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Research Article

Evaluation of Antifungal Activity of Oil Extracted from Two Varieties of Tomato Seeds against Fungal Organisms Causing Rot in Tomato Fruits

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ABSTRACT

Evaluation of the antifungal activity of oil extracted from two varieties of tomato seeds against fungal organisms causing rot in tomato fruits was carried out in Makurdi. The presence of decay caused by Fungi on tomatoes results in postharvest losses. Riogrande and Roma VF tomato seeds were washed, dried at 50˚C for 3 days in an oven, and milled. With the aid of a soxhlet extractor, using n-Hexane at 40-60°C for 6 hours, oil was extracted from the milled samples. Analysis of the oil revealed the oil is stable, translucent, and highly penetrating. A portion of tomatoes showing signs and symptoms of rot were detached, sterilized, and placed in a medium of Potato Dextrose Agar for one week for fungal growth. Fungi growths were identified macroscopically and microscopically and matched with standards. Fungi such as *Curvularia affinis, Aspergillus flavus, Aspergillus niger, and Penicillium waksmanii* were isolated. The pour plate method was used for fungi growth in the presence of the oil extracted. At days 3-7 the Riogrande seed oil media inhibit the growth of the isolated fungi with *Aspergillus niger* having the highest inhibition ranging from 1.333 to 1.733 mm and the Roma seed oil media inhibit the growth of *Curvularia affinis* with the highest inhibition ranging from 1.266 to 1.950 mm and while the control ranged from 3.533 to 8.100 mm. The results showed that numerous fungi are involved in rot of tomatoes and the extracted oil contains phytochemicals that are responsible for inhibiting fungal growth in the plates.

Keywords: Antifungal; Tomato seed; sodium hypochlorite; n- Haxane; Potato Dextrose Agar; Antioxidant

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INTRODUCTION

Fungi organisms are the most significant and major pathogens contaminating a wide range of fruits and causing tough and economic important losses of fruits during storage, transportation and marketing [Sommer, 2008]. Fungal pathogens are accountable for an over-all postharvest losses of tomato fruits for as high as 30 – 40% [Agrios, 2005] but this is much higher in unindustrialized nations like Nigeria due to lack of methods that prevent decay caused by these pathogens. In northern Nigerian where fresh tomato is the most cherished vegetable crop cultivated in large quantity due to the nature of the soil and accounts for about 18% of the average daily consumption of vegetables in Nigeria [Kator *et al*., 2018]. Sadly, about half of the yield of tomatoes produced in Nigeria at large are lost because of poor postharvest handling practices and lack of appropriate storage facility that can prevent fungi rot.

Oil extracted from tomato seeds composed mainly of glycerides (>98%) and unsaponifiable segment (1- 2%) that are quantitatively small but an important portion in which the negligible constituents are present [Giuffre' and Capocasale, 2016]. The unsaponifiable portion of the oil are filled with lycopene (carotenoid), phytosterols, and polyunsaturated fatty acids and

minerals which are liable for its antioxidant potential of the extracted oil [Ameh *et. al.,* 2023]. Therefore, the objective of the research was to evaluate the presences of phytochemicals and the antifungal activity of the oil extracted from seeds of tomatoes against the growth of fungal organisms causing rot in tomato fruits.

MATERIALS AND METHODS

Collection and Identification of Tomato Varieties

Riogrande and Roma VF tomato varieties seed used for this research work were collected from Azare, Bauchi state. The Riogrande and Roma VF tomato fruits were procured from an experimental farm in Makurdi, Benue State at beaker stage by hand picking and authenticated in Biological Sciences Department, Benue State University.

Experimental Location

The experiments were carried out in both laboratories of Chemistry and Biological Sciences, Benue State University, Makurdi, located in North central Nigeria along the Benue River, on latitude 07°43'N and longitude 08°35'E.

Sample Preparation

One kilogram (1kg) of each of the two varieties of the tomato seeds was collected as a by-product from Azare local government area, Bauchi State. The seeds were washed in clean water and sun dried temporary for two days before transporting it to Department of chemistry laboratory, Benue State University. In the laboratory, the seeds were dried in an oven at 50^{o}C for 72 hours and ground to powder using a mechanical grinder to obtain a larger surface area.

