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Efficacy of antimicrobial effect of *Venonia amygdalina* and *Tridax procumbens* in *in vitro* control of tomato (*Lycopersicum esculentum*) post harvest fruit rot.

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Abstract: The antimicrobial effects of extract of *Venonia amygdalina* and *Tridax procumbens* were determined on rot causing fungi. In the present study, the pathogenic fungi isolated from the infected tomato fruit parts and identified based on morphological and cultural characters were: *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporum*, G*eotrichum candidium*. Two different extractive solvents (water and ethanol) were used; aqueous concentrations of 80 and 60% as well as ethanol concentration of 30 and 20% were used in this study. All the plant extracts, both aqueous and ethanol showed significant reduction of mycelia growth of isolated pathogens. In aqueous extract, 80% of both *Venonia amygdalena* and *Tridax procumbens* had high inhibitory effect of 49.20% against *Geotrichum candidium* and 53.30% against *Aspergillus niger* respectively than 60% aqueous concentration of the test plant extracts, while in ethanolic extract, 30% ethanol extract of *Venonia amygdalena* and *Tridax procumbens* inhibited up to 65.20 and72.20% against *Fusarium oxysporum* respectively more than 20% ethanolic extracts. Higher concentration of both aqueous and ethanol favoured higher mycelia growth reduction. Plant extracts are accessible for controlling phyto diseases, non hazardous, eco-friendly, low cost and non-pollutant.

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Key word: *Venonia amygdalena*, tomato fruit *rot*, *Tridax procumbens* and biological control.

Introduction

Tomato is a native of Peru and Ecuador; tomato had spread in pre Columbia far north and Mexico. Tomato was introduced into Europe from Mexico by the Spanish in 1523. At first, it was little used for food because of its relationship with the deadly night shade family. Tomato was referred to as an object of affection and therefore known as love apple 'pommels d'amour' in French. In earlier times, tomatoes were consumed only in Italy and so it is since the second half of the 19th century that the crop has been widely appreciated for the fine food that it provides. From Europe, it was carried across the pacific into south eastern Asia before in1650, today it is widely grown throughout the warm temperate and tropical region of the world. Worldwide about 36million tones of tomatoes are produced annually mostly in Europe and North America. The major tomatoes producing countries in order of importance are: the United State, followed by Italy, the Soviet Union, Spain, Turkey, Egypt, followed by Greece, Romania and the Arabian country. Mexico, Japan, Brazil, India and Argentina are other important producers.

Tomato is a weak stem, trailing, much branch, short leaved perennial, but treated as an annual under cultivation. The leaves are both spirally arranged, unevenly impair pinnate compound. The flowers are bond in clusters on both the main axis and lateral branches, the fruits are freshly berries and are hairy when young but becoming smooth, juicy and shining when ripe. Tomato is a familiar ingredient of salad, being valued for its colour, destroyable flavor and pleasing acidic taste of the flesh, caned or preserved state. Fresh ripe fruit is refreshing and appetizing. Tomato is used also in the preparation of tomatoes soups, pickles (green tomatoes). Checkup, sources puree, paste, juice manufacture canning and pickling and other products. The seeds are flat and reniform, embedded in a tele-like mass of tissues containing large quantity of phosphorus. The seed contains about 24% of semi drying oil which is used as a salad oil and also in the production of margarine and soup. The residual mass or 'press cake' is used as a stock field and fertilizer.

The tomato fruits are largely water (about 94%) but have moderate quantities of soluble sugars or several organic acid (especially citric and malic acid) mineral salt and relatively large quantities of the vitamin C compared with oranges, tomatoes contain nearly 20 times as much vitamin A, the same amount of vitamin B, slightly more vitamin B2. Effective and efficient management of phyto diseases is generally achieved by the use of vitamin C and chemicals (Kiran *et al*. 2006). Due to increased awareness about the risks involved in the use of synthetic pesticides, much attention is being focused on the alternative method of pathogen control, the spiraling up cost of chemical fungicides particularly in those countries where pesticides are important, pollution to soil, water, air by the accumulation of obnoxious chemicals residues due to continuous use of fungicides and development of resistance races to these chemicals are there, now facing the scientists to look for methods which are ecologically friendly, safe and specific for pathogens. The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and to the existing human eco geographical conditions as some of those chemicals have already been proved to be either mutagenic, carcinogenic or teratogenic. Keeping in view the drawback of chemical management of plant, the use of plant extract in disease management is therefore gaining importance (Kiran *et al.* 2006; Okigbo, 2009). The objective of this study is therefore, to evaluate locally available plant extracts: *V. amygdalina* and T. *procumbens* to control tomato fruit rot pathogen.

