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CONTROL OF POST HARVEST BACTERIAL DISEASES OF TOMATO IN ABIA STATE, NIGERIA

Opara Emma[1], Njoku Theresa[2] And Ogbonna Ucheoma[1]
[1,]*Department Of Plant Health Management, Michael Okpara University Of Agriculture, Umudike, Abia State, Nigeria.*
[2]*department Of Crop Science, Imo State University, Owerri.*
**Corresponding Author.*

----------ABSTRACT----------
A laboratory experiment was conducted at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria to assess the effect of pre-heat treatments and some plant extracts in the control of bacterial soft rot (Erwinia carotovora) and fruit spot (Xanthomonas vesicatoria), the two major post harvest bacterial diseases of tomato fruit (Lycopersicon esculentum) in Nigeria. The design of the experiment was a randomized completely randomized block design (CRD) with 4 replicates. A batch of tomato fruits was subjected to hot water bath at different temperatures and time duration: 50, 60 and 70^{0}C at different time duration; 10, 20 and 30mins with cold water as the control. While another batch was treated with cold and hot water plant extracts consisting of; Azadirachta indica seed, Garcinia kola, Zingiber officinale, Piper guineense seed and Myristica fragrans seed at different concentrations (10, 20, 30%). Data obtained showed that subjecting tomato fruit to a temperature of 50^{0}C for 10mins recorded the longest shelf life (storage period) and was significantly different (P=0.05) from the control experiment. Increase in temperature regime above 50^{0}C to 70^{o}C resulted to decrease in shelf life/storage period. At higher temperatures and longer time duration of 60^{0}C to 70^{0}C for 30mins, the fruits became physiologically unstable accompanied with skin crumpling and cracks with leakage leading to rapid rot and decay of the fruits. Results also showed that all the five plant extracts were effective in inhibiting the growth of bacterial spot pathogen and the soft rot diseases of tomato in vitro (P<0.05) when compared with the untreated control. However, cold water extracts of A. indica, Z. officinale, P. guineense significantly inhibited bacterium growth by 76.26-78.92 % more than the hot water extracts.

***KEYWORDS**: Pre storage treatment, plant extracts, fruit rot.*

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I. INTRODUCTION

1.1.Symptoms on fruits: Bacterial spot incited by X*anthomonas campestris* pv. *vesicatoria* Doidge (Dye) is an important disease of tomatoes grown under warm humid conditions (Opara and Obani, 2009). Symptoms begin as small circular to irregular greasy spots most visible on the surface of the fruits. As these water- soaked region enlarge, coalesce, change from dark green to purplish – gray, accompanied by a distinctive black center. Affected fruits become thin and may crack. The infected regions may be surrounded by a white to yellowish halo. In wet weather, infected parts appear scorched. Sometimes fruit lesions begins as dark raised spots that become brown and sunken in the mature fruit and later give out scabby appearance (Venette, *et al*., 1996). The epidermis of the fruit finally ruptures and curl back from the center of the spot leading to secondary rot infection by *Erwinia carotovora*. The bacterial spot are very seldom deeper than half cm through the fleshy layer of the tomato (Jones *et al.*, 2000).

1.2.Pre-storage Heat Treatments: The use of pesticides in the control of agricultural pests and diseases has brought about some serious problems. These include harmful effect on human healthy, increases in diseases resistance, residues in food, negative effects on non target organism and depletory in the environment.

The desire to reduce the chemical residues in foods, soils and environments has led to alternative control measures. Thus, heat treatment becomes an alternative which may be applied in various ways, which have been strongly recommended for both disinfestations and disease control for fruit borne pathogens, the application of physical heat treatment for this disease control has been recommended by many authors. (Cheah *et al.*, 1991; Tsang and Shintaku, 1998; Olson and Simonne, 2004; Opara and Amadioha, 2008).

