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Antimicrobial Activities of *Allium sativum* on Microorganisms Isolated from Spoilt Tomatoes Sold in Awka Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tomato is a universally consumed vegetable crop. Its spoilage involves changes in which they become less palatable, less attractive to the eyes or even toxic to consumers. These changes may be accompanied by alterations in taste, smell, appearance or texture. The aim of this study is to

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identify the spoilage pathogens of tomatoes and to ascertain the antimicrobial potential of garlic ethanol extract on the isolates. Spoilt tomato samples were purchased from three markets (Eke Awka, Nnamdi Azikiwe temporary site (Temp. site) and Amenyi) in Awka, Anambra State. They were transported Alpha Laboratory, Awka in a sterile polythene bags for microbial isolation and analysis. The media used for the isolation were Nutrient Agar and Sabouraud Dextrose Agar, both were placed into a conical flask autoclaved at 121ºC at 15psi for 20 minutes and the plates were incubated at room temperature. The bacteria isolated from the tomato fruits were: *Pseudomonas sp, Bacillus sp, Staphylococcus sp and Escherichia coli* while the fungi isolates were *Aspergillus sp, Penicillium sp and Mucor sp.* The antimicrobial activity of ethanol garlic extract against these microorganisms isolated from spoilt tomatoes were determined using disc method. Three different concentrations (25%, 50%, 100%) of ethanol garlic extract were used to test for the antimicrobial activity. The result showed that at different concentrations the ethanol garlic extract possess antimicrobial properties on the selected organisms apart from *Pseudomonas sp* and *Penicillium sp* where there was no zone of inhibition. The presence of these microorganisms is hazardous to health. Therefore, garlic extract could be used as a beneficial substitute of synthetic chemicals.

Keywords: Tomatoes; microorganism; garlic; antimicrobial activity.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is an edible, often red berry fruit that belongs to the family Solanaceae. Tomato fruit is widely used in households for stews, soups, salads, sauce, puree, juices etc. It is a world known vegetable crop. Tomato is one of the most cultivated and extensively consumed horticultural crop [1]. In Nigeria, it is one of the most important vegetable grown for consumption in every home; it can be consumed raw or cooked, fresh and in paste form. It is a crop with high Vitamin A, C, E and minerals which protect the body against diseases [2]. Some of the minerals contained include iron, potassium, calcium, zinc; it contains fibre, protein, carotenoid, flavonoid and lycopene which is a pigment that is responsible for the characteristic red colour of tomato fruits when ripe.

Tomato plant grow up to 1-3 meters (3-10 ft) in height and have a weak stem that often sprawls over the ground. Tomato crop does well in warm climate. It requires rainfall ranging between 760mm - 1300mm and deep fertile loam soil that is well drained with high content of organic matter. The crop is grown either on an open field or under greenhouse technology [3].

Nigeria ranks the16th largest tomato producing nation in the world with over 48million tomato farmers across the country. Nigeria accounts for 65% of tomato produced in West Africa [4]. Pathogens easily penetrate and infect the crop, usually through lesions and injury caused by improper handling of the fresh produce [5]. The storage and preservation of tomato is vital to the economy of homes, farmers and country considering the important role played by tomato in health and food security [6]. Tomato fruits spoil easily after harvest and during storage which result to post harvest losses which can be as a result of microorganism attack, physiological breakdown as well as an ambient environment for microorganism growth. Postharvest losses are more severe in developing than in developed countries [7]. This is estimated to be 20 - 25% in developing countries [8]. Due to high perishable nature of tomato fruits, many spoil before they reach various areas where they are not cultivated and where their demand is high. Reducing postharvest losses improves the welfare of farmers and consumers thereby increasing tomato availability. In Nigeria, commercial food vendors, restaurants, some urban and rural dwellers use physically damaged or spoiled tomato fruits for their cooking intentionally, due to the fact that these tomatoes are cheaper than fresh intact ones [9].

Over the years, there is a need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it [10]. The plant world is a rich store house of natural chemical that could be exploited for use as biopesticides [11].

The aim of this study is to identify the spoilage pathogens of tomatoes and to ascertain the antimicrobial potential of garlic ethanol extract the isolates.

2. MATERIALS AND METHODS

2.1 Samples Collection

Plant samples of *Solanum lycopersicum* fruits were bought from three markets in Awka: Eke

Awka, Amaenyi and Temp site. Laboratory and other facilities used in the practical work were obtained from Alpha research laboratory Awka, Anambra state.

2.2 Fungal Isolation

2.2.1 Sabouraud dextrose agar media preparation

In 300 ml of distilled water, 5 g of the medium was suspended, heated over a Bunsen flame with frequent agitation, and allowed to boil for one minute to completely dissolve the medium. The solution was autoclaved at temperature of 121°C for 15 minutes, at a pressure of one (1) atmosphere (15 Psi). After removing from the autoclave, allowed to cool for 10 minutes. Five hundred (500 mg) streptomycin sulphate was added into the molten solution to serve as antibiotics.

2.2.2 Isolation of fungi

One gram of each sample was aseptically collected and serially diluted in normal saline to the fourth dilution using a ten-fold serial dilution. About 0.1ml aliquot of each dilution was inoculated onto a freshly prepared SDA agar and incubated at room temperature (37°C) for three (3) days.

2.2.3 Sub-culturing techniques

Resulting colonies were then sub-cultured onto Sabouraud Dextrose Agar (SDA), process was repeated whenever more than a single colony of fungi was observed in the petri-dishes, until pure cultures were obtained.

2.2.4 Identification of isolated fungi

All the various species of fungi isolated were identified, both macroscopic and microscopic features, and their various characteristics studied, (i.e) colour, texture, form of hyphae, form of conidia, presence of conidiophores, shape of conidial heads [12].The microscopic identification was aided by appropriate taxonomic keys.

