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Effects of field spray of selected botanicals on management of *Phytophthora colocasiae* induced taro cultivars cormels myco storage rots in Nsukka, Southeastern Nigeria

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Abstract: Anti-myco pathogenic effect of field spray of botanical extract from neem (*Azadirachta indica), Eucalyptus (Eucalyptus spp)* and siam weed (*Chromolaena odorata*) against *Phytophthora* leaf blight disease of taro cultivars at weekly spray intervals were determined *in vitro* on growth of post-harvest myco pathogens of stored taro cultivars cormels. The storage, isolation and identification of the isolates were done at the Department of Crop Science Taro barn and Plant Pathology Unit, University of Nigeria, Nsukka. Results showed that the following fungal isolates were identified; *Botryodiplodia theobromae*, *Sclerotium rolfsii, Aspergillus niger, Fusarium solani, Rhizopus stolonifer, Penicillium digitalium and Trichoderma viride* with *Botryodiplodia theohromae* being the most occurred among the pathogens in all taro stored samples. The inhibitory potential of extracts varied with cultivars and pathogens. The percentage (%) of occurrence of isolates was highest in the coco India cultivar with no treatment, while the least was in purple green taro/Nachi cultivar with Ridomil gold treated samples. However, all botanicals proved effective in reducing post-harvest rot organisms compared to control in all samples. The fungitoxic potential of field sprays of botanicals against myco-rot pathogens of stored taro cultivars cormels is highly recommended to farmers as alternative natural fungicides to common potential health danger synthetic commercial fungicides.

Keywords: Taro cormels, post-harvest rot organisms, botanicals, *Phytophthora colocasiae,* Nsukka.

INTRODUCTION

Phytopathogenic fungi are one of the most biotic agents in plants causing alterations during the different plant growth stages, post-harvest and even during storage. *Colocasia esculenta* (L.) popularly known as taro (Ugwuoke *et al*., 2008; Eze *et al.,* 2015) is an important edible herbaceous underground starch-rich tuber crop in many developing countries of Central and West Africa (CWA), Pacific (Brooks, 2005). It belongs to a member of the aroid family, Araceae (Ugwuoke *et al*., 2008). It is the third most important root and tuber crop after cassava and yam in Nigeria, second to cassava in Cameroon and first in Ghana (Echebiri, 2004), and the fourteenth vegetable crop in the world (Brooks, 2005). The consumption system of taro edible parts is transforming from poor-income resource families, low-income women, large families, scarcity food to all years round food, public ceremonial/social function foods to all poor and rich-income resource families and groups depending on product availability, preservation and storability (Omeje *et al.,* 2020). The consumption of the taro diet in the southeastern States of Nigeria is increasing exponentially both at home and public social functions among the low and rich-income resource classes due to its high delicacy, nutritional, medicinal, industrial and socio-economic/cultural benefits (Mwenye, 2009; Omeje *et al.,* 2020), as well as its increased versatility and utilization in various food formulations.

At present, *Phytophthora* taro leaf blight caused by *Phytophthotra colocasiae* and post-harvest rot causing pathogens are the most serious challenges among the taro production stakeholders, processors and marketers globally (Omeje *et al.,* 2020). The rotting of tubers is another major factor after *Phytophthora* taro leaf blight that affects the qualities and quantities of tubers for consumption and planting materials (Eunice and Osuji, 2008) and other taro agro-based industrial uses. The resulting rapid decline in taro production and post-harvest storage are threatening the survival and existence of the crop and extinction is obvious (Ugbajah and Uzoegbuna, 2012), thereby making nations' sustainable food production, nutritional, medicinal and socio-economic empowerment impracticable and less effective. In most instances, the management of post-harvest quality losses should start before harvest in the field during the production cycle. The first line of proper post-harvest rot management is the adoption of pre-harvest infection management especially against soil-borne pathogens. The field management of TLB and post-harvest losses still depends on the use of synthetic fungicides. This often leads to human and livestock poisoning, environmental pollution, the emergence of resistance and resurgence of pests and diseases and the presence of pesticide residues in agricultural produce (Sande *et al*., 2011), including heavy metal residues and even poisoning of the users (Pakora *et al*., 2017). More so, excessive application of inorganic fungicides creates minerals/nutrients imbalance and heavy metal and pesticide residue contamination (Kosalec *et al.,* 2009; Lu *et al*., 2015).

