* while the minimum effect of garlic shown on *Escherichia coli* with zone of inhibition of 8mm at 1000 ppm.

Maximum effect of clove was shown on *Salmonella typhi* with zone of inhibition 23mm at 2000 ppm and minimum effect of clove was shown on *Escherichia coli* with zone of inhibition of 7mm at 1000 ppm.

It was concluded that due to the indiscriminate use of antibiotics, antibiotic resistant strains of pathogenic bacteria are formed. Moreover usages of antibiotic have various side effects. Therefore as an alternative mode of treatment garlic and clove has antibacterial activity against these pathogens can be used. More investigations are needed in this field to confirm the present finding.

Acknowledgement

Authors are thankful to Prof. (Dr.) Rubina Lawrence (Head) Dept. of Microbiology & Fermentation Technology, SHIATS, Allahabad for her valuable suggestions and guidance during the course of investigation and also we wish to express our sincere thanks to Siddharth, Smita, Mukesh, Ritesh and Vineet for their kind co-operation and for having faith in us without which this work would not have been possible.

REFERENCES

1. Arora, D. and Kaur, J. Antimicrobial activity of spices. *International Journal of Antimicrobial Agents*, **12**: 257-262 (1999)
2. Burst, S.A. and Reinders, D.R. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Journal on Applied Microbiology*, **36(3)**: 162-167 (2003)
3. Durairaj, S. Srinivasan, S. and Lakshmanaperumalsamy, P. *In vitro* Antibacterial activity and stability of garlic extract at different pH and temperature. *Electronic Journal of Biology* **5(1)**: 5-10 (2009)
4. Gold, S.G. and Moellering, R.C. Antimicrobial drug resistance. *England Journal of Medicines* **335**: 1445-1453 (1996)
5. Hora, S.L. and Nair, K.K. Pollution of streams and conservation of fisheries. *Journal of environmental biology* **10**: 147-166 (1994)
6. Joe, M.M. Jayachitra, J. and Vijayapriya, M., Antimicrobial activity of some common spices against certain human pathogens. *Journal of Medicinal Plants Research* **3(11)**: 1134-1136 (2009)
7. Pandey, A. and Singh, P. Antibacterial activity of *Syzygium romaticum* (clove) with metal ion effect against food borne pathogens. *Asian journal of plant science and research*, **1(2)**: 69-80 (2011)
8. Saeed, S. and Tariq, P. 2008, *In vitro* antibacterial activity of clove against gram negative bacteria. *Pakistan journal of botany* **40(5)**: 2157-2160 (2008)