Brazilian Journal of Biological Sciences, 2020, Vol. 7, No. 17, p. 327-337.
ISSN 2358-2731
https://doi.org/10.21472/bjbs(2020)071708

Phytochemical screening and antifungal potency of *Vernonia amygdalina* (bitter leaf) extract against post harvest mycodeterioration of tomato (*Lycopersicum esculentum)*

U. N. Emiri[1] and E. B. Enaregha[2]

[1]Isaac Jasper Boro College of Education. Department of Agricultural Education. Sagbama. Bayelsa State. Nigeria. Email: ucheemiri@gmail.com
[2]Isaac Jasper Boro College of Education. Department of Biology Education. Sagbama. Bayelsa State. Nigeria.

Abstract. The study investigated fungi associated with post harvest tomato fruits sold in the open market in Port Harcourt Metropolis. The antifungal activity (bitter leaf) extracts against spoilage was studied using well-in-agar diffusion method. Results showed that mean percentage incidence of fungi isolated from tomato fruits were *Rhizopus stolonifer* (56%), *Aspergillus niger* (62%) and *Altermaria altermata* (35%). Aqueous and ethanolic extracts of *Vernonia amygdalina* at different concentrations (20%, 40%, and 60%) (w/v) were used against fungi isolates, while water and ketoconzole (0.5 mg/mL) served as negative and positive control, respectively. Aqueous and ethanolic extracts of *V. amygdalina* inhibited the growth of all three test fungi. There were significant differences ($p < 0.05$) in the mean inhibitory effects of plant extracts and control. Antifungal activity measured as diameter of zone of inhibition revealed that *V. amygdalina* acqueous extract at 60% was very active against *Aspergillus niger* (16.50 mm) and *Alternaria altermata* (16.00 mm), while being moderately active against *Rhizopus stolonifer* (13.00 mm). However, 60% ethanolic extract of *V. amygdalina* was very active against *A. niger* (19.00 mm), *A. altermata* (17.00 mm) and *Rhizopus stolonifer* (15.80 mm). Phytochemical screening of *V. amygdalina* revealed the presence of tannins, oxalate, saponnins, flavanoid, cynogenic glucoside, phytate and Alkaloids *V. amygdalina* could serve as a potentially viable alternative to chemical fungicides in the preservation of post harvest tomato fruits (*Lycopersicum esculentum).*

Keywords: Tomato; *Vernonia amygdalina*; Port harvest; Fungi; Phytochemical.

Received
September 14, 2020

Accepted
November 30, 2020

Released
December 31, 2020

Full Text Article

ORCID
0000-0003-2964-8970
U. N. Emiri

0000-0002-9213-6129
E. B. Enaregha

Introduction

Post harvest are diseases responsible for heavy losses of agricultural produce during storage, reduce food quality and render them unfit for human consumption (Doyle, 2007). Micro organisms, particularly fungi are adjudged the most notorious culprits, among other factors, responsible for post harvest diseases of crops (Ray et al., 2000). Some fungi implicated in post harvest diseases are able to produce mycotoxins known to be highly toxic, carcinogenic and are able to suppress one's immune system (Bankole et al., 2005). Seeking ways to effectively control post harvest spoilage of agricultural produce is therefore undeniably essential, especially for crops such as Tomato that are widely consume in Nigeria and other parts of the world.

Tomato (*Lycopersicum esculentum)* is a widely consumed fruit eaten in both raw and processed forms. (Moneruzzaman et al., 2008). It is rich in lycopene, vitamins A and C, carbohydrates, proteins, fats, fibres and potassium (Talvas et al., 2010).

The consumption of tomatoes throughout the world is believed to benefit the heart and other organs. It is on record that the richest source of lycopene is tomato and tomato-based products (Evangelia et al., 2005). Lycopene has been found to prevent prostrate cancer, improve the skin's ability to protect itself against the harmful ultra violet rays, decrease the risk of breast, lungs, 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 (Shidfar et al., 2010; Zdenka et al., 2010; Borguini et al., 2009; Zhang et al., 2009).