2.2.5 Determination of fungal frequency (%)

Fungal frequency will be determined location wise as well as culture media wise and later its correlation will be observed with the Percent Disease index calculated based on symptoms. The following formula will be used for fungal frequency percentage determination:

> Fungal Frequency (%) = Number of particular fungus colony observed in plates x 100 / Total number of colonies of all fungi

2.3 Bacteria Isolation

2.3.1 Isolation of bacteria

The spread plate method was used for isolation of bacterial pathogens from the sample using nutrient agar. One gram of each sample was aseptically collected and serially diluted in normal saline to the fourth dilution using a ten-fold serial dilution. About 0.1 ml aliquot of each dilution was inoculated onto duplicate set of Nutrient Agar, to determine total bacterial population. All plates were incubated at 35^0C for 24 hours.

2.3.2 Total Plate Count of Bacteria (CFU/ml)

Microbial load in each agar plate sample was determined as CFU/ml and was calculated using formula.

> Cfu/ml = {(No. of colonies X dilution factor) / volume of inoculums]

2.3.3 Purification of isolates

Single colonies of bacteria were randomly selected from different media plates based on their morphology. These bacterial cultures were subsequently isolated in pure forms by subculturing on nutrient agar plates incubated for 24hrs and used for microscopic characterization and biochemical analysis.

2.3.4 Identification of microorganisms

The isolated bacteria were identified on the basis of motility and Gram's-staining.

2.3.5 *In vitro* antimicrobial tests with plant extracts

Disc diffusion method adopted from Birhanu et al. (2014) was applied to the ethanolic extract. A 100 µL of prepared inoculum (10^4 CFU/mL) was spread uniformly onto SDA and Nutrient Agar using a sterile cotton swab. The sterile paper disc impregnated with 100, 50 and 25% of. extract then placed on top of the agar media.. The plate was then incubated upright for 48 hrs at 30°C. The clear inhibition zones surrounding

the disc were then measured in millimetre. Three replicates of five plates each were maintained for each treatment and inoculated plates were incubated for seven days at room temperature (28°C). The diameter of the radial growth of the bacteria and fungus was measured at the end of the incubation period and then used to determine the activity of the extracts using the formula:

$$\text{Percentage growth inhibition } (\%) = \frac{\text{dc} - \text{dt x } 100}{\text{dc } 1}$$

Where

dc = average diameter of fungal and bacterial colony in control treatment
dt = average diameter of fungal and bacteria colony with extract.

2.4 Statistical Analysis

Percentages and means of fungal colonies were calculated. Data obtained was subjected to Analysis of Variance (ANOVA), and Duncan Multiple Range Test (DMRT) was used to separate the treatment means when significant at 5% level of probability.

3. RESULTS

The research study showed the presence of microorganisms in all the tested samples. The pathogens that were isolated and identified were *Bacillus sp, Staphylococcus sp, Escherichia coli, Pseudomonas sp, Aspergillus sp, Mucor sp and Penicillium sp* (Table 1). They were found in association with spoilt tomato fruits sold at three different locations in Awka, Anambra State. The research also revealed the potentiality of garlic extract on the Isolated microorganisms. Among all the Isolates garlic gave the highest inhibition of *Aspergillus sp* and lowest of *Penicillium sp* and *Pseudomonas sp.* It should that the higher the concentration the more efficient it is in the control of the microorganisms. In comparison between the plant extract (*Allium sativum*) and the controls (streptomycin and fluconazole), the controls had more effect on all the pathogens by suppressing their growth.

Table 1 shows the Bacteria isolates from each collection sites.

It reveals that *Pseudomonas sp* was found on tomatoes from Nkwo Amaenyi only. *Escherichia coli* was isolated from tomato samples from Eke Awka and Temporary site, while, *Staphylococcus sp and Bacillus sp* were found on tomatoes from the three collection sites.

Table 2 shows Bacteria colonies from each location. Tomato samples from Eke Awka had the highest colonies ($6.10 \times 10^4 \pm 0.03^a$) while tomatoes from Temporary site had the lowest colonies ($4.71 \times 10^4 \pm 1.120^a$). Results are mean scores + Standard deviation of three replicates.

Data in the same column bearing different superscript differ significantly ($p < 0.05$).

Table 1. Prevalent bacteria isolates

Collection sites	Bacteria Isolates
Eke Awka	*Escherichia coli*
	Staphylococcus sp
	Bacillus sp
Temporary site	*Staphylococcus sp*
	Bacillus sp
	Escherichia coli
Nkwo Amenyi	*Bacillus sp*
	Staphylococcus sp
	Pseudomonas sp

Table 2. Total bacteria count of tomato

Collection Sites	Total Bacteria Count (cfu/ml)
Eke Awka	$6.10 \times 10^4 \pm 0.03^a$
Temporary site	$4.71 \times 10^4 \pm 1.120^a$
Nkwo Amaenyi	$5.64 \times 10^4 \pm 0.101^c$

Table 3. Frequency of occurrence of fungi isolates

Sample site	Fungi Isolates
Eke Awka	*Aspergillus sp*
	Mucor sp
Temporary site	*Aspergillus sp*
	Mucor sp
	Penicillium sp
Nkwo Amaenyi	*Aspergillus sp*
	Mucor sp

Table 3 shows the Fungi isolates from each site.

Aspergillus sp and *Mucor sp* occurred in tomato samples from the three locations while *Penicillium sp* occurred in samples from Temporary site.

Table 4 shows Fungi count for the three locations.

Nkwo Amaenyi had the highest fungi colony ($4.50 \times 10^4 \pm 0.00^a$) while Eke Awka had the lowest colony count ($2.70\times10^4 \pm 0.200^c$).

Results are mean scores + Standard deviation of three replicates.

Data in the same column bearing different superscript differ significantly ($p < 0.05$).

Table 5 shows the Invitro antibacterial activities of ethanol garlic extract on the isolated bacteria strains.

The garlic extract had the highest inhibition on *Staphylococcus sp* at 100% and 50% (19.00 ± 0.112 and 10.00 ± 0.030) among other isolates, at 25% the garlic extract inhibited the growth of *Escherichia coli* (5.00 ± 2.00) only while *Pseudomonas sp* showed the least susceptibility to the plant extract (0.00 ± 0.00). When compared with the plant extracts, the control had higher rate of inhibition on all bacteria isolates.

