Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

ISOLATION OF MICROORGANISMS IN SPOLAGE OF HARVESTED TOMATO FRUITS AND ANTIBIOTIC SENSITIVITY

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ABSTRACT

The purpose of this investigation was to study the microorganism associated with the spoilage of fresh fruits of tomato, *Lycopersicum esculentum* obtained from Tenkasi, kadayanallur, in Tamilnadu, India. A total of some species of bacteria isolated and identified by the different morphological identification of organism by Gram staining technique and Hanging drop technique. Biochemical tests such as Catalase test, Coagulase test, Indole test and Suger fermentation test was characterized to find out the enzymes which are released by the above microorganisms. The antibiotics sensitivity nature of the above microorganisms was done by Disc diffusion method by using narrow and broad spectrum standards. The above identified microorganisms has highest resistance and less sensitivity towards mentioned antibiotics standards. It gives the intimations for us about the consumption of spoiled tomato fruits.

KEYWORDS

Tomato Fruit, Microscopic method, Biochemical method, Antibiotics sensitivity and Disc diffusion method.

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INTRODUCTION

Tomato, *Lycopersicum esculentum*, is annual plant, is a common vegetable eaten raw as salad or for garnishing various cooked food in India as well as in many parts of the world. The fruit contains high amount of carbohydrates, fat, organic acids, water, minerals, vitamin and pigments[1-4]. It is estimated that ripe tomato fruits contain approximately 94% water, 4.3% carbohydrates, 1% protein, 0.1% fat, 0.6% fiber and vitamins. It is rich in vitamins including vitamin A and vitamin C, carbohydrates, proteins, fats, fibres and potassium. The consumption of tomatoes throughout the world is believed to benefit the heart and other organs. It is

rich in lycopene which has many beneficial health effects[5-7]. The richest source of lycopene is tomato and tomato-based products Lycopene has been found to prevent prostate cancer, improve the skin's ability to protect itself against the harmful ultra violet rays, decrease the risk of breast, lung, stomach, bladder, uterine, head and neck cancers, protect against neurodegenerative diseases, lower urinary tract infections and reduce the cardiovascular risk associated with type 2 diabetes[8-10].

The nutrients support the growth of microorganisms such as neutral media, which product enzymes that degrade the nutrients. Tomato fruit contain a lot of water which makes them more susceptible to spoilage by microorganisms[11-14]. The average fungal count range between 1.3 x 10^3 and 2.0 x 10^3cfu/ml, the fungal isolates were *aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oysporum*, *Saccharomyces cereviae, Alternaria alternate, Penicillium digitatum* and *Geotrichum candidum* were found[15-18]. Also the high water content makes storage and transportation of this vegetable difficult. The microorganisms reduce not only the nutritional value but also the market value of tomato fruits[19-23].

A total of nine species of bacteria isolated and identified were: *B. cereus, B. aureus, Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis* and *Staphylococcus aureus: Pathgenic Bacteria Pseudomonas fluorescens, micrococcus varians* (*Listeria monocytogenus, Micrococcus varians*) were isolated[24,25] on the 1st day and 2nd day in the sample. *Lactobacillus fermenti* came in the second day. One hundred and fifty tomato fruit samples indifferent stages of spoilage from three different markets showed of sixteen bacteria and eleven fungi including yeasts were associated. Further morphological studies were done to know the fungal member responsible for the spoilage. Among the fungi, it was found the Aspergillus niger and Fusarium were found in most of the spoiled samples with a few samples containing Penicillium[26,27]. Our study is morphological identification of organism followed by the antibiotics sensitivity nature of the microorganisms was performed by disc diffusion method by using standard antibiotics.

MATERIAL AND METHODS

Collection of Samples

All samples of tomato fruits were collected from Kadayanallur, Tenkasi in Harvested land. The ripened tomato fruits selected were fresh, undamaged, firm and healthy. The samples were taken to the laboratory in sterial pack and washed and drained of water. The fruit samples were kept free from dust and insects at room temperature for up to 14 days to undergo a natural process of spoilage before being used in this study.