Tomato contains large amount of water which makes it more susceptible to spoilage by the action of micro-organisms. (Bai and Lindhout, 2006). These micro-organisms attack is one of the limiting factors that influence tomato economic value as well reduce its shelf life.

Fungi have been reported as the source of spoilage of most of the tomato samples accessed than bacteria. (Gosh, 2009). Earlier workers reported fungi implicated in tomato spoilage. Some of which include *Aspergillus phoenics*, *Trichoderma spp*, *Alternaria altermata*, *Fusarium* spp, *Aspergilus niger*, *Mucor* spp, *Rhizopus stolonifer, Penicillium* spp, and *Geotrichum* spp. (Etebu et al., 2013, John et al., 2016; Chuku et al., 2010). Some of these fungi have been recognized as a source of potential health hazard (Bankole et al., 2005).There is therefore the need to seek ways that would control the proliferation of potential harmful spoilage fungi associated with the fruit.

The use of synthetic chemicals in the preservation post-harvest agricultural produce in storage has proven over the years to be very effective in controlling pathogenic fungi (Manczinger et al., 2002). However, their use is increasingly becoming undesirable because they are themselves carcinogenic, teratogenic, highly toxic with long degradation periods and are able to induce chemical poisoning, as well as fungal resistance (Adegoke et al., 2002). As a result, the search for post-harvest control strategies has recently been directed towards the use and implementation of natural preservatives that may have a positive effect on human health (WHO, 2002).

Amongst natural preservatives, the use of natural essential oils obtained from plants, particularly medicinal plants has been promising. They have been shown to reduce microbial and chemical spoilage among agricultural produce with no proven detrimental effect on human and the environment even at high concentration (Pessoa et al., 2002).These botanicals of medicinal importance have been proven to be very effective against fungal infection even where treatments with synthetic antibiotics failed (Oshim et al., 2016). One of such proven botanicals is *Vernonia amygdalina* (bitter leaf).

Bitter leaf is a shrub that grows abundantly throughout all African countries, and possibly in the torrid zones of the Caribbean Islands. It is efficacious as a medicine, it is an antidote for malaria, it is also anti-bacteria and anti-parasites (Challand and Willcox,

2009). The roots and the leaves when taken in any form detoxifies the blood, prevents rheumatism, indigestion, scurvy and counters the effects of excess sugar in the blood stream (Challand and Wilcox, 2009). Fashola et al. (2011) reported that *V. amygdalina* has hypoglycaemic activity. They observed a close dependent reduction in fasting blood sugar level in alloxan induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts.

Extracts of bitter leaf have been shown by numerous workers to control microbial infections and aflatoxin contamination of food commodities (Suleiman et al., 2008; Audu et al., 2016; John et al., 2016; Onyeani et al., 2020). In this work, fungi associated with post harvest tomato fruits sold in the open market in Port Harcourt metropolis is being studied and the efficacy of inhibitory properties of *Vernonia amygdalina* (aqueous and ethanolic) leaf extracts against spoilage fungi isolated from tomato was also studied.

Materials and methods

Experiment 1: Survey of post harvest quality of tomato fruits

Ten tomato fruits samples were purchased from Woji market in Port Harcourt, Rivers State, Nigeria. They were transported to the Microbiology Laboratory of Rivers State University, Port Harcourt, in sterile polythene bags for fungal isolation. The samples were left for five days for spoilage to occur. The ten partially rotted tomato fruits were used for the study (Figure 1).

Figure 1. Healthy tomato fruits (left) and fungal infected tomato fruit (right).

Samples processing

Each of the partially rotted tomato fruits was carefully cut with the aid of a sterile scalpel and enriched in sterile Sabouraud Dextrose Broth for 24 h. Ten fold serial dilutions of the sample were thereafter carried out.

Isolation of fungi

The pour plate method was used. One milliliter of the serially-diluted sample was dispensed into a conical flask containing sterile Sabouraud Dextrose Agar (SDA) and two percent chloramphenicol to inhibit bacterial growth. The contents were properly mixed and dispensed aseptically into sterile petri-dishes,

incubated at 28 °C for five days. The colonies that developed were counted and sub cultured repeatedly on Sabouraud Dextrose Agar plates to obtain pure cultures.