Extraction of Oil from the Ground Tomato seeds

The grounded tomato seeds (Ten grams) were poured into a permeable thimble and placed in the soxhlet extractor and set for extraction. The temperature of the hearting mantel was set at 60°C and using 300 ml of n-Hexane as extracting solvent in the soxhlet extractor set up, the extraction was allowed to run for six (6) hours repeatedly. The extracted oil were then collected from the round bottom flask of the soxhlet extractor into a beaker of 500 ml. Using water bath at 70°C, excess n-Hexane was removed from the crude oil and refrigerated at 4˚C until required for analysis [AOAC, 2005].

Phytochemical Screening of the Extracted Oil

The phytochemical screening of the oil were conducted to determine the presence or absence of the various phytoconstituents in the extracted oil as described by Campbell *et al*. [2004]. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the important phytochemicals with diverse biological activities determine with colour changes as present or absent.

Isolation and Identification of fungi organism causing rot in Tomato fruits

Preparation of Potato Dextrose Agar (PDA) Media

Fungal microorganisms causing rot were isolated from rotten tomato fruits using potato dextrose agar (PDA) media. The PDA media were prepared according to the manufacturer's instruction and sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes. After sterilization, the media was allowed to cool to room temperature and pouring into sterile Petri dishes.

Collection of infected fruit samples and isolation of pathogens

Decaying and rotten tomato fruit samples were recognized by physical examination and then collected randomly from the Wuruku market, Makurdi. Five (5) tomato fruits each from the two varieties with various rot symptoms were collected in a plastic containers of 500 ml, covered and transported via motor car to the Biological Science laboratory, Benue State University for analysis. About 0.5-1cm portion of the rotten tomato fruits were cut off and sterilized in 1% sodium hypochlorite solution for thirty seconds and rinsed in four different distilled water (250 ml beaker). It was then placed on a solidified Potato Dextrose Agar (PDA) medium gently in the petri dish and allowed to remain on the workbench in the laboratory at room temperature for microbial growth. Replicas of each samples were made for more observations of microbial growth. As reported by Liamngee *et al.,* [2015] to acquire pure isolates from the numerous fungal organisms causing rot, sub-cultures were performed after 5-7 days of growth in the petri dish.

Identification of Fungi organisms

After 5-7 days growth, the pure isolates of the fungal microorganisms were identified based on their macroscopic and microscopic organizations. The colony characteristics of each specific fungal microorganisms such as their appearance, change in medium colour and growth rate were identified macroscopically on the Petri plates. For the microscopic identification, a 5–7 day old cultures of the isolated fungal microorganisms were inoculated aseptically on a sterilized glass slide with the aid of a sterile inoculating loop and a drop of lactophenol cotton blue was added and the mixture covered with a cover slip. The glass slide were placed under the stage of a light microscope and viewed under a 40x objective. Shape of the conidia and conidiophores were noted, observed and compared with standards described by Barnett and Hunter [1972] and Booth [1971].

Pathogenicity Studies of fungal microorganism on ripped tomato fruits

The procedure described by Kator *et al*. [2018] on the pathogenicity studies of the fungal microorganism were

used. The washed tomatoes were surface sterilized by dipping the fruits in 1% Sodium hypochlorite solution for thirty seconds and rinsed in three different distilled water in a 500 ml beaker. With the aid of a cork borer, cylindrical plugs were used to create holes of about 5 mm across the fruits and the tomatoes were infected through the opening with fungal isolates from the five day old culture. Then the sterile PDA was use to sealed off the holes on the tomato fruits and allowed to remain on the workbench for 5–6 days of post inoculation. Three replica for each tomato fruits infected with the pure fungal isolates was arranged. The control tomato fruits were inoculated with sterile PDA only. At day 6 of the inoculation, signs and symptoms of the disease incidence and severity of the fruits were verified by hand feel and visual inspection of both the exterior and interior of the tomato fruits. Also, the morphological appearances and growth configurations observed on the infected tomato fruits were compared with those of the original isolates on the petri dish. Using the rate-limiting scale described by Bowen [2004], the infected tomatoes disease severity was determined in 0 = no disease symptom, 1 = 1 – 20% severity level on infected fruits, 2 = 21 – 40%, 3 = 41 - 60%, 4 = 61 – 80% and 5 = 81 – 100%. The results of the rate limiting scale was use in the formula suggested by Akhtar and Alam [2007].

Disease incidence % (DI) = X/N × 100

Where X= number of infected fruits and N = total number of fruits sampled.

Disease severity % (DS) = Σ (a + b) / N.Z × 100

Where, Σ (a + b) = Sum of symptomatic fruits and their corresponding score scale, N = Total number of fruits sampled and Z = highest score scale.