Materials and Methods

Collection of tomato fruits

Tomato fruits with symptoms of rottenness were randomly collected from the market stalls at Ado-Ekiti, Nigeria. Softness of tissue of tomatoes was identified as being biologically deteriorated. Fresh and healthy tomatoes were also collected and packed into a sterile polythene bag already lined with soft paper and taken to the laboratory for further studies.

Collection of plant materials

Venonia amygdalena and *Tridax procumbens* were collected in the premise of the University of Ado-Ekiti. These plants were taken to the herbarium unit of the University for Identification.

Isolation of spoilage fungi form rotted tomato fruit

Pieces of tomato were washed in a running tap, these pieces were cut from the periphery of a rot tomato, these were surface sterilized in the plate with 70% ethanol for just 1minute, dropped on sterile soft paper and cultured out on PDA mixed with streptomycin. A minimum of four replicates pieces from each of the rots were cultured out. The Petri-dishes were incubated at 28 ± 2^0c for five days and observed for fungal growth. Fungi associated were re cultured to obtain pure culture and the pure isolates stored in slant for further use. The frequency of occurrence was determined using the method of Okigbo and Ikediugwu (2000).

Pathogenecity Test

Cylindrical cores of 1cm deep were taken away from different spots of a fresh and healthy tomato fruits with the aid of sterile 5mm cork borer and then disk of 4mm discs taken from the periphery/core of a colony of five days old test fungus was placed downward into each hole in the tomato fruit. The core of the tomato fruit was placed after 2mm pieces have been cut off to compensate for the thickness of the agar inoculums and then replaced core sealed with Vaseline (jelly). Sterilized PDA was used in place of the culture discs in the control set up.

Preparation of leaf extract

Venonia amygdalina and *Tridax procumbens* were collected and washed thoroughly under running water and allow to air dried for 7 days. These were grinded separately. Thirty grams of each sample was added to 15ml of distilled water in separate flasks. This was vigorously shaken and left to stand for 24hours. The sample was filtered with 3 layer cheese cloth and filtrate extract preparation of 80 and 60% concentration were used as the extract. The same procedure was used for 30% and 20% ethanol extract.

Effect of plant extracts on fungi mycelia growth.

The approach of Amadioha and Obi (1999) was used to evaluate the allelopathic effect of the extract on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicated the centre of the plates. This was done before dispensing PDA into each of the plates. The extracts were poured into the flask plugged with cotton and heated for about 10 minutes to avoid contamination (Madari and Singh 2005). About 2 ml of the extract of various plant materials was separately introduced into the Petri-dish containing the media (poisoned food method) Nene and Thalpiyal (2000) and placed on the extract in (4mm diameter) of the pure isolate each was placed in the extract in plate with PDA at the bottom of the plate.

Control experiments were without addition of any plant extract but sterile distilled water. Fungitoxicity was determined in term of percentage colony inhibition %: $\frac{DC - DT}{DC} \times \frac{100.}{1}$

Where DC - Average Diameter of fungal colony in control

DT - Average diameter of fungal colony with treatment.

Results

Table 1:-Percentage reduction of mycelia growth of spoilage fungi cultured in PDA incorporated with aqueous plant extracts of 60% and 80% concentrations.

	Plant extracts (% reduction of mycelia growth)				
	Venonia amygdalina		Tridax *procumbens*		
Rot Fungi	60%	80%	60%	80%	Control
Aspergillus niger	40.20a	46.00a	46.60ab	53.30a	14.8
Fusarium oxysporum	40.00a	40.00b	40.00c	42.60b	16.00
Rhizopus stolonifer	43.90a	46.60a	50.70a	50.50a	12.00
Geotricum candidium	40.80a	49.20a	43.40bc	50.50a	10.00

Each of the data is a mean of three replacements. Each data followed by the same alphabet along the columns not significantly different at P= 0.05, using (DMRT) Duncan Multiple Test to separate the means.