It is necessary to investigate the plant-based pesticides that could become an alternative strategy against TLB, an improvement on post-harvest qualities and shelf life of taro cultivars. It has been reported that pesticide extracts of plant origin are specific, cheap, readily available, highly biodegradable and environmentally friendly (Kumar *et al.,* 2008). It could be used as an alternative to inorganic fungicides so that the environmental pollution, chemical pesticides, and heavy metal residues in agricultural products/soils can be minimized. Nevertheless, many plant-derived pesticides have been extensively studied as alternative treatments against plant diseases in order to avoid all limitations of synthetic fungicides by pathogenic organisms (Timothy *et al*., 2011; Idowu *et al*., 2005**)**. Many botanicals and their phytochemical compounds have been proven by many researchers to have promising fungicidal values of which Neem (*Azadirachta indica)* (A. Juss), Siam weed (*Chromolaena odorata)* and *Eucalyptus* (*Eucalyptus spp)* plant leaves extracts are inclusive.

Azadirachta indica (A. Juss) of the mahogany family. Meliaceae is one of the two species of the genus *Azadirachta*. It is an evergreen plant whose leaves shedding and re-generation evergreens do not shade their leaves, flowering and seeding occur concurrently around December- January months. It is commonly used in many traditional medicines as a source of therapeutic agents in Indian culture and can be used for the treatment of diabetes (Maragathavalli *et al*., 2012), soil amendment, insecticidal storage pesticides and fungicides (Akhtar and Manhood, 1997). *A. Indica* (A. Juss) leaves and seeds have been reported earlier for their possession of anti-fungal, insecticidal, anti-feedant, diuretic, anti-bacterial, and nematicidal properties due to the presence of many bioactive compounds.

Chromolaena odorata L. is a tropical shrub of flowering shrubs in the sunflower family, Asteraceae, which originated from North America. It adapts to most soils and is a prolific weed found in abundance on open wasteland along road sites (Ling *et al.,* 2007). *C. odorata* is used as an anti-bacterial, anti-inflammation, anti-hypertensive, astringent, anti-plasmodic and anti-protoza potential, anti-hepatotropic, trypanosomal, anti-fungal agent (Phan *et al*., 2001; Akinmoladun *et al*., 2007), nematicidal (Agaba *et al*, 2015) and anti-stomach ulcer. Owolabi *et al*. (2010) have reported the inhibition of *Aspergillus niger* with some extract of siam weeds. Plant extracts have played a significant role in the inhibition of seed-borne pathogens, *Fusarium oxysporium* (wet rot) and in the improvement of seed quality and emergence of plant seeds (Nwachukwu and Umechuruba, 2001).

Eucalyptus spp is a genus of over seven hundred species of flowering, evergreen trees, shrubs or mallees in the myrtle family, Myrtaceae. *Eucalyptu*s *spp* along with other tribes of *Eucalyptecae* are commonly referred to as Gums or *Eucalyptus*. The genus *Eucalyptu*s have bark that is either smooth, fibrous, or hardy, always scaling annually and the leaves with oil glands. *Eucalyptu*s *spp* oils extracted from leaves are reported to have some biological and pharmaceutical properties (Sameza *et al,* 2014). *Eucalyptus spp* oils have been used in the pharmaceutical, cosmetic, food and medicinal industries (Hur *et al*.,2000) due to their natural compounds that are disinfectants, insecticidal and anti-microbial potentials. *Eucalyptus spp* leaves especially *E. globulus* leaves have been used to protect post-harvest agro products against bacteria, fungi, and insect damage among farmers in Cameroon (Sameza *et al*., 2014). Although the effectiveness of these plants has been confirmed scientifically based on their antifungal activities and have been severally reported as possessing anti-microbial and insecticidal properties against a wide range of plant pathogens. However, documented reports of the effect of *Azadirachta indica* (A. Juss), *Chromoleana odorata* and *Eucalyptus spp* leaves extracts on-field management of *Phytophthora* leaf blight disease and their residual effect on the improvement of post-harvest qualities of taro cultivars are at the infant stage.

The dwindling availability of fungicides (Better Crops,1998), together with increasing public concern for the environment means that the use of plant materials to manage both field and post-harvest pathogen are attracting increased attention. Therefore, there is a need to compare a synthetic fungicide with botanical extracts for the improvement of post-harvest rot of taro cultivars cormels. The study will provide better management of post-harvest myco rot disease strategies and improvement on post-harvest qualities of taro cultivars that will meet the needs of all classes of taro stakeholders and all prospective farmers. Thus, the specific objective of this study is to determine the effect of botanicals against *Phytophthora colocasiae* management on the occurrence of myco-rot pathogens in stored taro cultivars cormels.