Isolation of microorganisms

The fruit samples were ground using a sterile mortar and pestle. A homogenate of each sample was made by blending one gram in 9ml of sterile water and shaking them together. Serial dilutions of up to 10^4 of the homogenate was made in sterile test tubes. 1ml of the serially diluted tomato sample was pipette. Into each serially marked petri dish. The total microbial count was carried out on the spoiled tomato fruit samples using the pour plate method. In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette Nutrient agar is used for bacteria. The plates were subsequently incubated at 37^0C for 24 hours for bacteria at the end of incubation, developed colonies were counted and colonies forming units per unit gram of tomato fruit. Sample were calculated and recorded.

Characterization and Identification of Isolates

Discrete colonies that developed after incubation, were subcultured to obtain pure cultures which were stored at 4^0C and used subsequently for microscopic characterization and biochemical analyses. The distinct colonies that developed in the pure culture plates were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, colour and transparency. The isolates were further characterized and identified following biochemical procedures as described by these included catalase, coagulase, indole and sugar fermentation tests.

CHARACTERISATION OF ORGANISM: MICROSCOPIC METHOD

Gram Staining Technique

Preparation of the Smear

The first consideration is the correct preparation of the smear. Make a thin film of the material on a clean glass slide, using a sterile loop or swab for viscous specimens. Air dry, then heat fix the slide by passing it several times through a flame (the slide should not become too hot to touch). Failure to follow these directions may cause staining. To be visible on a slide, organisms that stain by the Gram method must be present in concentrations of a minimum of10^4 to 10^5 organisms/ml of unconcentrated staining fluid. At lower concentrations, the Gram stain of a clinical specimen seldom reveals organisms even if the culture is positive. Smears that are not properly fixed tend to be washed away during staining and washing resulting in the absence of stained bacteria. In special situations, the following guidelines may be helpful: When cerebrospinal fluid contains only a few organisms, they are more likely to be found if a concentrated "thick smear" is examined. To prepare a "thick smear" the specimen is spun at high speed and a large drop of sediment (or multiple drops, drying in between each drop) is placed in the center of the slide and allowed to air dry. The cytocentrifuge may prove to be useful in concentrating bacteria as well as in preserving cell morphology. When fluid specimens such as urine or CSF seem to vanish after the staining procedure, a wax mark, placed near the smeared area on the slide (same side) after the staining procedure (to avoid introducing wax artifacts) will reduce frustration in locating the specimen under the microscope. The wax mark can be used for quick focussing. In a grossly bloody specimen, it may prove difficult to distinguish microorganisms from artifacts. After air-drying and heat-fixing this type of specimen, the added preparatory step of covering it with distilled water, waiting five minutes, and then rinsing, may cause the red blood cells to lyse and float off.

Gram Staining Procedure

The difference between Gram-positive and Gram-negative bacteria is in the permeability of the cell wall to these "purple colored iodine-dye complexes" when treated with the decolorizing solvent. While Gram-positive bacteria retain purple iodine-dye complexes after the treatment with the decolorizing agent, Gram-negative bacteria do not retain complexes when decolorized. Flood slide with crystal (or gentian) violet- 60 seconds. Flood with Gram's iodine - 180 seconds. Carefully decolorize with 95% ethanol until thinnest parts of the smear are colorless. (Wash with water).This third step is the most critical and also the one most affected by technical variations in timing and reagents. Flood with safranin (pink color) (10% Fuchsine) - 60 seconds. (Wash with water).Air dry, or blot with absorbent paper.

Preparation of a Hanging Drop Slide

A hanging drop slide allows you to view live bacteria under microscope. This method allows organisms to maintain their natural shape and makes it possible to observe their behavior, but keeps the organisms well contained. If an organism is motile, its activity is clearly apparent, and directional, place-to-place locomotion will be observed. Non-motile organisms can exhibit Brownian Movement, a vibration or oscillation caused by the action of water molecules striking the microorganism; this must be distinguished from true locomotion.