Mean percentage incidence of fungi was calculated using the formula:

$$\text{Mean percentage} = \frac{Total\ number\ of\ occurence\ \ of\ a\ particular\ \ fungi}{Total\ number\ of\ \ plated\ sample}\ X\ \frac{100}{1}$$

Characterization and identification of the isolates

The pure cultures of the fungi were identified on the basis of their colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination. The identity of each fungus was confirmed with the aid of a mycological atlas.

Pathogenicity test of the isolates

The procedures of Agrios (2005) was used. Five healthy tomatoes were properly washed with tap water, rinsed with distilled water and surface-disinfected with ethanol. Sterile cork borers were used to bore holes in each of the tomato fruits. Each of the isolated fungi was thereafter inoculated into the fruits after which the cores of the fruits were replaced. Sterile petroleum jelly was used to seal the holes of the fruits to prevent contamination. Five tomatoes fruits wounded with the cork borers but were not inoculated with the fungi served as controls.

The inoculated tomato fruits and the control were placed in sterile polythene bags (one fruit per bag). Each of the fruits was moistened with wet balls of absorbent cotton wool to create a humid condition. The fruits were thereafter incubated at 28^0C for five days and observed for spoilage. The fungi were re-isolated from the fruits and compared with the original isolates. The fungi isolated from the partially rotted tomato fruits were *Rhizopus stolonifer*, *Aspergillus niger* and *Alternaria altermata.*

Experiment 2: Anti-fungal potency of *V. amygdalina* (bitter leaf) extracts against fungal spoilage of tomato fruits

The inhibitory effect of aqueous and ethanolic leaf extract *V. amygdalina* on growth of three test fungi; *Aspergillus niger, Rhizopus stolonifer* and *Alternaria altermata* previously isolated from post harvest tomato fruits was studied, using the well-in-agar diffusion method. Leaves of *V. amygdalina* were collected from a garden in Port Harcourt of Rivers State, Nigeria and washed thoroughly under running water and further with sterile distilled water, after which they were air dried for 10 days. The leaves thereafter were ground into powder using a vegetable blender.

Sample extraction (aqueous extract)

Three quantities (20 g, 40 g, and 60 g) of the leaf powder were measured and each dissolved in 100 mL of sterile distilled water to obtain 20%, 40% and 60% (w/v) aqueous extracts and mixed vigorously after which the plant residue was filtered through a sterile muslin cloth and the obtained filtrate was further sterilized by filtration through the membrane filter. The sterile extracts obtained were then stored in sterile capped McCartney bottles and refrigerated at 4 °C until use

Extraction using ethanol (ethanolic extract)

20 g, 40 g and 60 g of the leaf powder were each dissolved in 100 mL of 70% ethanol for 24 h, to obtain 20%, 40% and 60% (w/v) ethanol extract at room temperature with

occasional stirring. The content was filtered with muslin cloth and evaporated to dryness in a water bath at 78 °C. The extracts were collected and stored in sterile McCartney bottles and refrigerated at 4 °C until required for use.

Phytochemical analysis

The phytochemical analysis of the *V. amygdalina* leaf was done according to the procedure of AOAC (2005).

Sterility test of plant extracts

Each of the extracts (aqueous and ethanol) was tested for sterility after sterilization by inoculating l ml of each extract on sterile Sabouraud Dextrose Agar (SDA) and incubated at 37 °C for 24 h. The plates were observed for growth. No growth was observed after incubation, which indicates the extracts were sterile.

Determination of anti-fungal potency of various crude extract

Sabouraud Dextrose Agar (SDA) was prepared according to manufacturer's prescription, integrated with chloramphenicol. Three media plates were separately inoculated with suspension of the test organisms; *Rhizopus stolonifer, Aspergillus niger* and *Alternaria altermata* respectively, to obtain pure culture of each by spread plate method in three replicates, incubated at room temperature for 7 days. Thereafter, a sterile cork borer (8 mm diameter) was used to bore hole in each of the pure culture plates. Thereafter 1 mL each of the 20%, 40% and 60% *V. amygdalina* aqueous plant extract was dispensed into the hole on each plate. The plates were allowed to stand for 30 min for diffusion of the extract to occur and then incubated at room temperature for 5 days. This procedure was repeated for ethanolic plant extracts, distilled sterile water and ketoconazole which served as negative and positive control, respectively.