The beneficial effects of conditioning fruits with mild heat stress have received much attention and have been found to be more effective and safe for fruits than other methods of fruits preservation in storage (Couey 1989; Aborishade and Oguitimhin, 2005). Williams *et al.,* (1994) also stated further that, gradual heating process of the fruits improved with disinfestations was more successful in maintaining fruit quality, than the surface sterilization by hot water immersion for 12 min at 53^{o}C, and that heat treatment on fruit showed enhanced color development. According to Williams *et al.* (1994) the pre-storage heat treatment reduces pathogenicity of *Penicillium expansum* in apple fruit, which is a main post harvest pathogens of apples. They also noted that heating of apples with inoculated fungus for 98 hours at 38^{o}C completely inhibit disease development. The fruits held for 24 hours at 43^{o}C showed reduced decay after additional 14 days incubation at 20^{o}C, when compared with untreated fruits. Mycelia growth and percentage spore germination were inversely proportional to length of time of exposure to various temperatures.However, heat treatment has not been fully tested on the tomato fruit storage, against bacterial soft rot and bacterial spot as it has been demonstrated on tomato seeds (Opara and Amadioha, 2008). Therefore, this study is aimed at exploring how bacterial post harvest rot can be reduced through wet heat treatment.

1.3.Use of Botanicals*:* The use of plants materials as traditional protectants of both stored products and for field crops is an old practice used all over the world (Golob and Webley, 1980). This tradition has been largely neglected by farmers, after the Second World War, with the advent of synthetic chemicals of petroleum based pesticides. However, the ecological consequences and the increase resistance to pesticides led to a search for new classes of control measures with lower mammalian toxicity and a low persistence in the environment. Recently, the research emphasis has been to find some alternative pesticides of plant origin (Olajede *et al.,* 1993). *Azadirachtin* extracted from *Azadirachta indica* (neem tree) is the most famous compound of this kind (Rembold, 1989 ; Saxena , 1989) Studies on the microbial activity of plant extract have shown the importance of natural chemicals as possible sources of non –phytotoxic systemic and easily biodegradable alternative pesticides (Singh, 1994; Quasem and Abublan, 1996, Amadioha , 1998, 2000, Amadioha and Obi, 1998, 1999) with a view to countering obvious pollution problem in the environment and avoiding the toxic effects of synthetic chemicals on non target organisms , investigation on exploiting pesticides of plant origin are becoming increasingly important in the field of plant pathology (Olajede *et al*., 1993; Amadioha, 2003) pesticides of plant origin are readily available and cost effective in the countries where synthetic pesticides are expensive and difficult to obtain apart from hazard involved in using these chemicals (Synthetic some of them are beyond the reach of resource poor farmers who produce over 98% of the food consumed (Olayide, *et al*., 1980) development of pesticides of plant origin will be cheap and readily available to resources poor farmers (Amadioha, 2004).

However, botanicals have been found to be highly potent in controlling most plants and pests and diseases with no traceable side effect on beneficial organisms and environment although beneficial, Taluker and horse, (1965) reported that insect control agents of plant origin are easily processed. Finney, (1990) stated that insecticide desired from higher plant are desirable because they are safe to the environment, to users and for consumers, broad spectrum in activity and relatively specify in the mode of action, easy to process and use.
With increasing problems of toxicity and resistance of target organism to the synthetic compounds currently in use, interest in natural pesticides or botanicals has been reviewed. Botanicals for pest and diseases control provide environmentally friendly method of control through the naturally curing substances in plants or application of compounds derived from plant (Scott and Jones, 1986). Okonkwo (2001) reports that the most promising species for consideration as potential grain protectants belongs to the generae: *Citrus, Dennettia, Ocimum, Capsicum frutescens*, *Azadirachta indica*, *Garcinia kola* and *Piper guineense* In the tropics, large numbers of these plants species have properties, which are traditionally were used as human medicine and other medicinal uses.

II. MATERIALS AND METHODS

2.1.Pre-Storage Heat Treatment:

This experiment was conducted in the laboratory of Michael Opkara University, Umudike, in which sixty freshly harvested and ripe tomato fruits were subjected to a hot water bath heat treatment at five different temperature regimes as follows: 30, 40, 50, 60 and 70^{o}C and at three different time duration; 10, 20 and 30 minutes. The tomato fruits variety used were Roma f and each treatment was replicated four times and the heating was done using a thermo hot-plate regulator with the fruits immersed in a water bath container when the water bath has attained the required temperature. After the heat treatment the fruits were allowed to dry and cool down to normal room temperature (28^{o}C$\pm$2 ^{o}C), stored on the shelf and observed for firmness or rottenness on weekly intervals for four weeks on the following ratings:

1 = fruit surface firm and intact, no weakness
2 = fruit surface shows signs of weakness but no leakage
3 = fruit surface shows signs of weakness and leakages
4 = fruit surface shows signs of leakages and decaying
5 = fruit surface completely collapsed or rotten

2.2.Isolation of pathogen from diseased fruits:

Some infected tomato fruits were collected from some local farms in Umuahia and its surroundings. Isolation from diseased fruits was carried out by scraping a small piece of infected spot or lesion from the tomato fruit with sterile scalpels after sterilization with alcohol (70% absolute ethanol). The tissue was washed thoroughly three times in sterile water after surface sterilization with 70 % absolute alcohol and allowed to stay for 30 minutes in a drop of sterile water. Before the culturing, the small piece of infected fruit was teased apart with glass rod (Bradbury, 1975).

2.3.Preparation of Culture Medium

The medium for Isolation was nutrients agar (NA) and this was prepared by weighing about 7.5 grams of nutrient agar powder into 250ml conical flask with the aid of a sensitive balance and dissolved in 250ml sterile water, thoroughly shaken and melted over a hot plate followed by autoclaving at 120^{o}C for 15lbs/pressure for 15mins. After it was allowed to cool down to 45-50^{o}C before dispensing 15ml into glass Petri dishes and then allowed to solidify. The culture plates were kept under room temperature to dry after which suspension of the bacterium (*Xanthomonas campestris* pv. *vesicatoria*) was inoculated on the nutrient agar medium and incubated in the laboratory at 28^{o}C for 24hrs.

2.4.Inoculation of the bacterium

The inoculation chamber was mopped, both inside and outside severally with 70% absolute alcohol to prevent contamination. After which the infected fruit portion was cut from the advancing margin, washed thoroughly in three exchanges of sterile water and then been teased apart with sterile needle and allowed to stay for 30minutes before inoculation. A loopful of bacterial suspension was streaked on a prepared nutrient agar in Petri dish and incubated for 24-48 hrs. A pure culture was obtained by sub culturing three or more times.

2.5.Preparation of aqueous plant extracts

The plants extracts used were from neem seed (*Azadirachta indica*) bitter kola seed (*Garcinia kola*), ginger stem (*Zingiber officinale*), black pepper seed (*Piper guineense*) and nut meg (*Myristica fragrans*) while streptomycin sulphate (antibiotic) and sterile water served as checks.The plant materials were first air dried and grinded into powder using a hand grinder. After which 10, 20, 30% concentration were made from the plant extracts by weighing out 10, 20, 30g respectively and first soaked in 100ml of sterile cold and also hot water separately and left for 24hrs. The suspension of each plant extract was filtered using a sieve cloth, 5ml of the extracts was used per 15ml of nutrient agar in a Petri dish while for streptomycin sulphate, and only 1g of powder was used in 100ml of sterile water after which 5ml was also used per 15ml nutrient agar as stated above.

2.6.Inoculation of the pathogen onto the extract culture

A young culture of 24hr old containing single colonies of bacterium (*Xanthomonas campestris* pv. *vesicatoria*) was used in the inoculation. A cork borer disc of 4mm was used to introduce the bacterium inoculum into the center of the medium containing the plant extract in Petri dish and also the control experiments (streptomycin and sterile water), each treatment was replicated four times. This was applied in the three concentrations (10, 20, and 30 %). The inoculated Petri dishes were incubated at a temperature of 28±2^{o}C and allowed to stay for 48hrs (Amadioha, 2002). Hot water was also used as extractant following the same procedure as those described above for cold water extracts. All data collected were statistically analyzed and treatments means separated using least significant difference (LSD) and standard Errors of means at 5% probability according to Gomez and Gomez (2004).

III. RESULTS AND DISCUSSION

3.1.Pre-Storage Heat Treatment

The data obtained showed that out of the five different temperature regimes (30, 40, 50, 60 and 70 ^{o}C) it is only at 50^{o}C for 10mins that recorded the best shelf life during storage after the 4 weeks of the trial and this is significantly different from the other treatments including the control which had the least storage capacity. At temperature of 60^{o}C, heating for 10mins proved better than heating for 20mins or for 30mins. Similarly, heating for 10mins at temperature 70^{o}C proved be damaging as was with heating for 20 or 30mins at 70^{o}C. However the control did not last long at time of storage on the shelf and by the 7th day unlike those heated at different

temperature regime. It was also noted that the disease severity was least at 50^{o}C/10min which was better than at 60^{o}C for 10min. Then at 70^{o}C it was noted that rot occurred at all the 10, 20 and 30min levels of treatments including the control at room temperature.