Place a coverslip on the desk and with a toothpick place a small dot of Vaseline in each corner of the coverslip. Sterilize an inoculating loop and obtain a loopful of the broth culture being used. Transfer a loopful of culture to the center of the coverslip. Resterilize the inoculating loop. Obtain a depression slide, invert it and gently press it against the coverslip so that the depression is over the drop of culture. Invert the slide so that the drop may hang free from the coverslip into the space of the depression. Microscopically examine the preparation. After completion of observation, place the entire slide into the tray of disinfectant provided on the front table. Because these slides contain living organisms they must be autoclaved (steam and pressure sterilized) before being cleaned.

BIOCHEMICAL METHODS

Catalase Test

Tube Method

Pour 1-2 ml of hydrogen peroxide solution into a test tube. Using a sterile wooden stick or a glass rod, take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution. Observe for immediate bubbling.

COAGULASE TEST

Tube Test (to detect free coagulase)

Dilute the plasma 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline). Take 3 small test tubes and label as T (Test), P (Positive Control) and N (Negative Control). Test is 18-24 hour broth culture, Positive control is 18-24 hr S. aureus broth culture and Negative control is sterile broth. Pipette 0.5 ml of the diluted plasma into each tube. Add 5 drops (0.1 ml) of the Test organisms to the tube labelled "T", 5 drops of S. aureus culture to the tube labelled "P" and 5 drops of sterile broth to the tube labelled "N". After mixing, incubate the three tubes at 35-37 Degree Celsius. Examine for clotting after 1 hours. If no clotting has occurred, examine at 30 minutes intervals for up to 6 hours.

Indole Test

Take a sterilized test tubes containing 4 ml of tryptophan broth. Inoculate the tube aseptically by taking the growth from 18 to 24 hrs culture. Incubate the tube at 37°C for 24-28 hours. Add 0.5 ml of Kovac's reagent to the broth culture. Observe for the presence or absence of ring.

Sugar Fermentation Test

Take three different tubes containing three different types of sugar broth and invert a Durham's tube in it and screw cap the tubes. Further the three sugar broth tubes are sterilized by autoclaving. After sterilization, the tubes are cooled down to room temperature and inoculated with a cell suspension in aseptic condition. The tubes are inoculated at $37^{O}C$ for 24 hours. After incubation, the tubes are examined for acid and gas production and results are noted down.

ANTIBIOTIC SENSITIVITY TEST

Disc Diffusion Method

The standardized disc diffusion method and the zone size interpretation chart are used for the determination of the bacterial sensitivity to the various antibiotics selected. The following commercially prepared paper discs impregnated with the various antibiotics were assessed against the isolates: gentamycin (10µg/ml), streptomycin (10µg/ml), septrin (30µg/ml), chloramphenicol (30µg/ml), ciproflaxacin (10µg/ml), amoxycilin (30µg/ml), augumentin (10µg/ml), ampiclox (30µg/ml), erythromycin (10µg/ml) and ampicilin (30µg/ml). Each inoculum of the bacterial isolates was grown in separate tubes at 370C in Mueller-Hilton broth (agar plates) for 18 hours, with shaking and subsequently diluted to an optical density of 0.1 (0.5 McFarland standard) and stored at 40C. The paper discs were gently but firmly placed on the inoculated plates using sterile forceps. The plates were incubated at 370C for 24hours after which zones of inhibition are measured and interpreted according to Results obtained and shown in table.

RESULTS AND DISCUSSION

This Study is related to Spoilage of tomato fruits on after hervesting and suring handling,storage condition distrbution Marketing Practice and Microorganism can able to intect the tomato, here we are study and concentrate about the Bactrial. Microorganism so to identify the bacteria which inact the spoilt tomato fruits we have todo the different works such as cultural morpological and biochemical characteristic and antibiotic sensitivity test the result are shown in following pages in a different following tables.

There are number of isolate are obtained from the colonies which was mde under sterial condition. The each and every colony has to be identified in various process and their reports are noted. The culture characteristic may be the margin,colour,shape of the different species are shown in Table No.1. The morphological means identification of gram's nature and the mobility of organism has to be noted in Table No.2. The biochemical reaction such as catalase, and sugar fermentation test are allso worked out in a sutable

proceture noted above. The reports of each test is shown in Table No.3.