Results are mean scores + Standard deviation of three replicates.

Data in the same column bearing different superscript differ significantly ($p < 0.05$).

Table 6 shows the Invitro antifungal activities of ethanol garlic extract on the fungi pathogens. The garlic extract gave the highest inhibition on *Aspergillus sp.* 100% of the concentrations had the highest inhibition effect (18.4±1.00). *Penicillum sp* showed the lowest inhibition effect of the garlic extract (0.00±0.00). The control had effect on the three Fungi isolates and showed to be stronger and effective than the plant extract. The control had the highest effect on the growth of *Mucor sp* (41.60±1.06) while *Aspergillus sp* showed the least susceptibility to the control (22.33±0.11).

Table 4. Mean fungi count for tomato samples in SDA

Sample site	Mean total fungi count (cfu/g)
Eke Awka	$2.70\times10^4 \pm 0.200^c$
Temporary site	$3.15 \times 10^4 \pm 0.100^b$
Nkwo Amenyi	$4.50 \times 10^4 \pm 0.00^a$

Table 5. *In vitro* antibacterial activities of ethanol extract of garlic

Extract concentrations	*Staphylococcus sp*	*Escherichia coli*	*Bacillus sp*	*Pseudomonas sp*
Garlic 100%(mm)	19.00 ± 0.112	17.00 ± 3.01	9.000 ± 0.55	0.000 ± 0.00
Garlic 50%(mm)	10.00 ± 0.030	7.50 ± 1.110	0.000 ± 0.00	0.000 ± 0. 00
Garlic 25%(mm)	0.000 ± 0.000	5.00 ± 2.00	0.000 ± 0.00	0.000 ± 0.00
Control (Fluconazole) 30 ug/ml	34.833 ± 1.110	34.83 ± 0.300	19.16 ± 1. 00	34.833 ± 0.200

Table 6. *In vitro* anifungal activities of ethanol extract of garlic

Extract Concentrations	*Aspergillus sp*	*Penicillium sp*	*Mucor sp*
Garlic 100%(mm)	18.4 ± 1.00	0.000 ±0.00	18.13±2.00
Garlic 50%(mm)	13.67 ± 0.01	0.000 ± 0.00	12.20 ± 1.00
Garlic 25%(mm)	12.00 ± 0.30	0.000 ± 0.00	9.86 ± 0.20
Control (Streptomycin) 30ug/ml	22.33 ± 0.11	34.833 ± 0.15	41.60 ± 1.06

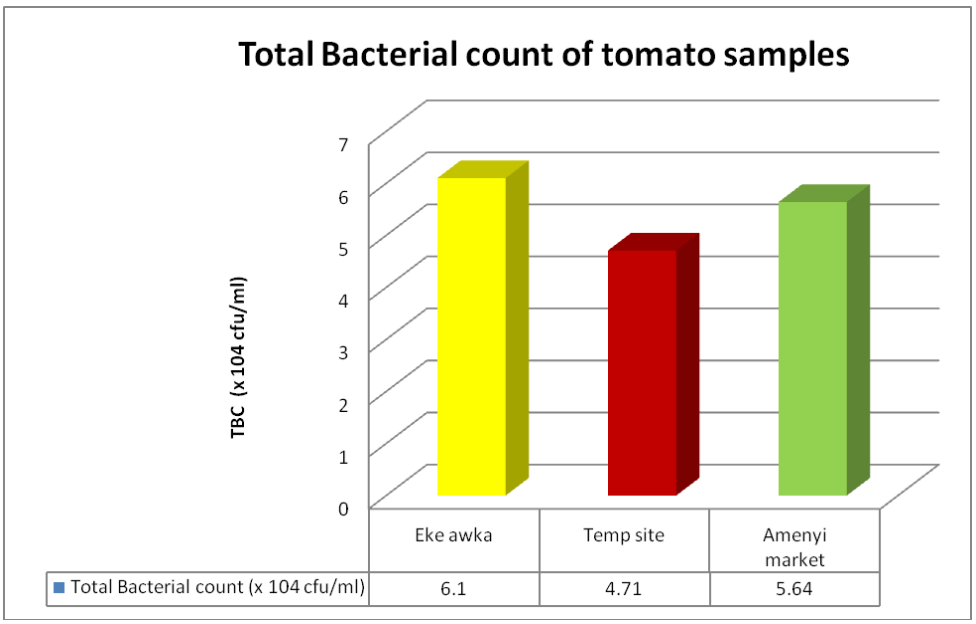


Fig. 1. Shows the total bacteria isolate count from tomatoes tested. Eke Awka had the highest count (6.1 ml), while Temp site was with the lowest bacteria count (4.71 ml)

