IN VITRO AND *IN VIVO* ANTIBACTERIAL EFFECT OF AFRICAN STAR APPLE (*Chrysophyllum albidum* Linn.) EXTRACTS ON BACTERIAL WILT OF TOMATO

BY

LAWANI Yetunde Olamiposi
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……

Prof. (Mrs) A. O. Salami

Supervisor

……

Dr. (Mrs) A. O. Oluduro

Co-Supervisor

……

Dr. B. J. Amujoyegbe

Head of Department

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ABSTRACT

This study was carried out to investigate the antibacterial activity of leaf and stem bark extracts of African star apple, *Chrysophyllum albidum* on *Ralstonia solanacearum*, the causative organism of bacterial wilt of tomato; the antibacterial effects of leaf and stem bark extracts of *Chrysophyllum albidum* on pathogenicity of *Ralstonia solanacearum* infecting tomato and to determine the phytochemicals present in the extracts. This was with a view to providing information on the antibacterial effects of the extracts on *R. solanacearum*.

The sensitivity assay was carried out using agar well diffusion method at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml of each extracts and 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml of streptomycin. The minimum inhibitory concentration was determined using broth dilution method while the minimum bactericidal concentration was determined using spread plate technique. The effect of the extracts on the pathogenicity of *R. solanacearum* on tomato was carried out in the screenhouse using randomized complete block design with fifteen treatments in four replicates and two trials. The treatments included two levels of each extract (1000 mg and 500 mg), 20 mg streptomycin, inoculated control and uninoculated control. The data obtained were subjected to analysis of variance using SAS statistical package. Significant means were separated using LSD at 5% probability level.

The results obtained showed that the extracts have antibacterial effect on the test organism *in vitro* except for aqueous leaf extract at 25 mg/ml. Methanolic stem bark extract compared favourably with streptomycin, a synthetic bactericide. The minimum inhibitory concentration of the extracts ranged from 6.25 mg/ml to 50 mg/ml while the minimum bactericidal concentration ranged from 25 mg/ml to 50 mg/ml. The extracts significantly reduced the extent of pathogenicity of the test organism on tomato in comparison with the inoculated control. For the concentration of the extracts used, 1000 mg gave a lower disease severity in comparison with 500 mg. For the diluents used in extraction, ethanolic extracts gave the lowest disease severity

followed by methanol and then water. For the plant part used, stem bark extracts gave a lower disease severity in comparison with leaf extracts. The extracts reduced the severity of the disease on tomato. Tomato plants treated with the extracts gave a higher shoot weight in comparison with the inoculated control. Phytochemicals such as saponins, tannins, flavonoids, terpenoids, steroids, glycosides and alkaloids were found in African star apple extracts.

In conclusion, the extracts had both *in vitro* and *in vivo* antibacterial effect on *Ralstonia solanacearum* and this can be attributed to the phytochemicals in the extracts.

CHAPTER ONE

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae. It is native to the Andes region of South America from where it spreads to Central America (Naika *et al.*, 2005). Villareal (1990) reported that the crop originated from tropical parts of central and southern America, was domesticated in Mexico and later taken to Europe and across the pacific by the early Spanish explorers and Portuguese traders. An intensive breeding programme has since produced the wide range of tomato cultivars available throughout the world today (UNCTAD, 2012). According to Uguru (1996), tomato is a short lived herbaceous annual with weak trailing, much branched stems covered with hairs at the juvenile stage of development.

Tomato is a warm season crop that requires a temperature range of 65^{o}F to 85^{o}F for optimum yield. Tomato plants react to temperature variation during the growth cycle for seed germination, seedling growth, flower and fruit set and fruit quality (Shankara *et al.,* 2005). Yield can be reduced due to poor fruit-setting caused by high temperatures and disease problems (Fajinmi and Fajinmi, 2010).