So Based on the above cultural, morphological and biochemical characters of the identified microorganism we were assumed the identified organism are various species of bacterial organisms. These are may be due to our assumption. *Bacillus subtilis, Bacillus cereus,Bacillus aureus, Escherichia coli, Saphylococcus aureus, Klebsiella aerogens, Pseudomonas aeruginosa, Salmonella thyphi, Proteus mirabilis*.

The sensitivity Pattern of bacterial isolates to different antibiotics and there concentration such as gentamycin (10µg/ml), streptomycin (10µg/ml), septrin (30µg/ml), chloramphenicol (30µg/ml), ciproflaxacin (10µg/ml), amoxycilin (30µg/ml), augumentin (10µg/ml), ampiclox (30µg/ml), erythromycin (10µg/ml) and ampicilin (30µg/ml) are shown in Table No.3.

As in final assumption we have concluded the number of species of bacteria can make the detoriotraction on the hardested tomato fruits. And based on the sensitivity test we have to concluded the organism like *bacillus subtilius* recorded the highest sensitivity to all the antibiotics and had no resistance to any of the antibiotic species with the exception of bacterial sensitivity *psudomonas aueruginosa* and *salmonella typhi* has the highest and of the resistance Eight all the antibiotic used resistance to the used antibiotics. The presence of bacterial isolate with multiple antibiotic resistant in the spoiled tomato fruit samples. Product the highest the risk intimation to mate the effective treatment against the infectious disease in the sunsuption of such fruits.

Table No.1: Description of Isolates

CHARACTERISTICS	**Description of Isolates**								
CULTURAL									
Margin	Smooth	Smooth	Smooth	Entire	Smooth	Entire	Entire	Smooth	Entire
Color	White	White	White	Pink	Yellow	White	Creamy	Creamy	White
Shape	Small and irregular	Small	Small	Small	Mediu m	Large	Large	Medium	Large

Table No.2: Morphological

Morphological									
Cell type	Rod	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single	Cluster	Single	Single	Single	Single
GRAM REACTIONS	+	+	+	–	+	–	–	–	–
MOTILITY TEST	+	+	+	–	–	+	+	–	+
SUGAR FERMENTATION TEST									
Glucose	A	A	A	AG	A	AG	A	A	A
Lactose	–	–	–	+	+	+	–	–	–

Table No.3: Bio Chemical Test

BIO CHEMICAL TEST									
Coagulase	–	–	–	–	+	–	–	–	–
Catalase	+	+	+	+	+	+	+	–	+
Oxidase	–	–	–	–	–	–	+	–	–

Indole	_	_	_	_	_	_	_	_	_
Probable Microorganism	Bacillus Subrilis	B.cereus	B.aureus	Escherichia coli	Staphylococcus aureus	Klebsiella aurogenes	Pseudomonas aeruginosa	Salmonella typhi	Proteus mirabilis

	ANTIBIOTICS										
BACTERIAL ISOLATES	GE	ST	SE	CH	CP	AY	AU	AX	AN	TOTAL	
	Disc diffusion method results variables									S No(%)	R No(%)
Bacillus subtilis	SSSS	SSSS	SSSS	SSSS	SSSS	SSSS	SSSS	SSSS	SSSS	36(100)	0%
B.cereus	RRSS	RSSS	SRSR	RSSS	RSRS	SSSR	RSSR	SSRR	RRSR	19(53)	17(47)
Escherichia coli	SSSSR	RSSR	SSSS	SRRS	SSSS	SSRR	RRSS	SSRR	SSSS	27(75)	9(25)
Staphylococcus aureus	SSSS	SSRR	RRRS	RSSR	SSSS	SSRR	RSSR	RSRS	RSSR	21(58)	15(42)
Klebsiella aerogenes	SRRR	RSRS	SSRR	RSSR	SSSS	SRRS	SSRR	RRSR	SRSR	18(50)	18(56)
Pseudomonas aeruginosa	SRRR	SRRR	RSRR	RSSR	RRSR	RRRR	SRRS	RRRS	RRSR	9(25)	27(75)
Salmonella typhi	RRRR	RSRR	RRRS	RRSR	RRRS	RSRS	RSRR	RRSR	SRRR	9(25)	27(75)
Proteus mirabilis	SSSR	SSSS	SSSR	RSSS	SSSR	RRRS	SRRR	RSSS	RSRR	23(6)	14(39)
B.aureus	SSSR	RSRR	SRRR	RRSS	RSSR	SSRR	RSSR	RSSR	SRRR	16(44)	20(56)