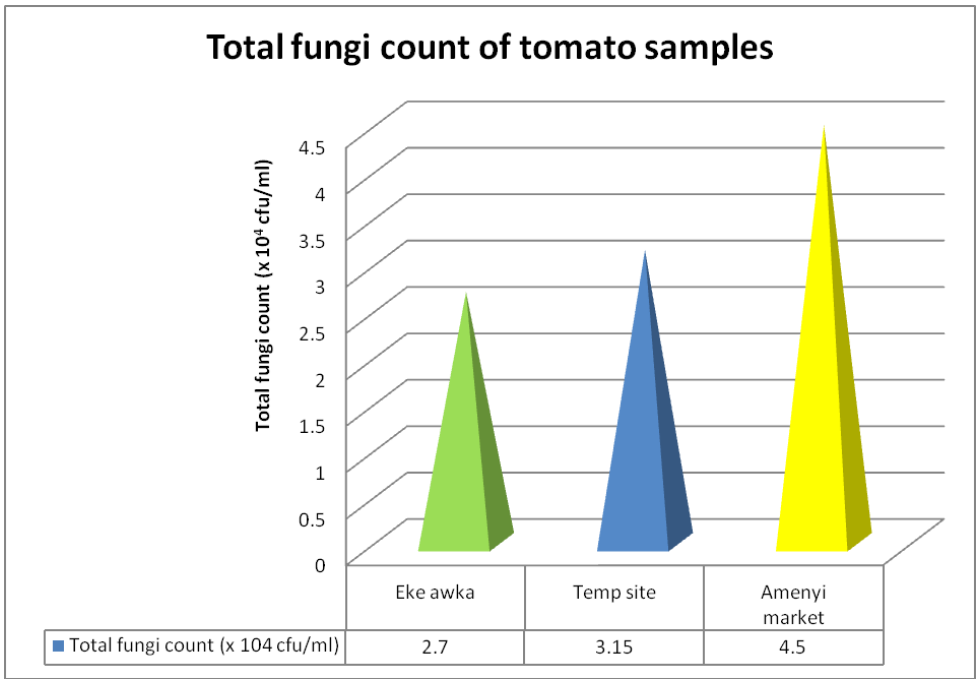
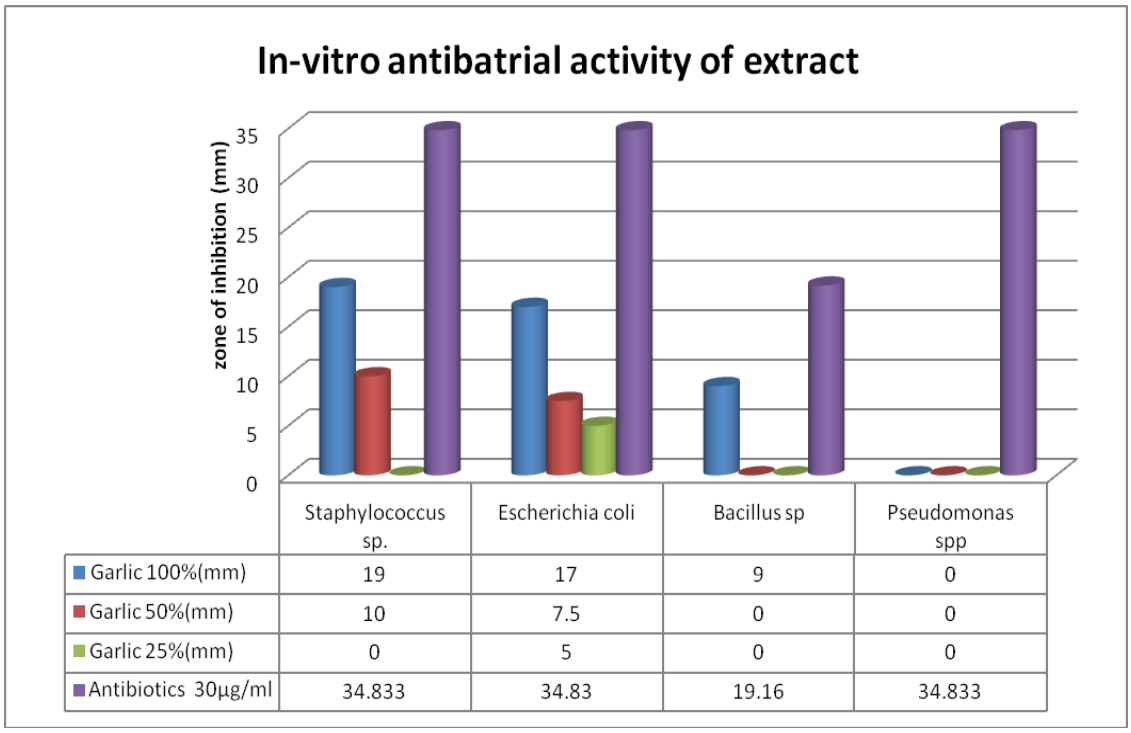
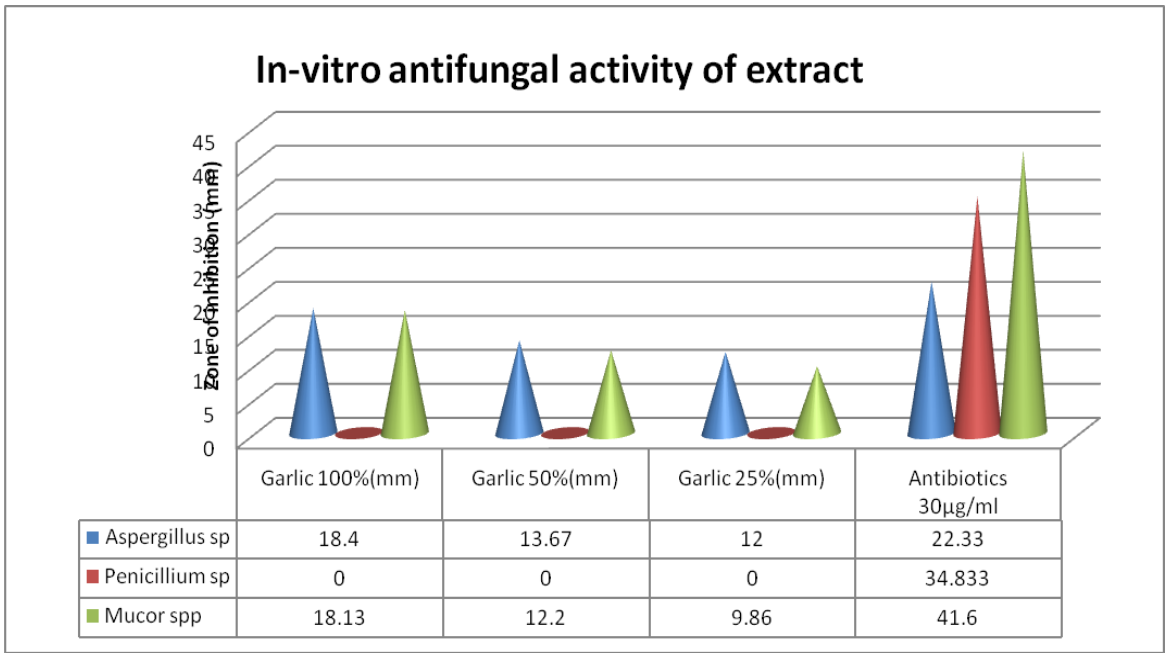