The inhibitory effect of the test plant extracts and control treatments were ascertained by a clear zone of inhibition of fungal growth around the well after 5 days of incubation. Data obtained were subjected to statistical analysis using ANOVA. Mean diameter of zones of inhibition were further subjected to LSD means separation test.

Results

Tomato fruits sold in Port Harcourt, Rivers State Nigeria, were infected by postharvest spoilage fungi (Table 1).

Table 1. Mean percentage incidence of fungi isolated from tomato fruit.

Fungal isolated	Tomato (*Lycopersicum esculentum)*
Rhizopus stolonifer	56 ± 0.37
Aspergillus niger	62 ± 0.30
Alternaria altermata	35 ± 0.32

The results on effect (inhibitions) of different concentrations of aqueous extracts showed there was significant increase in zone of inhibition as the concentration of the extracts increased. *R. stolonifer* had the least inhibition while *A. niger* had the highest diameter of inhibition across the different rates of concentration of plant extract (Table 2).

Table 2. Comparative mean effect (inhibition) of different concentrations of aqueous extracts of *V. amygdalina* on fungal growth.

Treatment concentration	Diameter of zone of inhibition (mm)		
	R. stolonifer	***A. niger***	***A. altermata***
Extract (20%)	11.00	12.00	11.50
Extract (40%)	11.80	14.00	13.00
Extract (60%)	13.00	16.50	15.00
Water	0.00	0.00	0.00
Ketoconazole (0.5 mg/mL)	75.00	80.00	80.00
LSD ($P \leq 0.05$)	1.08	0.79	1.28

Means are significantly different LSD at $p \leq 0.05$.

Similar observation was made with ethanolic extracts, *R. stolonifer* showed least inhibition compared to other fungal isolates (Table 3).

Table 3. Comparative mean effect (inhibitions) of different concentrations of ethanol extracts of *V. amygdalina* on fungal growth.

Treatment concentration	Diameter of zone of inhibition (mm)		
	R. Stolonifer	***A. sniger***	***A. altermata***
Extract (20%)	11.90	15.00	13.50
Extract (40%)	14.00	17.00	16.00
Extract (60%)	15.80	19.00	17.00
Water	0.00	0.00	0.00
Ketoconazole (0.5mg/mL)	75	80	80
LSD ($p \leq 0.05$)	0.76	0.92	1.00

Means are significantly different LSD at $p \leq 0.05$.

The results of the phytochemical constituents of *V. amygdalina* leaf is presented in Table 4.

Table 4. Phytochemical constituents of *Vernonia amygdalina* leaf.

Fungal isolated	Tomato (*Solanum lycopersicum)*
Tannins	10.26 ± 0.03
Oxalate	3.08 ± 0.12
Saponins	6.17 ± 0.05
Flavonoid	4.69 ± 0.08
Cynogenic glucoside	2.11 ± 0.02
Phytate	4.43 ± 0.03
Alkaloids	5.75 ± 0.06

The phytochemical screening revealed that the leaf of *V. amygdalina* has appreciable amount of tannins, saponins, alkaloids and flavonoid relatively higher than

phytate, oxalate, and cynogenic glucoside. However, ethanolic extracts appeared to have more inhibitory effect on all test fungi, relative to aqueous extracts.

Discussion

Results on the fungi implicated with the mycodeterioration of post-harvest tomato fruits sold in the open market in Port Harcourt, Nigeria revealed *Rhizopus stolonifer, Aspergillus niger*, *Alternaria altermata*. These fungi have been reported by earlier workers to be consistently associated with post harvest rot of tomatoes during storage (Chuku et al., 2010; Etebu et al., 2013; Ugwu et al., 2014; John et al., 2016). Deductions from the incidence of these organisms revealed that *A. niger* had higher incidence followed by *R. stolonifer,* while *Alternaria altermata* had the least incidence. The high incidence recorded by *A. niger* and *R. stolonifer* was not unexpected as fungi thrive in moisture, hence tomato fruit (rich in moisture) provided a good substrate for the fungi. *Aspergillus species* are known to produce several toxic metabolites, mycotoxins which is a very important toxin known to pose hazard on human and animal health (Bankole, 2005).