Evaluation of antifungal activity of Tomato seed oils on fungi causing rot in tomato fruits

The evaluation of antifungal activity of tomato seed oils on fungi causing rot in tomato fruits were investigated using the pour plate method. Two (2) ml of each sample of tomato seed (Riogrande and Roma VF) oil varieties were dispensed into the sterile petri dishes, followed by addition of about 18 ml of molten PDA media and swirled mildly on the worktable for a suitable mixture. The mixture were allowed to solidify after 30 minutes. The control samples of only molten PDA media of about 20 ml was prepared. Thereafter, 4 mm mycelia plug of the isolated fungi from 4 – 5 day old cultures was placed centrally on the petri dish and incubated at room temperature for 10days. Antifungal activity of the Tomato Seed Oil was evaluated at 3 days interval using the formula proposed by Liamingee *et al.,* [2015]. Where:

Growth incubation = $\frac{(R1-R2)}{R1} \times 100$

Where R_1 = Rate of fungi growth in control

R_2 = Rate of fungi growth in treated media

Statistical Analysis

Data were presented as mean value ± standard deviation and analyzed by multiple factor analysis of variance (ANOVA) using SPSS 10.0 software (SPSS, Chicago, IL, USA). Statistically significant between means were determined by Duncan's multiple range tests at $P \leq 0.05$. T-Test was used to determine significant differences in the pathogenicity of fungi isolate

RESULTS

Phytochemical composition of Roma VF and Riogrande Seed Oil

The results of the phytochemical composition of Roma VF and Riogrande tomato seed oil varieties were determined in table 1, where cholesterol, flavonoids, Terpenoids, phonels and steroids were all present in the extracted oil.

Identification of fungal microorganism causing rot of tomato fruits

Identification of fungal microorganisms causing rot of tomato fruits were identified macroscopically and microscopically and *Aspergillus flavus, Penicillium waksmanii, Curvularia affinis and Curvularia affinis are* some of the fungal microorganisms identified and presented in table 3 below.

Pathogenicity studies on Incidence and Severity of decay

The results of Pathogenicity studies on Incidence and Severity of decay of the tomato fruits inoculated with the isolated fungal microorganisms are presented on table 3 and 4 below.

Effect of the extracted oil on the growth of the isolated fungal microorganisms

The results on the efficacy of the oil on the growth inhibition of the fungal isolates are presented in table 5 and 6 below.

Table 1. Phytochemical composition of Roma VF and Riogrande Seed Oil status

Metabolite	Roma VF	Riogrande
Carbohydrate	-	-
Alkaloid	-	-
Cholesterol	+	+
Flavonoids	+	+
Terpenoids	+	+
Phenol	+	+
Anthraquinone	-	-
Steroid	+	+

KEYS: -Ve = Not present and + = Present

Table 2 Macroscopic and Microscopic identification of fungal microorganisms causing rot in tomato fruits

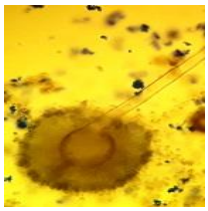
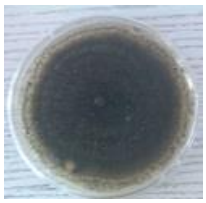
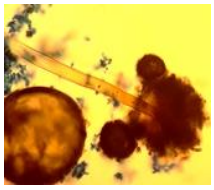
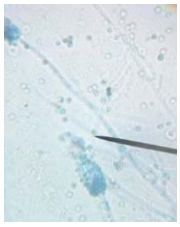
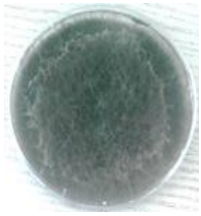
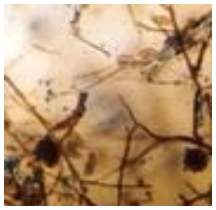
Macroscopic Characteristics	Microscopic characteristics	Appearance on potato dextrose agar media	Photomicrograph	Possible fungi
The colony coloration were yellowish-green, and bounded by a white circle and the borders were intact. The reverse was hyaline and growth of the colony was adequate to hasty.	Biseriate conidia covered the entire vesicle. Conidiophores were thick-walled, colourless, and roughed, and possess a vesicles that is round with a radiated head.			*Aspergillus flavus*
The colony coloration is dark brown/gray tones or dark drown to black. The colony growth are slow, initially white but changes to black after a few days producing conidial spores.	The hyphae are septate and hyaline and conidial heads are black and radiate with tendency to split. Conidia are globose to subglobose and brownish with warts, spines or irregular ridges.			*Aspergillus niger*
A dark green to greyish colony coloration with a powdery and compact with white border. The reverse was light orange and the growth rate was moderate.	Hyaline hyphae with unicellular conidia which were round, smooth and in chains. Brush like clusters (Penicilli) were found at the ends of conidiophores which were smooth.			*Penicillium waksmanii*
A colony of blackish brown, cottony and spread loosely. Conidiophores arise singly and in groups and are septate.	Conidia are curved broadly fusiform to ellipsoidal			*Curvularia affinis*