Fig. 2. Shows the Fungi isolates count. The collection site that had the highest fungi count is Nkwo Amaenyi (4.5 ml) whilt the site with the lowest count is Eke Awka (2.7 ml)



	Staphylococcus sp.	Escherichia coli	Bacillus sp	Pseudomonas spp
Garlic 100%(mm)	19	17	9	0
Garlic 50%(mm)	10	7.5	0	0
Garlic 25%(mm)	0	5	0	0
Antibiotics 30µg/ml	34.833	34.83	19.16	34.833

Fig. 3. Reveals the *In vitro* antibacteria activities of garlic at various concentration. *Staphylococcus sp* was highly susceptible at 50% and 100% (10mm and 19mm) at 100, 50 and 25 % Escherichia coli w inhibited (17, 7.5, 5mm) while *Pseudomonas sp* showed the least susceptibility to the garlic extract (0.00). The control (Streptomycin) had a great inhibitory effect on the bacteria strain over the plant extract



	Garlic 100%(mm)	Garlic 50%(mm)	Garlic 25%(mm)	Antibiotics 30µg/ml
Aspergillus sp	18.4	13.67	12	22.33
Penicillium sp	0	0	0	34.833
Mucor spp	18.13	12.2	9.86	41.6

Fig. 4. Shows the inhibitory effects of garlic extract on the Fungi isolates. The garlic extract had positive effect on *Mucor sp* (18.13, 12.2, 9.86mm) and *Aspergillus sp* (18.4, 13.67, 12mm) and negative effects on *Penicillium sp* (0.00) at various concentrations used in the study