This work is similar to that reported by Couey (1989) which shows that hot water bath resulted to reduce diseases incidence in fruit during storage. Also Cheah *et al*, (1991) rightly observed that submersion of tomato fruits in water bath allows for a more sufficient and homogenous heating of fruit in control of *Botrytis* fungi. Gracia *et al*., (1995) also applied post harvest heat treatment on Spanish strawberry fruits and discovered that heating strawberry fruit at different storage temperature helped to preserve the fruits for a longer period than when the fruits are stored without any heat treatment or at room temperature. Klein and Lurie (1991) noted that post harvest heat treatment improved fruit quality and helped to prevent fungi and bacterial infections. However, Paul and Chean (1990) applied heat shock response in *Papaya* fruit and noted that heat treatment only increase ripening of *Papaya* followed by fruit rot. In this work however, post harvest heat treatment did not lead to quick fruit rot when compare with the control which did not enhance storage at room temperature during the four weeks of test.

Optimum ripening and storage condition for tomato ranges from 36 to 40^{o}C and relative humidity of 85 to 95% as observed by Sargent *et al*., (2003). Shelf life can be extended and quality retained if tomato is first ripened under the optimum conditions and then held at a reduced temperature of 23^{o}C. Storage at a temperature below 18^{o}C, both in the market place and in the home, is a major contributor to poor tomato fruit quality (Sargent *et al*., 2003). But in this study heating tomato fruit at 50^{o}C/10 minutes before storage recorded the best shelf life temperature than either at 40 ^{o}C and below or at 60^{o}C and above irrespective of the time duration. Sargent *et al*., (2003) also observed that tomato is susceptible to water loss, mainly through the stem scar. Shriveling symptoms may become evident with as little as 3% weight loss; storage in a high relative humidity (85 to 95%) will minimize this weight loss. In their study, they found out that waxing provides some surface lubrication which reduces chafing during handling and transit and that care should be taken to prevent heavy waxing of the stem scar because the wax can also be block gas exchange and interfere with ripening. However in our own study there was no noticeable water loss at normal room temperature (28$\pm$2^{o}C) and 75-85% relative humidity.

Mahovic *et al*., (2004) and Olson (2004) reported that *Rizopus* rot is distinguished from bacterial soft rot by the presence of coarse mold that can be seen by gently pulling apart the diseased tissue, under humid conditions the mold may grow out over the lesions. Mahovic *et al*., noted that high relative humidity (90-95%), such as that found in ripening rooms or in cartons, promote the survival of these bacteria as well as their ability to infect wounds. Also free water on wound surfaces absolutely promotes infection. High fruit temperature above 30 to 35^{o}C as associated with a rapid development of bacterial soft rot, such that the period between inoculation and visible soft rot may be less than 18 hours. This was also observed in this work where rot and decay were rapid at a temperature of 30 and 40^{o}C (control). Therefore heat treatment of tomato fruits at mild temperatures and time duration is aimed at eliminating bacterial soft rot while maintaining the fruit quality and resistance to microbial pathogens even during storage (Bartz. 1982; Williams *et al*., 1996).
From this work results showed that increase in temperature regime from 60^{o}C to 70^{o}C leads to a decrease in storage period and enhance rapid fruit rot and decay. Heating the fruit for 20 to 30 minutes at 60 or 70^{o}C proved not be significant ($P\leq0.05$) in terms of disease control while non treated fruits at these temperatures are more susceptible to fruit rot. In this work no clear difference was found between fruits treated at 50^{o}C for 10 and at 20 or b30 minutes at $P\leq0.05$.