Antibiotics: GE=Gentamycin ST=streptomycin SE=septin CH=chlorophenicol CP=Ciprofloxacin AM=Amoxicillin AU=Augumentin AX=ampiclox AN=ampicilin
Test results: S=sensitivity R=resistant

Figure No.1: Catalase test- positive

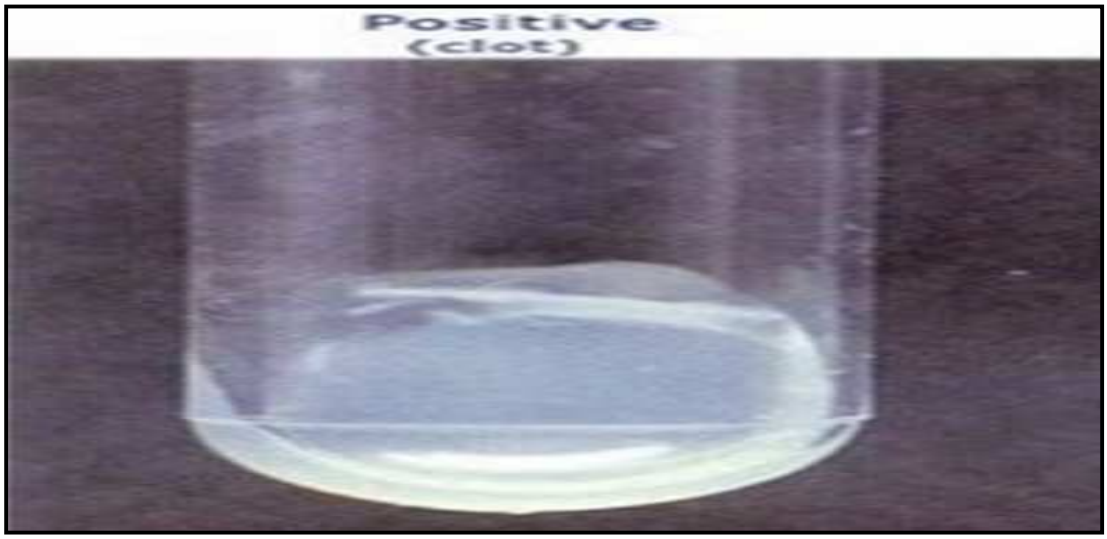

Figure No.2: Coagulation test

CONCLUSION

Several genera of bacteria have been identified in the study as being associated with the spoilage of tomato fruit. Therefore concerned effort people those are involved in production and tomato fruits and should be made by the relevant health workers to discourage or stop the display and sales of tomato fruit in local markets. The general public should also be enlightenet about the health risks that may be associated with the conception of relatively cheaper but spoilt ripe tomato fruits, as these could be agent in food borne diseases.

ACKNOWLEDGEMENT

The author wish to express their sincere gratitude to Sri Ram Nallamani Yadava College of Pharmacy, Kodikurichi, Tenkasi, Tirunelveli, Tamilnadu, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Maddox D A. Implications of new technologies for seed health testing and the worldwide movement of seed, *Seed Science Research,* 8(2), 1998, 277-284.
2. Butler E E. Pathogenicity and taxonomy of *Geotrichum candidum*, *Phytopathology,* 50(9), 1992, 665-672.
3. Wokoma E C W. Preliminary report on disease of tomatoes in Choba, *Rivers State. J, Appl. Sci.Environ.,* 12(3), 2008, 178-121.
4. Eni A O, Ibokunoluwa O, Oranusi U. Microbial quality of fruits and vegetables, *African Journal of Food Science,* 4(5), 2010, 291-296.
5. Holt J G, Krieg N R, Sneath P H A, Stanley J T, Williams S T. Bergey's Manual of Determinative Bacteriology, *Williams and Wilkins, Baltimore,* 9th Edition, 1994, 787.
6. Bauer A W, Kirby W M, Strerris J C, Turk M. Antibiotic susceptibility testing by a standard single disk method, *American Journal of Clinical Pathology,* 45(4), 1999, 493-496.
7. Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testing, Wayne, PA. USA, 4(1), 2012.
8. Beuchat L R. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables, *Microb. Infect,* 4(4), 2002, 413-423.
9. Goldoni J S, Cavestre R C, Kurozawa C, Roca R C, Bonassi I A. Occurrence of spoilage bacteria ontomato fruit