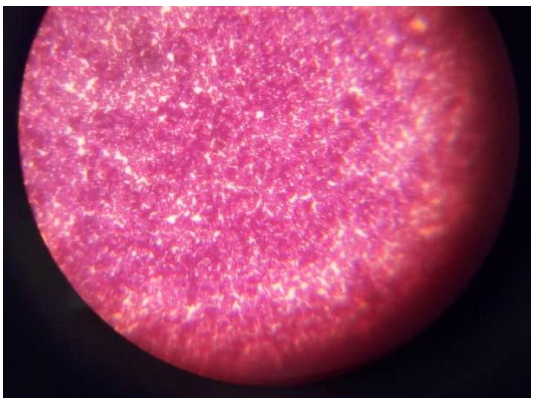


Plate 1. Microscopic Picture of *Pseudomonas sp*

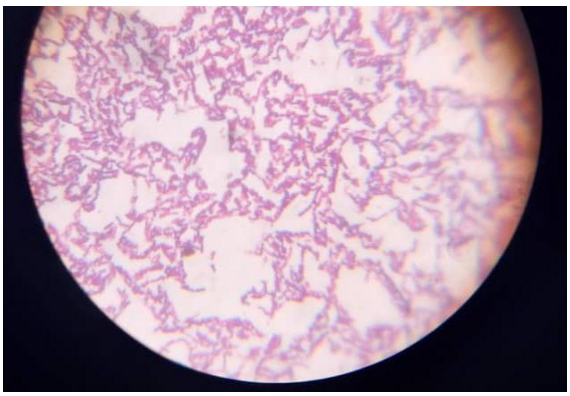


Plate 2. Microscopic picture of *E. coli*

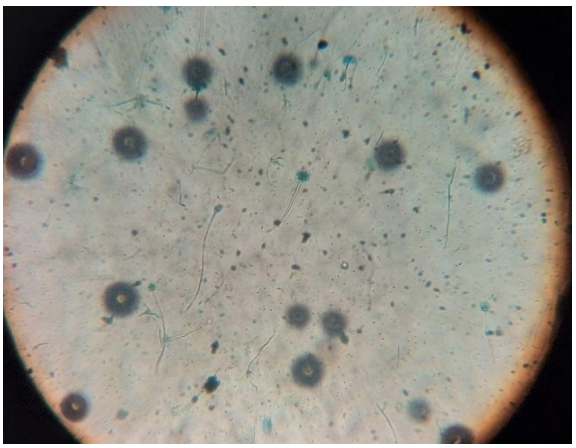


Plate 3. Microscopic picture of *Penicillum sp*

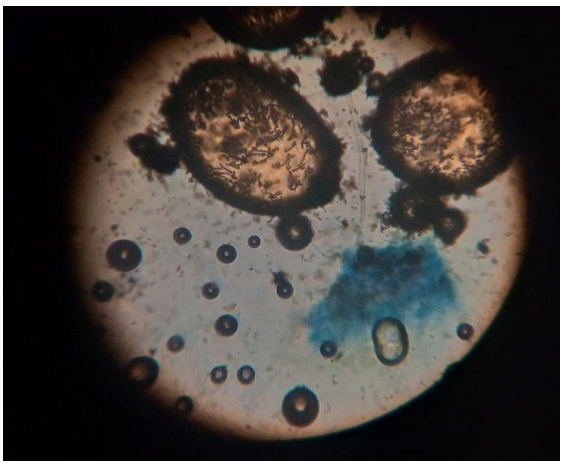


Plate 4. Microscopic picture of *Aspergillus sp*

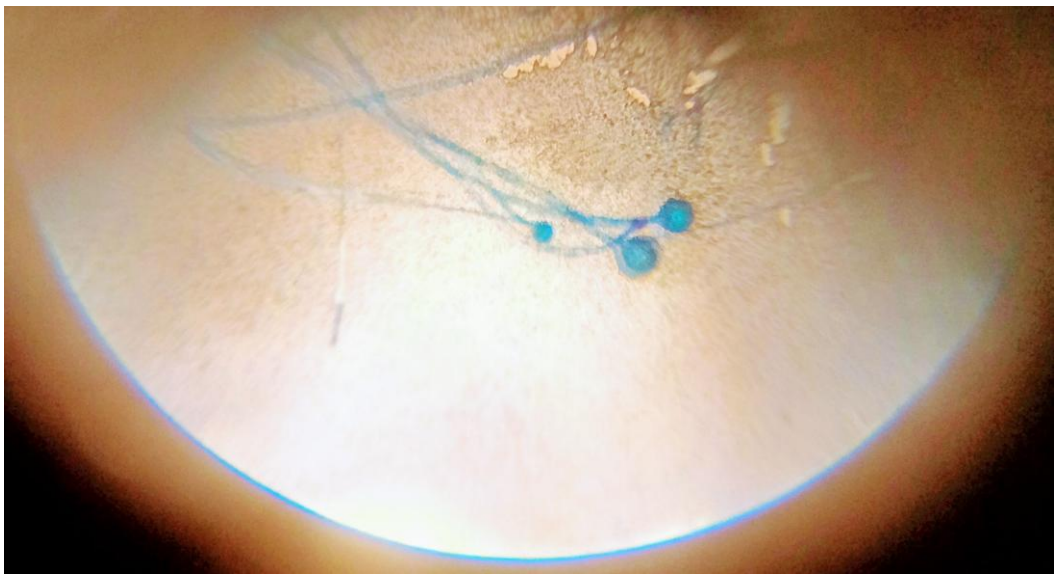


Plate 5. Microscopic picture of *Mucor sp*

4. DISCUSSION

The research study was based on Isolation of pathogens causing spoilage on tomatoes and antimicrobial properties of garlic extract. The pathogens associated with spoilt tomato fruits were studied and the results reviewed the presence of a teeming population of microorganisms. Spoilt tomatoes from Eke Awka had the highest bacterial count (6.1×10^4 cfu/ml) while those from Temporary site had the lowest bacterial counts (4.7×10^4 cfu/ml). Spoilt tomatoes from Eke Awka had the least fungal count (2.7×10^4 cfu/g) while samples from Amaenyi had the highest fungal count (4.5×10^4 cfu/g). The research showed seven microbial pathogens in association with spoilt tomatoes bought from three locations in Awka. The Bacteria isolated were *Escherichia coli, Staphylococcus sp, Pseudomonas sp* and *Bacillus sp.* While the Fungi Isolates were *Aspergillus sp, Penicillium sp and Mucor sp.* Some of these microorganisms had been reported previously by other researchers as pathogens and are responsible for spoilage of tomatoes [13,14]. A similar study revealed the high level of *Bacillus sp, Staphylococcus sp and Escherichia coli* in Lagos State Nigeria [15]. Also, some of the fungal organisms that were isolated and identified in this research were also reported by other researchers in the past. In Annual Research and Review in Biology 2018, 5 fungi species were identified after Isolation from tomato samples which include *Aspergillus sp, Penicillium sp, Botryodiplodia theobromae, Fusarium sp, and Colletotrichum sp* [16]. Mohammed and Kuhiyep, [17], reported that *Staphylococcus sp, Escherichia coli and Salmonella sp* were isolated from tomatoes sold in Kaduna. Garlic bulb ethanol extract was used for the antimicrobial assay. The garlic extract was able to inhibit the growth of the isolated pathogens. The antimicrobial properties of garlic have been reported to be due to the presence of a variety of alkaloids, flavonoids, terpenoids, tannins and saponins [18]. The *Allium sativum* antimicrobial activities at different concentrations against the bacteria and fungi pathogens were evaluated. The concentrations of the garlic extract used: 25, 50 and 100%. Zone of inhibition obtained varies based on concentrations. In almost all of the concentrations, the garlic extract gave antimicrobial activity against all the pathogens isolated. For the bacteria isolates, the garlic bulb extract gave the highest inhibition on *Staphylococcus sp* and the lowest of *Pseudomonas sp*. While for the Fungi isolates, the garlic extract gave the highest inhibition of *Aspergillus sp* and the lowest of *Penicillium sp.* Several research carried out has shown the efficacy of garlic in suppressing the growth of pathogenic microorganism. A similar result was observed by other researchers using garlic extract against tomato diseases. The result agrees with earlier work of Mugao, [19]; Tijjani et al. [20] and Ikon et al. [13] on the inhibition of growth and sporulation of microorganism pathogens on *Solanum lycopersicum* by *Allium sativum.* A similar study reported garlic to be a significant antimicrobial agent (Rehman et al. 2021). Ashraf et al*.,* [21], reported that ethanol extract of garlic suppressed the growth of *Escherichia coli, Bacillus so* and *Aeromonas hydrophila*. Two different studies revealed no inhibitory effect of garlic on *Mucor sp* [22] and *Aspergillus sp* [3] is in contrast with the findings which might be as a result of method of extract preparations and extraction and solvent used. According to Senhaji et al. [23], the efficiency of any plant extracts against pathogenic organisms depends on the nature and quantity of the active ingredients it contains as well as the mode of extraction. *Aspergillus sp* largely exist as saprophytes (obtain their nutritional needs from dead and decaying leaves, fruits, vegetables). They are ubiquitous causing a wide range diseases. *Mucor sp* belongs to the order Mucorales and comprise mainly saprobes, endophytes, parasite of plants and human pathogens causing mucormycosis [24]. *Penicillium sp* are wide spread attacking various fruits and vegetables especially during storage and often producing a variety of mycotoxins. Harmful mycotoxins and carcinogenic compounds such as citrinine, patulin, penicilic acid and other secondary metabolites are produced by *Penicillium sp* [25]. *Staphylococcus sp* is a gram positive bacteria that colonizes 30% of healthy individuals from different body parts [26]. Its susceptibility to the plant extract is a huge relief since it plays a significant role in causing infections ranging between simple to life threatening infections [27]. *Escherichia coli* was found susceptible to the plant extract. It has been reported to cause a serious food poisoning which can be transmitted to humans primarily through consumption of contaminated foods such as contaminated raw vegetables and sprouts [28]. According to Chaudhuri and Henderson, [29], *Escherichia coli* has been associated with a large number of infections in healthy and immunocompromised persons (diarrhea, pneumonia, urinary tract infections, wound infections, sepsis and meningitis). *Pseudomonas*

sp is a ubiquitous gram negative bacterium belonging to the family Pseudomonadaceae and is able to survive in a wide range of environments [30], is the highest resistant microorganism among the four bacteria Isolated. Its high resistance to the garlic extract could be its restricted outer membrane permeability, efflux systems that pump antibiotics out of the cell, production of antibiotics inactivating enzymes such as B lactamases, horizontal transfer of resistance genes or mutational changes [31]. Its resistance is a problem to the world as it has been reported to be associated with nosocomial infections and ventilator associated pneumonia [32].