Table 3 Pathogenicity studies on Incidence of decay of tomato fruits infected with the isolated fungal microorganisms

Treatment	*Aspergillus flavus*	*Aspergillus niger*	*Penicillium waksmanii*	*Curvularia affinis*
Inoculated	100.00	100.00	100.00	100.00
Control	0.00	0.00	0.00	0.00
-value (0.05)	3.46^{xx}	3.46^{xx}	3.46^{xx}	3.46^{xx}

Significant at 1 % and 5 % level of probability.

Table 4 Pathogenicity studies on severity of decay of tomato fruits infected with the isolated fungal microorganisms

Treatment	*Aspergillus flavus*	*Aspergillus niger*	*Penicillium waksmanii*	*Curvularia affinis*
Inoculated	31.230±1.687[b]	31.493±3.743 [b]	39.130±11.822[b]	30.530±2.723 [b]
Control	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]
p-value	0.001	0.001	0.001	0.001

Values are mean ±standard deviation

Table 5 Effect of Riogrande variety of TSO on growth inhibition of fungi isolates

Fungi spp	Day 3	Day 5	Day 7
A. niger	1.333±0.144	1.450±0.050	1.733±0.160
CTRL	4.143±0.011	6.500±0.050	7.850±0.050
T-test	0.001	0.001	0.001
A. flavus	1.416±0.115	1.650±0.100	1.983±0.160
CTRL	3.750±0.050	6.250±0.050	7.750±0.050
T-test	0.001	0.001	0.001
Curvalaria	1.400±0.132	1.633±0.256	1.950±0.180
CTRL	2.950±0.050	4.550±0.050	6.250±0.050
T-test	0.001	0.001	0.001
Penicillium	1.833±0.076	2.066±0.076	2.266±0.076
CTRL	4.700±0.100	6.950±0.050	7.950±0.200
T-test	0.001	0.001	0.001

Values are Mean ± Standard deviation
Significant at 1% and 5% level of probability
A. niger = Aspergillus niger placed on PDA treated with tomato seed oil
A. flavus = Aspergillus Flavus placed on PDA treated with tomato seed oil
Curvularia = Curvularia affinis placed on PDA treated with tomato seed oil
Penicillium =Penicillium waksmanii placed on PDA treated with tomato seed oil
CTRL = fungi sample placed on PDA without treatment in sterile petri dish

Table 6 Effect of Roma variety of TSO on growth inhibition of fungi isolates

Fungi spp	Day 3	Day 5	Day 7
A.niger	1.800±0.100	2.366±0.332	2.533±0.057
CTRL	4.033±0.057	6.516±0.076	7.833±0.125
T-test	0.001	0.001	0.001
A. flavus	1.866±0.057	2.316±0.144	2.666±0.144
CTRL	3.833±0.076	5.900±0.100	7.800±0.100
T-test	0.001	0.001	0.001
Curvalaria	1.266±0.057	1.666±0.057	1.950±0.050
CTRL	3.533±0.152	6.200±0.100	8.100±0.100
T-test	0.001	0.001	0.001
Penicillium	1.766±0.152	2.233±0.160	2.500±0.200
CTRL	4.250±0.150	6.700±0.200	8.116±0.076
T-test	0.001	0.001	0.001

Values are Mean ± Standard deviation
Significant at 1% and 5% level of probability
A. niger = Aspergillus niger placed on PDA treated with tomato seed oil
A. flavus = Aspergillus Flavus placed on PDA treated with tomato seed oil
Curvularia = Curvularia affinis placed on PDA treated with tomato seed oil
Penicillium =Penicillium waksmanii placed on PDA treated with tomato seed oil
CTRL = fungi sample placed on PDA without treatment in sterile petri dish

DISCUSSION

The results of phytochemical screening of the oil extracted from the tomato seed using n-hexane solvent) have shown the presence of flavonoids, steroids, terpenoids, cholesterol, phenols in the plant extract. Several studies have shown that plant extract such as tomato seed oil is rich in carotenoids particularly flavonoids [Engelhard *et al*., 2006]. The high carotenoids that are mainly phenolic compounds in the oil could therefore be attributed to the antioxidant activates inherent in the tomato seed.