Table 2: Percentage reduction of mycelia growth of spoilage fungi cultured in PDA incorporated with ethanol plant extract of 20% and 30% concentrations.

Rot Fungi	*Venonia amagdalina*		*Tridax procumbens*		Control (mm)
	20%	30%	20%	30%	
Aspergillus niger	46.00b	61.00ab	48.00a	68.20a	18.00
Fusarium oxysporum	50.00ab	65.20a	50.00a	70.20a	16.00
Rhizopus stolonifer	44.00b	56.20b	46.00a	59.20c	14.00
Geotricum candidium	56.00a	49.00c	48.00a	60.00bc	16.00

Each of the data is a mean of three replacements. Each data followed by the same alphabet along the columns not significantly different at P= 0.05, using Duncan Multiple Test (DMRT) to separate the means.

The isolated fungi were identified on the basis of cultural and morphological features as *Aspergillus niger*, *Fusarium oxysporum*, *Goetrichum candidium* and *Rhizopus stolonifer*. The leaf extracts were prepared with distilled water at 80 and 60% concentration, also 30 and 20% ethanol

concentrations and their effects were studied. The aqueous leaf extracts of both V. *amygdalina* and T. *procumbens* inhibited as well as ethanol leaf extracts, only that, at higher aqueous concentrations in both test plants, more mycelia reduction was recorded; the same thing was applicable to ethanol concentrations. The maximum mycelia growth was observed in the control (untreated).

Conclusion

The inhibitory effect of the plant extracts could be alluded to the presence of antimycelia substances. Greater inhibition of fungal growth was observed at higher concentrations of the aqueous and ethanolic extracts. *R. stolonifer*, *F. oxysporum*, *G. candidium* and *A. niger* are common pathogenic fungi which cause tuber rot, fruit and vegetable rot. The results of the present investigation are vivid indications for the potential of plant extracts to control fungal pathogens. It is also clear from the result that both the test plant extracts significantly reduce the radial growth of isolated fungi. It seems that the antifungal and the antimicrobial effects are the results of many compounds acting synergistically (Bangamolla *et al.* 2004). Plant extracts belonging to 12 families Russel and Muss (1977) and *Prosopis Juliflora* Raghavenda *et al* (2002) were used to control *Fusarium* specie. Fokunang *et al* (2000) reported the efficacy of antimicrobial effects of *V. amygdalina* extract on the growth of *Collectrotrichum gleosporiedis* causing cassava anthracnose disease. Bajwa *et al* (2001) reported antifungal activities of *Asteraceae* on the growth of *Aspergilli*. This can be formulated and successfully devised as fungicides with easy process of minimal instrumentation together with little chemical agents which can be applied as both seed and foliar spray. 80% aqueous extract of *V. amygdalina* inhibited *G. candidium* to 49.20%, while the highest of 60% aqueous extract concentration was 43.90% against *R. stolonifer*. In *T. procumbens*, 80% aqueous extract reduced the mycelia growth of *A. niger* to 53.30%, in ethanolic extract of both plants, *V. amygdalina* and *T. procumbens* inhibitory effect of 65.20 and 70.20% were recorded against *Aspergillus niger* respectively.

Thus, it can be recommended that the use of *V. amygdalina*, *T. procumbens* against tomato rot pathogens: *F. oxysporium*, *R. stolonifer*, *G. candidium* and *A. niger* would have better results as they are biologically based and environmentally safe. It can be inferred also that plant extracts are effective antimicrobial agents against food spoilage and post harvest fungal pathogens without residual effects. The plants used are not hazardous, non pollutant, safe cost, easy to formulate and apply; also it does not affect ecological balance. The results of this work can as well be further explored for designing integrated pest management strategy for tomato crop. More studies can be done to determine the regard for isolation and characterization of antimycotic moieties and subsequently recommend in field applications.

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