5. CONCLUSION

Despite tomato being fruit it is generally prepared and eaten like a vegetable. At the end of this study, it was observed that there are microbial organisms that causes postharvest diseases on tomatoes sold in Awka. The consequences of microbial contamination and growth of these pathogens on tomatoes causes spoilage, mitigate sensory appeal and lead to great economic losses and wastage of products. The study revealed that garlic possesses antimicrobial properties since it was able to suppress the growth of tested microbial isolates. Therefore there is need for further improvements and research on the plant extracts for effective control of microbial isolates. Also garlic can be used by pharmaceutical industries and manufacturing industries in production of drugs against tested isolates and in developing plant based biofungicides and bio -bactericide for the management of tomato diseases since the plants are always available, cheap and environmental friendly with lower risk of hazardous residues.

6. RECOMMENDATION

1. Farmer should employ control measures at every point in postharvest chain to mitigate contamination.
2. Government should sponsor research scientist in this discipline for isolation, extraction and purification of the active compounds in the medicinal plants that could be used in producing drug against several diseases.
3. More research should be carried out to find plant alternatives for *Pseudomonas sp* and *Penicillium sp* other than chemicals and higher concentrations of plant extract should be used which might serve as a perfect substitute of chemicals used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Arbrar S, Abera D, Simegne K, Ali M. Effect of storage conditions and packing materials on shelf life of tomato. Food Sci Qual Manag. 2016;56:2224-6088.
2. Hosea ZY, Liamngee K, Owoicho AL, David T. Effect of Neem leaf powder on postharvest shelf life and quality of tomato fruits in storage. International Journal of Development and Sustainability. 2017; 6(10):1334-49.
3. Mohammad A, Abdullahi A, Nasiru AM. Effects of neem leaves and garlic bulbs on fungi causing post-harvest spoilage of tomatoes in Sokoto North Western Nigeria. J Agric Vet Sci. 2021;14(16):8-14.
4. Eno-Abasi S, Gbenga A, Joke M, Abdulganiu A. A long way to self-sufficient in tomato production. Sunday Mag. 2018; 50:7-8.
5. Gustavsson J, Cederberg C, Sonesson U, Vanotterdijk R, Meybeck A. Global food losses and food waste- Extent, Causes and Prevention; 2011.
   Available:https://scholar.google.com/scholar?q=Gustavsson+J.+Cederberg+C.+Sonesson+U.+van+Otterdijk+R.+Meybeck+A.+Global+Food+Losses+and+Food+Waste%E2%80%93+Extent,+Causes+and+Prevention+2011+Food+and+Agriculture+Organization+of+the+United+Nations+(FAO)+Rome,+Italy
   Accessed 2nd January 2022.
6. Irokaanulo EO, Egbezien IL, Owa SO. Uses of Moringa oleifera in the preservation of fresh tomatoes. J Agric Vet Sci. 2015;8(2):127-32.
7. Enyiukwu DN, Awurum AN, Nwaneri JA. Efficacy of plant derived pesticides in the control of myco induced postharvest rots of tubers and agricultural products. Net J Agric Sci. 2014;2(1):30-46.
8. Neeta NP, Gol B, Ramana R. Effect of post-harvest treatment on physicochemical characteristic of and shelf life of tomato fruits during storage. Am Eurasian J Agric Environ Sci. 2010;9:470-9.
9. Agwaranze DI, Kalu AC, Sabo IA, Aso RE, Okachi MA. Mycologicalassessment of deteriorated tomato fruits sold in Wukari, Nigeria. J Biotechnol Res. 2020;6(7):84-9.