Four different fungal microorganisms were identified out of the decaying fruits samples. They are *Aspergillus flavus, Aspergillus niger, Penicillium waksmanii, and Curvularia affinis* as presented in table 2. It was observed from the table that numerous fungal microorganisms are often connected with rot of tomato fruits which may lead to post-harvest losses. Some of these fungi have been earlier isolated from tomatoes by Ogo-Oluwa and Liamngee [2016], and were labelled pathogens of tomato fruits. The existences of these fungi in different fruits and crops shows that they are not host specialized but are only found anywhere nutrients are presents at an advantageous condition. The research was in agreement with the study carried out by Liamngee *et al.* [2015] who also identified similar group of fungi from Garri, a cassava processed food product.

The Tables 3 and 4 showed the results of pathogenicity test of the fungi infected into tomato fruits. The results revealed 100% capability of the isolated fungi to cause infection in uninfected tomato fruit after 6 days of incubation with numerous percentages of severity while the control displays no rot sign. Table 3 show severity of rot on the tomatoes infected with the isolated fungal, with *Penicillium waksmanii* produced the higher rot diameter of 39.130 mm while *Curvularia affinis* produced the lower rot diameter of 30.530 mm in the tomato fruits. The control samples (tomatoes infected with sterilized PDA) shown no sign and symptom of decay. The pathogenicity test confirmed that the pathogenic fungi infected in the tomato fruit causing rot were as a result of the isolated fungal microorganisms' ability to utilize nutrients inherent in the tomato fruits as substrate for growth and development at room temperature [Liamngee *et al*., 2015a].

The effect of the extracted oil on the isolated fungi growth were examined using the pour plate method in table 5 and 6. In table 5 where the effect of Riogrande variety of the TSO treatment on growth inhibition for the four isolated fungi has *Aspergillus niger* with the highest growth inhibition/ poor growth at day 3 to day 7 (1.333 to 1.733 mm) and the control (with no treatment) possess a high growth rate (4.143 to 7.850 mm). *Penicillium waksmanii* has the least growth inhibition in the plate (1.833 to 2.266 mm) compare to both *Aspergillus flavus, and Curvularia affinis*. In table 6 where the effect of Roma variety of TSO treatment on growth inhibition for the four isolated fungi has *Curvularia affinis* with the highest growth inhibition at day 3 to day 7 (1.266 to 1.950 mm) and the control (with no treatment) have a high growth rate, ranging from 3.533 to 8.100 mm, and *Aspergillus flavus has* the least inhibition ranging from 1.866 to 2.266 mm from days 3 to 7 respectively. The inhibitory effect exhibited by the TSO in the presence of PDA substrate in table 5 and 6 on the fungi growth could be as a result of the extracted oil from tomato seed packed with phenolic compounds [Engelhard *et al*., 2006], that are present as phytochemicals in biological samples. Many of these phytochemicals present in the oil extracted from tomato seed exhibit antioxidant ability naturally [Ameh *et. al.,* 2023a], and suppresses the development of microorganisms like viruses, protozoa, bacteria, fungi, yeast and mold etc. The phenolic compounds present in the extracted oils (Table 1) are responsible for the low growth of the fungi organisms observed in the plates containing TSO compared to the control. The unsaponifiable matter of the TSO that contains the phytochemical compounds are insignificant in volume but are made up of the vital fraction in which the active components (phytochemicals) are present and are liable for the antifungal ability of the extracted oil. Most of this natural antioxidant present in plants are generally circulated in plants and concentrated in the seeds of the plants which are highly active in delaying or inhibiting oxidation of oxidizable substrates at low concentration and can delay microbial growth when incorporated in a substrate [Tenore *et. al.,* 2023].

CONCLUSION

Based on the above experimental results, it can be established that both varieties of tomato seed oil (Riogrande and Roma VF), possess phenolic compounds that have antioxidant potentials which are liable for the growth inhibition observed in the plates while the control sample (without the TSO) exhibited high growth rate. The oil extracted from tomato seed (a byproduct) contain natural antioxidant that suppresses or inhibit the growth of fungi causing rot in tomato fruits and can be used to control postharvest losses by coating or pretreating tomato fruits and other perishable fruits with the oil.

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