It has been revealed from this study that the plant extract possess anti-fungal properties, however ethanol extract appeared to be more potent than aqueous extracts because ethanol extracts produced higher diameter of inhibition among all the test fungi as shown in Table 2 and 3. It thus suggests that ethanolic extract possess more anti microbial activities than aqueous extract. *R. stolonifer* had the lowest diameter of inhibition, while *Aspergilus niger* had the highest diameter of inhibition, which suggest that *A. niger* is more suspectible to the plant extract especially ethanolic extract. This observation is in consonance with the report of Adetunji et al. (2013) and Audu et al. (2018). However, this result negates the assertion of John et al. (2016) who reported that *R. stolonifer* was more susceptible to ethonolic *V. amygdalina* extract. The potency of ethanolic extract over aqueous extract could be attributed to the better solubility of the active components in organic solvents (de Boer et al., 2005; Doughari et al., 2007). The presence of phytochemicals, tannins, oxalate, saponins, flavonoids, cynogenic glucoside, phytate, and alkaloids in the extracts of *V. amygdalina* is comparable to the findings of Audu et al. (2018) and Udochukwu et al. (2015). These petrochemicals in *V. amagdalina* extracts may explain the reason for its anti-microbial actions, as have been previously documented (Nenaah, 2013; Jasim et al., 2015; Al-Habi et al., 2017; Jin et al., 2017).

The fungal inhibitory effect of aqueous and ethanolic extracts of *V. amygdalina* in this study was observed to be dose dependent. This pattern of correspondingly greater diameter of zone of inhibition with respect to increase in concentration of the plant extract was similar to the results reported by several other workers (Ijato, 2011; Etebu and Emiri, 2016; John et al., 2016; Chuku and Emiri, 2019). These workers separately reported that an increase in the concentration of an antimicrobial plant extract achieved a better result in the control of pathogenic fungi.

In a similar development, antifungal effectiveness of some tropical plants extracts in controlling several plant pathogens have been reported by several researchers (Amadioha, 2000; Okigbo and Ikediugwu, 2000; Okigbo and Nmeka, 2005). Results from this work agrees with the report of Etebu and Emiri (2016) which showed the inhibitory effect of *Ocimum gratissimum* against *Aspergilu* spp. Ijato et al. (2011) reported that *Chromolaena odorata* (leaf), *Tridax procumbens* (leaf), *Vernonia amygdalina* achieved an inhitory effect of 54.58% on the radial growth of rot fungi. Ugwuoke et al. (2008) reported the use of *V. amygdalina* in the control of *Fusarium solani* causing tuber rot on cocoyam. The observations of these researchers agree with this present study.

The result from this work suggest that *V. amygdalina* may be more effective if used in the control of post harvest diseases occasioned by *Aspergillus spp* than by those caused by *Rhizopus stolonifer.*

Johnson and Case (1995) posited than an organism could be considered resistant if diameter of zone of inhibition is < 100 mm, intermediate (moderately susceptible/ resistant) if diameter of zone of inhibition is between 11-15 mm and susceptible if the zone of inhibition is > 16 mm. Going by this assertion, 60% *V. amygdalina* aqueous extract and 40% ethanolic extratcs could be said to be very active against *Aspergillus niger* and *Altermaria altermata* while being moderately active against *Rhizopus stolonifer*. However, 60% ethanol extract is very active against all test fungi.