Tomato is one of the most important vegetables and one of the most widely cultivated crops worldwide (Seleim *et al.*, 2011). It is a good source of minerals, vitamins and health acids, with global production estimate of 124.75 million tonnes (FAO, 2007). Tomato is grown for its edible fruits and thousands of cultivars have been selected with varying fruit types and for optimum growth in different environmental conditions. Naika *et al.* (2005) reported that tomato is a relatively short duration crop that gives a high yield, it is economically attractive and the area under cultivation is increasing daily. Tomatoes contribute to a healthy, well-balanced diet. They are rich in minerals, vitamins, essential amino acids, sugars and dietary fibres. Tomato contains

much vitamin B, the antioxidant C, iron and phosphorus, lycopene and β-carotene. It was reported by Ajayi (2013) that red raw tomatoes has the following nutritional value for-100 g; Energy 20 Kcal-80 KJ; Carbohydrates- 4g; Sugar-2.6g; Dietary fiber-1g; Fat-0.2g; Proteins-1g; Vitamin C (13mg) and Water 95g. Tomato fruits are harvested and consumed fresh in salads or cooked in sauces, soups and meat or fish dishes. They can be processed into purées, juices and ketchup. Canned and dried tomatoes are economically important processed products. Beecher (1998) reported that tomato fruits are significant source of nutrition for substantial portions of the world's human population because the crop is widely cultivated and consumed extensively as both fresh vegetable and concentrated processed products.

However, sustainable tomato production is constrained worldwide by pest and diseases and more than a hundred of them have been reported on tomato (Dairo and Akintunde, 2012). Champoiseau and Momol **(**2009) reported that the production of tomatoes is being limited by pests and diseases which cause serious losses, among diseases bacterial wilt caused by *Ralstonia solanacearum*, formally *Pseudomonas solanacearum* has been reported to cause reduction in yield and economic losses of tomatoes. In commercial tomato production, presence of bacterial wilt may result in significant yield reduction and complete loss under favourable disease conditions (Boshou, 2005). Amusa and Odunbaku (2007) reported that tomato cultivation in the humid forest of western Nigeria has been abandoned due to the activities of tomato wilt caused by *R. solanacearum*. Control of bacterial wilt has been difficult, at present the disease is controlled by application of various methods including sanitation, chemical treatment, selection of resistant cultivars and soil management (Abo-Elyousr and Asran, 2009).

Chemical control through soil fumigation and use of antibiotics (Streptomycin, Ampicillin, Tetracyclin and Penicillin) have shown little suppression of *Ralstonia solanacearum* (Abo-

Elyousr and Asran., 2009). Also, incessant and extensive use of these synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity (Campos *et al*., 2005). A large number of synthetic pesticides have been banned in the western world because of their undesirable attributes such as high and acute toxicity, long degradation periods, accumulation in the food chain and an extension of their power to destroy both useful and harmful pests (Ortelli *et al*., 2005). Most of these chemicals have preventive action only and cannot cure infected plants. Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative means for management of pathogenic microorganisms (Mahajan and Das, 2003).

The use of plant extracts is found to be an effective way of controlling plant diseases compared to synthetic chemical as plant extracts have several advantages over it (Jai *et al*., 2011). Plant-derived compounds are major area of interest to source for safer and more effective antibacterial agents (Mann *et al*., 2008). Green plants are found to be reservoir of bioactive molecules and can provide valuable sources for the discovery of natural pesticides (Akhtar *et al*., 1997). Many reports of antibacterial activity of plant extracts against human and animal pathogens and their pharmaceutical application are available (Bylka *et al*., 2004; Kilani, 2006). Vudhivanich (2003) also reported that there are many reports on the use of herbal extract in plant disease control but only few of them are on phytopathogenic bacteria.

African star apple is one plant that has been reported as useful in treating various diseases (Adebayo *et al*., 2010). It is reported as an excellent source of vitamins, irons, flavours to diets and raw materials to some manufacturing industries (Adisa, 2000; Bada, 1997). The fruits of African star apple are widely eaten in southwestern Nigeria and have been found to have a very high content of ascorbic acid with 1000 to 3,300mg