(*Lycopersicum esculentum* Mill), *Acta Hort (ISHS),* 301(36), 1992, 285-292.

10. Ibrahim A D, Musa K, Sani A, Aliero AA, Yusuf B S, Microorganisms associated with the production of volatile compounds in spoilt tomatoes, *Research in Biotechology,* 2(2), 2011, 82-89.
11. Jushi A P, Patel S P. Microbiological analysis of fresh fruits and vegetables and effects of antimicrobial agent on microbial load, *Journal of Food Protection,* 14, 2008, 23-24.
12. Ghosh A. Identification of microorganisms responsible for spoilage of tomato (*Lycopersicum esculentum*) fruit, *Journal of Phytology,* 1(6), 2009, 414-416.
13. Akinmusire O O. Fungal species associated with spoilage of some edible fruits in Maiduguri, northeastern Nigeria, *Adv. Environ. Biol.,* 5(1), 2011, 157-161.
14. Al-Hindi R R, Al-Najada A R, Mohamed S A. Isolation and identification of some fruit spoilage fungi: screening of plant cell wall degrading enzymes, *African Journal of Microbial Research,* 5(4), 2011, 443-448.
15. Dennis C, Davis R F. Fungal spoilage of fresh tomatoes, *Biennial Report of Field Research Institute,* 78, 1980, 44-45.
16. Hanashiro A, Morita M, Matte G R, Matte M H and Torres E A F S. Micropil quality of selected street foods from a restricted area of sao Paulo city, *Brazin. Food cont,* 16(5), 2005, 439-444.
17. Tripathy S N. Food Biotechnology, *Dominant Pulblishers and Distributors, New Delhi,* 2004.
18. Aneja K R. Experiments in Microbiology, Plant Pathology and tissue culture, *Vishwa Prakashan, New Delhi, India,* 1993, 471.
19. Deshmukh A M. IN handbook of Media, Stains and reagents in Microbiology, *PAMA Publications. New Delhi, India.*1997.
20. Sambrook and Russell. Molecular Clonning: A Laboratory Manual, *Cold Spring Harbor Laboratory Press,* 3rd Edition, 2001.
21. Janerthanan S, Vincent S. Practical biotechnology: Method and Protocols, *Universities Press (India) Private Limited, Hyderabad, India,* 2007.
22. Nei M. Estimation of average hererozygosity and distance from small number of individuals, *Journal of Genetics,* 89(3), 1978, 583-590.
23. Naravaneni R and Jamil K. Rapid detection of food born Pathogens by using molecule techniques, *J Med Microbiol,* 54(Pt 1), 2005, 51-4.
24. Brackett E, Robert. Change in the Microflora of Packed Fresh Tomatoes, *J.Food Qual,* 11(2), 1988, 89-105.
25. Williams J G K. DNA polymorphisms amblified by arbrtrary primers are useful as genetic markers, *Nucl.Acid.Res,* 18(22), 1990, 6531-6535.
26. Brackett E, Robert. Change in the Microflora of Packed Fresh Tomatoes, *J. Food Qual.,* 11(2), 1988, 89-105.
27. Williams J G K. DNA polymorphisms amblified by arbrtrary primers are useful as genetic markers, *Nucl.Acid.Res,* 18(22), 1990, 6531-6535.

Please cite this article in press as: Seenikani K *et al.* Isolation of microorganisms in spolage of harvested tomato fruits and antibiotic sensitivity*, Asian Journal of Research in Pharmaceutical Sciences and Biotechnology,* 6(3), 2018, 56-63.