Conclusion

Aqueous and ethanolic extracts of *V. amygdalina* inhibited the growth of *Aspergillus niger,Altermaria altermata* and *Rhizopus stolonifer*. *V. amygdalina* 60% aqueous concentration, and 40% ethanolic concentration were considerably active against *Aspergillus niger* and *Altermaria altermata* while being moderately active against *Rhizopus stolonifer*. However, 60% ethanolic extracts was very active against all test fungi This botanical would serve as a viable alternative for chemical fungicides being readily available and possess little or no threat to our environment. It would be effective in the control of post harvest spoilage of tomato fruits in storage, especially spoilage occasioned by *Aspergillus* and *Alternaria spp.*

Conflict of interest

The authors declare that they have no conflict of interest.

References

AOAC - Association of Official Analytical Chemists. **Official methods of analysis of AOAC International**. 15. ed. Washington, D.C.: AOAC, 2005.

Adegoke, G. O.; Gbadamosi, R.; Evwoerhurhima, F.; Uzo-Peters, P.; Falade, K.; Itiola, O.; Moody, O.; Skura, B. Protection of maize (*Zea mays)* and Soybean (*Glycine max*) using *Aframomum danielli*. **European Journal of Foods Research and Technology**, v. 214, p. 408-411, 2002.

Agrios, G. N. **Plant pathology**. 5. ed. Cham: Elsevier Academic Press, 2005.

Al-Harbi, R.; Al-Wegaisi, R.; Moharram, F.; Shaaban, M.; El Rahman, O. A. Antibacterial and anti-hemolitictivity of tannins from Pimentadioica against methicillin resistant *Staphylococcus aureus*. **Bangladesh Journal of Pharmacology**, v. 12, p. 63-68, 2017.

Amadioha, A. C. Fungi toxic effect of some leaf extracts against *Rhizopus oryzae*causing tuber rot of potato. **Archives of Phytopathology and Plant Protection**, v. 33, n. 6, p. 499-507, 2000. https://doi.org/10.1080/03235400109383372

Adetunji, C. O.; Olaniyi, O. O.; Ogunkunie, A. T. J. Bacterial activity of crude extracts of *Vernonia amygdalina* on clinical isolates. **Journal of Microbiology and Anitimicrobials**, v. 5, p. 60-64, 2013.

Audu, I.; Sanusi, S. B.; Linatoc, A. C.; Mainassara, M. M.; Awawu, J. J. Phytochemical analysis and actimicrobial activity of bitter leaf (*Vernonia amygdalina*) collected from Lepai, Niger State, Nigeria, on some selected pathogenic micro organisms. **Science World Journal**, v. 13, no. 3, p. 15-18, 2018.

Bai, Y.; Lindhout, P. Domestication and breeding of tomatoes: What have we gained and what can we gain in the future? **Annals of Botany**, v. 100, no. 5, p. 1085-1094, 2006.

Bankole, S. A.; Joda A. O.; Ashidi J. S. The use of powder and essential oil of *Cymbopogon citratus* against mould deterioration and aflatoxin contamination of 'egusi' melon seeds. **Journal of Basic Microbiology**, v. 45, p. 20-30, 2005.

de Boer, H. J., Kool, A.; Broberg, A.; Mziray, W. R.; Hedberg, I.; Levenfors, J. J. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. **Journal of Ethnopharmacology**, 96, p. 461-469, 2005. https://doi.org/10.1016/j.jep.2004.09.035

Borguini, R. G.; Torres, E. A. F. S. Tomatoes and tomato products as dietary sources of antioxidants. **Food Review International**, v. 25, no. 4, p. 313-325, 2009. https://doi.org/10.1080/87559120903155859

Challand, S.; Wilcox, M. A clinical trail of the traditional medicine *Vernonia amygdalina* in the treatment of uncomplicated malaria. **The Journal of Alternative Complementary Medicine**, v. 15, no. 11, p. 1231-1237, 2009. https://doi.org/10.1089/acm.2009.0098

Chuku, E. C.; Emiri, U. N. Assessment of the proximate, phytochemical and antifungal potency of crude leaf extract of *Asystasia gangetica*. **Nigeria Journal of Mycology**, v. 11, p. 182-195, 2019.

Chuku, E. C.; Osakwe, J. A.; Daddy-West, C. Fungal spoilage of tomato (*Lycopersicon esculentum*) using garlic and ginger. **Scientia Africana**, v. 9, no. 2, p. 41-48, 2010.