10. Akinyele BJ, Akinkunmi CO. Fungi associated with the spoilage of berry and their reaction to magnetic field, Microbiology. Biotechnol Food Sci. 2012; 2(2):701-12.
11. Nicosia D, Pangallo S, Raphael G, Pomeo F, Strano M, Rapisard P, Droby S, Schena L. Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract. Postharvest Biology and Technology. 2016;114:54-61.
12. James CJ, Natalie S. Microbiology. A Laboratory Manual. 2013;43.
13. Abasiubong G, V, Amadi C. Ikon. Adv Microbiol. Antimicrobial activity of garlic extract On organisms isolated from tomato rot. 2017;7(2):1-10.
14. Wogu M, Ofuase O. Microorganisms responsible for the spoilage of tomato fruits sold in markets in Benin City Southern Nigeria. Sch Acad J Biosci. 2014;2(7): 459-66.
15. Ogundipe FO, Bamidele FA, Adebayo-Oyetoro AO, Ogundipe OO, Tajudeen OK. Incidence of bacteria with potencial pubic health impications on raw Tomato sold in Lagos State, Nigeria. Niger Food J. 2012; 30(2):106-13.
16. Liamgee K, Iheanacho A, Aloho K. Isolation, identification and pathogenicity of fungi causing postharvest spoilage of tomato fruits during storage. Annu Res Rev Biol. 2018;26(6):1-7.
17. Mohammad D, Kuhiyep C. Bacteria and fungi co-biodeterioration of selected fresh tomatoes sold within Kaduna. Sci World J. 2020;15(1):48 55.
18. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H et al. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). Foods. 2019;8(7):246.
19. Mugao LG. In-vitro activity of selected plant extract on post-harvest pathogens causing tomato fruit rot. J Phytopharmacol. 2021;10(4):236-41.
20. Tijjani A, Adebitan S, Gurama A, Aliyul M, Haruna S, Mohammad G et al. In vitro and in vivo efficacy of some plant extracts for the control of tomato fruit rot caused by Aspergillus flavus; 2014.
21. Ashraf Z, Ali Q, Rashid M, Malik A. Antibacterial, antifungi and anticancer activities of multistress affected *Allium sativum* plant extracts. Plant Cell Biotechnol Mol Biol. 2020;21:55 13 22.
22. Saravanasingh K, Frdrik G, Ramamurthy M. A study on antibacteria and antifungal activities of extract of medicinal plant Aegle marmelos. Int J Adv Res Biol Sci. 2016; 3(2):321-8.
23. Ben Senhaji B, H, Salghi R. Asteriscus imbricatus extract: antifungal activity and anticorrosion inhibition. Int J Electrochem Sci. 2013;8:6033-46.
24. Wagner L, Stielow JB, de Hoog GS, Bensch K, Schwartze VU, Voigt K et al. A new species concept for the clinically relevant Mucor circinelloides complex. Persoonia. 2020;44:67-97.
25. Abramson D, Lombaert G, Clear RM, Sholberg P, Trelka R, Rosin E. Production of patulin and citrinin by Penicillium expansum from British Columbia (Canada) apples. Mycotoxin Res. 2009;25(2): 85-8.
26. Oliveira D, Borges A, Simões M. Staphylococcus toxins and their molecular activity in infectious diseases. Toxins. 2018;10(6):30-8.
27. Bitrus A, Peter O, Abbas M, Goni M. Staphylococcus: A review of antimicrobial resistance mechanisms. Veterinary sciences research and reviews. 2018;4:2-10.
28. World Health Organization. Escherichia coli; 2018
[Cited Feb 2, 2022].
Available: https://www.who.int/news-room/fact-sheets/detail/e-coli.
29. Chaudhuri RR, Henderson IR. The evolution of E. coli phylogeny. Infect Genet Evol. 2012;12(2):214-26.
30. Silby MW, Winstanley C, Godfrey SA, Levy SB, Jackson RW. Pseudomonas genomes: diverse and adaptable. FEMS Microbiol Rev. 2011;35(4):652-80.
31. Breidenstein EB, de la Fuente-Núñez C, Hancock RE. Pseudomonas: all roads lead to resistance. Trends Microbiol. 2011; 19(8):419-26.
32. Barbier F, Andremont A, Wolff M, Bouadma L. Hospital acquired pneumonia and ventilator associated pneumonia, recent advances in epidemiology and management. Curr Opin Pulm Med. 2013; 19(3):216-28.

APPENDIX

Table 1a. Nature of bacterial growth in nutrient agar media for tomatoes samples

Sample	Nature of growth
Eke Awka Sample + NA	Moderate growth
Temp site Sample + NA	Moderate growth
Amenyi market Sample + NA	Heavy growth

Table 2a. Morphological and biochemical characteristics bacteria isolates

Parameters	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Colony Characterization	**Milky circular with flat elevation**	**Whitish irregular shape with flat elevation**	**Yellowish irregular shape with flat elevation**	**Milky irregular shape with flat elevation**
Cell characterization	Short rods in singles	Rods in clusters	Cocci in clusters	Cocci in clusters
Gram's Test	Positive	Negative	Positive	Negative
Motility Test	Negative	Positive	Positive	Positive
Catalase	Positive	Negative	Positive	Positive
Coagulase	Negative	Negative	Positive	Positive
Citrate	Negative	Positive	Negative	Positive
Indole	Positive	Negative	Negative	Positive
Oxidase	Negative	Positive	Negative	Positive
Urease	Negative	Positive	Negative	Positive
Probable organism	***Bacillus spp***	***E- coli***	***Staphylococcus spp***	***Pseudomonas spp***

Table 3a. Nature of fungal growth in SDA for onion samples

Sample	Nature of growth
Eke awka Sample + SDA	Heavy growth
Temp site Sample + SDA	Heavy growth
Amenyi market Sample + SDA	Heavy growth

Table 4a. Identification of Fungi

Isolate code	Description	Probable identity
SD1	They are typically powdery black, Conidiophores arising from long, broad, thick-walled, sometimes branched foot cell, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed.	*Aspergillus sp*
SD2	Colonies are whitish to olivaceous-buff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipsoidal, pyriform or subgloblose. Chlamydospores absent.	*Mucor sp.*
SD3	Colonies are fast growing conidiophores in fresh isolate typically loosely synematous, giving the colony a zonate appearance. Colonies are light green, reversed colourless, yellow-brown conidiophores usually smooth walled, pencilli 2-3 staged branched with numerous usually oppressed mutulae, conidia sub-globose to ellipsoidal smooth-walled,.	*Penicillium sp.*

Table 5a. Zone of inhibition

Extract	*Staphylococcus* sp.	*Escherichia coli*	*Bacillus sp*	*Pseudomonas spp*	*Aspergillus sp*	*Penicillium sp*	*Mucor spp*
Garlic 100%*(mm)*	19.00	17.00	9.00	0.000	18.40	0.000	18.13
	21.00	17.70	9.00	0.000	18.80	0.000	18.00
	18.50	16.30	9.40	0.000	18.00	0.000	18.300
Garlic 50%*(mm)*	10.00	7.50	0.000	0.000	13.67	0.000	12.20
	10.00	7.50	0.000	0.000	13.60	0.000	12.50
	10.00	7.50	0.000	0.000	13.72	0.000	12.00
Garlic 25%*(mm)*	0.000	5.00	0.000	0.000	12.00	0.000	9.86
	0.000	6.00	0.000	0.000	12.00	0.000	9.88
	0.000	4.00	0.000	0.000	12.00	0.000	9.85
Antibiotics 30µg/ml	34.83	34.83	19.16	34.83	22.33	34.00	41.60
	34.80	5.00	19.20	34.00	22.30	36.00	41.60
	34.88	33.80	19.15	34.50	22.36	30.00	41.60

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