Table 1: Effect of Heat treatment on Incidence of Tomato Fruit Rot

Temp °C	Time(min)	Rep	Mean Dis. severity	Std. Error
30	10	4	5.28	0.99
	20	4	6.25	0.50
	30	4	6.00	1.41
40	10	4	6.70	0.20
	20	4	6.50	0.08
	30	4	6.47	0.41
50	10	4	1.56	0.11
	20	4	2.23	0.25
	30	4	3.23	0.25
60	10	4	2.72	0.38
	20	4	2.70	0.60
	30	4	4.75	0.28
70	10	4	3.67	1.04
	20	4	4.50	1.29
	30	4	5.95	0.61

3.2.Isolated Pathogen

The most frequent bacterium isolated from the sample of tomato fruit spots was consistently *Xanthomonas campestris* pv. v*esicatoria* further test showed that the culture was yellow colonies while from the fruit rot pathogen isolated was *Erwinia carotuvor* (Table2 and Table3).

Table 2: Cultural Characteristics of the pathogen

Tests	Characteristics
Colour and texture of colonies	Muciod, yellow, creamy and pale yellow
Gram staining Test	Gram negative rods
Microscopic Examination	Motile, no spores or capsules
Colony Characteristic and shape	Mucoid and convex

Table 3: Biochemical Characteristics

Tests	Results
Gram reaction	-
Starch hydrolysis	+
Catalase activity	-
Gelatin hydrolysis	+
Oxidase activity	-
Acid Production from:	
Glucose	+
Mannose	+
Arabinose	+

***** + = positive**;** - = negative

3.3.Cold and hot water Extracts:

All the cold plant extracts tested in culture inhibited the growth of the bacterium (*X. campestris* pv. *vesicatoria*) when compared with sterile water (Table 2), especially *A. indica*, *Z. officinale* and *P. guineense* were more effective to the organism than *G. Kola* and *M. fragrans* (P>0.05).

The hot water extracts also inhibited the pathogens, though *A. Indica* and *P. guineense* were more effective than the rest extracts (*Z. officinale*, *M. fragrans* and *G. kola).*All the concentrations of cold-water extracts inhibited the bacterial growth, there was no much significantly different in all the concentration in 10, 20, 30% tested when compared to the control (sterile water). From the result, it can be said that at 20 and 30% concentrations were more effective than the 10% concentration.

Table 4: Effect of Cold Water and Hot Water Plant Extracts on the Pathogen

Extracts	Mean %inhibition (Cold Water Extract)	Mean %inhibition (Hot Water Extract)
A. *Indica*	78.92	76.73
G. *Kola*	74.36	71.86
Z. *officinale*	77.09	61.99
P. g*uineense*	76.29	76.69
M. *fragrans*	72.96	65.60
Streptomycin	99.67)	90.00
Control (water)	6.273	15.00
S.E.	2.03	4.17

The result obtained from this study showed the bactericidal potential of *A. indica* (seed), G. *Kola*, Z. *Officinale*, P. *Guineense*, and M. *Fragrans.* This is in conformity with similar work done by Okonkwo (2001), which reported that the most promising species for consideration as potential grain, protectants belongs to the generae: *Azadrichta* and *Piper*. Also Jacobson (1989) and Al – Abed *et al*., (1993) observed that the most promising botanical pesticides were to be found amongst wild plants including the *Annonaceae*, *Asteraceae*, *Canllaceae*, *Lobiataceae* and *Rutaceae*.

From the results of the study, it shows that all plants extracts (cold or hot water) tested significantly reduced or inhibited the growth of the bacteria when compared with the control both in the cold and hot water extracts. Also the result obtained, showed that the concentrations were all effective against the pathogen's (*Xanthomonas campestris* pv. *vesicatoria*) growth, although 20% and 30% were more effective when compare d with 10%. The potential use of higher plants in controlling diseases has been emphasized (Pandey *et al*., 1982, Renu *et al* 1980), but very little work has been done to investigate natural plant product as bactericides.

This study further showed that the extracts have a highly significant effect on the bacterial spot of tomato, which could lead to increase in yield. Therefore resources poor farmers can go for this plants extract in control bacterial spot disease of tomato instead of using synthetic chemicals in other to improved yield, reduced cost of production, reduced disease incidence and make more profit.

Some all the plant extract, at different concentration inhibited the growth of bacterium, as to compared to streptomycin sulphate a synthetic chemical, it should therefore recommended to poor farmers in replacement of synthetic chemicals, which causes pollution of the ecosystem and some times are harmful to man and animals when used carelessly or indiscriminately.

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