Doughari, J. H.; Elmahmood, A. M.; Manzara, S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. **African Journal of Microbiology Research**, v. 1, p. 37-41, 2007.

Doyle, M. **Microbial food spoilage**: Losses and control strategies. FRI BRIEFINGS, 2007. Available from: <https://fri.wisc.edu/files/Briefs_File/2017-07-18_0857_FRI_Brief_Microbial_Food_Spoilage_7_07.pdf>. Accessed on: July 13, 2020.

Ebiowei, E.; Emiri, U. Post harvest quality of commercial *Irvingia kernels* and the potential use of *Ocimum gratissimum* (scent leaf) against fungal spoilage. **Research Journal of Food Science and Quality Control**, v. 2, no. 1, p. 1-11, 2016.

Etebu, E.; Nwauzoma, A. B; Bawo, D. D. S. Postharvest spoilage of tomato *(Lycopersicum esculentum*) and control strategies in Nigeria. **Journal of Biology, Agriculture and Healthcare**, v. 3, no. 10, p. 51-63, 2013.

Evangelia, M.; Stefania, O.; Gizzi, S.; Ruggero, R.; Stefano R. Passion flower fruit: A new source of lycopene? **Journal of Medicine and Food**, v. 81, no. 1, *p.* 104-106, 2005.

Feshola, M.; Merli, E.; Boncampagni, E.; Ranpa, A. Monitoring heron populations in Italy, 1972-2010. **Journal of Heron Biology and Conservation**, 1:8, 2011.

Ghosh, A. Identification of microorganisms responsible for spoilage of tomato *(Lycopersicum esculentum)* fruit. **Journal of Phytology**, v. 1, no. 6, p. 414-416, 2009.

Ijato, J. Y. Inhibitory effect of two indigenous plant extracts of *Zingiber officinale* and *Ocimum gratissimum* on post-harvest yam (*Dioscorea rotundata)* rot *in vitro*. **Journal of American Science**, v. 7, no. 1, p. 43-47, 2011.

Jasim, H.; Hussein, A. O.; Hameed, I. H. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). **Journal of Pharmacognosy and Phytotherapy**, v. 7, p. 56-72, 2015.

Jin, Z.; Gao, L.; Zhang, L.; Liu, T.; Yu, F.; Zhang, Z.; Wang, B. Antimicrobial activity of saponins produced by two novel endophytic fungi from *Panax notoginseng.* **Natural**

Product Research, v. 31, p. 2700-2703, 2017. https://doi.org/10.1080/14786419.2017.1292265

John, W. C.; Anyanwu, N. C. J.; Ayisa, T. Evaluation of the extract o *Vernonia amygdalina* on fungi associated with infected tomatoes (*Lycopersicum esculentum*) in Jos North Local Government Area, Plateau State, Nigeria. **Annual Research and Review in Biology**, v. 9, no. 4, p. 1-8, 2016.

Johnson, T.; Case, C. **Chemical methods of control, adopted from Laboratory Experiments in Microbiology**: Brief edition. 4. ed. Redwood City, CA: Benjamin, Cummings Publishing, 2006.

Manczinger, L.; Antal, Z.; Kredics, L. Ecophysiology and breeding of mycoparasitic *Truchoderma* strains (a review). **Acta Microbiologica et Immunologica Hungarica**, v. 49, p. 1-14, 2002. https://doi.org/10.1556/amicr.49.2002.1.1

Moneruzzaman, K. M.; Hossain, A. B. M. S.; Sani, W.; Saiffudin, M. Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato. **American Journal of Biochemistry and Biotechnology**, v. 4, no. 4, p. 336-344, 2008.

Nenaah, G. Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. **World Journal of Microbial Biotechnol**, v. 29, *p.* 1255-1262, 2013.

Okigbo, R. N.; Ikediugwu, F. E. O. Studies on biological control of post harvest rot of yam (*Dioscorea spp*) with *Trichoderma viride*. **Journal of Phytopathology**, v. 14, no. 8, p. 351-355, 2000.

Onyeani, C. A.; Osunlaja S. O.; Oworu O. O.; Joda A. O. Evaluation of effect of aqueous plant extract in the control of storage fungi*.* **International Journal of Science Technology Research**, v. 1, no. 6, p. 76-79, 2012.

Oshim, I. O.; Desmond, C. O.; Anyi, R.; Nwobu, U.; Ezugwu, U. M.; Umana, E. U. Kinetics of minimum inhibitory concentration, minimum bacterial concentration and minimum fungicidal concentration of *Vernonia amygydalina* (bitter leaf) on micro-organism isolates from wound infections. **International Journal of Surgical Research**, v. 5, p. 8-14, 2016.

Pessoa, L. M.; Morais, S. M.; Bevilaqua C. M. L.; Luciano J. H. S. Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemomchus contortus.* **Veterinary Parasitology**, v. 109, no. 1/2, p. 59-63, 2002. https://doi.org/10.1016/s0304-4017(02)00253-4

Ray, R. C.; Nedunzhiyan M.; Balangopalan, C. Micro-organisims associated with postharvest spoilage of yams. **Annals of Tropical Research**, v. 22, no. 1/2, p. 31-40, 2000.

Shidfar, F.; Froghifar, N.; Vafa, M.; Rajab, A.; Hosseini, S.; Shidfar, S.; Gohari, M. The effects of tomato consumption on serum glucose, apolipoprotein B, apolipoprotein A-I, homocysteine and blood pressure in type 2 diabetic patients. **International Journal of Food Science and Nutrition**, v. 62, no. 3, p. 289-294, 2010. https://doi.org/10.3109/09637486.2010.529072

Suleiman, M. N.; Emua, S. A.; Taiga, A. Effect of aqueous leaf extracts on a spot fungus (*Fusarium* sp) isolated from cowpea. **American-Eurasion Journal of Sustainable Agriculture**, v. 2, no. 3, p. 261-263, 2008.

Talvas, J.; Caris-veyrat, C.; Guy, L.; Rambeau, M.; Lyan, B.; Minet-Quinard, R.; Lobaccaro, J.-M. A.; Vasson, M.-P.; Georgé, S.; Mazur, A.; Rock, E. Differential effects of lycopene consumed in tomato paste and lycopene in the form of a purified extract on target genes of cancer prostatic cells. **American Journal of Clinical Nutrition**, v. 91, no. 6, p. 1716-1724, 2010. https://doi.org/10.3945/ajcn.2009.28666

Udochukwu, U.; Omeje, F. I.; Uloma, I. S.; Oseiwe, F. D. Phytochemical analysis of *Vernonia amygydalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. **American Journal of Research Communication**, v. 3, no. 5, p. 225-235, 2015.

Ugwu, O. C.; Chukwuezi, F. O.; Ozougwu, V. E. O. Microbial agents of tomato spoilage in *Onitsha metropolis*. **Advances in Biological Research**, v. 8, no. 2, p. 87-93, 2014.

Ugwuoke, K. I.; Onyeke, C. C.; Tsopmbeng, N. G. R. The efficacy of botanical protectants in the storage of cocoyam (*Colocassia esculenta* (L.) Shott). **Agroscience Journal of Tropical Agriculture, Food Environment and Extension**, v. 7, no. 2, p. 93-98, 2008.

WHO - World Health Organization. Reducing risks, promoting healthy life. Geneva: OMS, 2002. (World Health Report). Available from: <http://www.int/entity/whr/2002/en/whr02_en.pdf>. Accessed on: Jan. 22, 2020.

Zdenka, P.; Peter, S.; Hana, D.; Milan, H. Antimutagenic effects of lycopene and tomato Puree. **Journal of Medicine and Food**, v. 13, no. 6, p. 1443-1450, 2010.

Zhang, C.-X.; Ho, S. C.; Chen, Y.-M.; Fu, J.-H.; Cheng, S.-Z.; Lin, F.-Y. Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. **International Journal of Cancer**, v. 125, no. 1, p. 181-188, 2009. https://doi.org/10.1002/ijc.24358