MATERIALS AND METHODS

Experimental area

The experiment was conducted at the taro storage barn and Pathology laboratory of the Crop Science Department, University of Nigeria, Nsukka. Nsukka is located in the derived savannah zone at latitude 6°52'' North and longitude of 07°24'' East with an altitude of 447.26 meters above sea level (m.a.s.l). The area has a mean annual rainfall range of 1500-2000 mm. The rainfall pattern is bimodal starting from March/April - October/November with short periods of low rainfall and relative humidity stress popularly referred to as August break, and peak in June and September.

Source of botanical samples

Neem leaves were collected from the tree stands in Enugu Ngwo along Milkin hill 9th Miles corner, Enugu State, Nigeria. Siam weed leaves were collected along the roads and wastelands in Nsukka area, Enugu State, Nigeria, while the leaves of *Eucalyptus spp* were collected from tree stands at the school environment of Enugu State Polytechnic, Iwollo, Enugu State, Nigeria.

Preparation of botanical extracts

The collected fresh plant leaves were washed thoroughly with tap water and then distilled water separately. They were air-room dried for a week to a point where they would be dry enough for milling. The dried leaves were separately ground in a laboratory Mill after which the ground samples were sieved to obtain the processed powder for extraction. The processed powers were kept in a sealed airtight container. The cold solvent extraction method was adopted (Doughari *et al.,* 2007). The 100 g of each processed powdered sample was mixed with a litre of distilled water solvent separately in a large conical flask bottle to produce 100% concentrations, respectively and were allowed to stand for 72 hours.

The extracts were sieved through four layers of sterile cheese cloth filter and stored in a sterile conical flask for the study. The concentration was calculated by dividing the 100 g of sample separately by 100 ml of sterile distilled water. The extraction and sprays were on weekly schedules at 60–150 days after planting.

Source of taro cultivars cormels

Taro cormels from Nigeria *Colocasia esculenta* (Nce) cultivars, Coco India/Ugwuta (Nce 001) and Nachi/purple green taro (Nce 003) which are highly susceptible and fairly resistant to taro leaf blight epidemic with symptoms of postharvest decay, respectively (Omeje et al., 2015) were selected in the barn of the Department of Crop Science, University of Nigeria, Nsukka, Southeastern Nigeria. Samples of infected cormel parts in the stored taro barn were collected and taken to the pathology laboratory. Each sample was properly washed with tap water and then rinsed two times with sterile distilled water to remove surface contamination before surface sterilization with 0.2% commercial bleaching agent (sodium hypochlorite) for five minutes and rinsed in three changes of sterile distilled water (SDW). The segments of the infected cormel materials were dried between sheets of sterile filter paper followed by cutting sections of approximately 2 mm cubes from the junction between the healthy and infected position of cormels with surface-sterilized blades from the different treatments. They were inter–plated on water agar which was acidified with acetic acid and incubated for 7 days at room temperature 27±2°C. With a flame and sterile cork borer, 3 mm agar plugs were collected from the actively growing cultures of the suspected fungal pathogen and transferred into potato-Dextrose Agar (PDA) medium in 9 mm Petri dishes. The culture was incubated for 7 days at room temperature of 27±2°C and then examined daily for the growth and development of fungi pathogens. When the growth had been established, a subculture was prepared using inocula from the different organisms in the mixed cultures to obtain a pure culture. This was done by transferring hyphal tip from the colonies of the mixed cultures to a fresh plate of PDA using flame sterilized blade. After sub-culturing, the plate was inoculated at 27°C until the pure culture was obtained. The petri dish of pure cultures of isolated fungus was sealed with paraffin to prevent contaminations. The resulting pure cultures were used for characterization and subsequent identification of the fungi isolates with the aid of compound microscopes and identification guides (Sutton, 1980). Fungal frequency of occurrence/ isolated organisms was calculated from the isolation plates by using the formula as outlined by Okigbo *et al*. (2009) as below:

$$\text{Percentage frequency of fungal occurrence (\%)} = \frac{\text{Nf}}{\text{Na}} x100$$

Where Nf = Number of isolates of each fungus and Na = Total number of all isolates.

RESULTS

Fungi pathogens isolated in this study include; *Botryodiplodia theobromae*, *Sclerotium rolfsii, Aspergillus*

Table 1. Fungi isolates and percentage of occurrence on rotten taro cultivars cormels with botanicals against *Phytophthora colocasiae* stored 120 days after harvest.

Samples	Fungi isolates	% Occurrence
UNLE	*Botryodiplodia theobromae*	19.20
	Sclerotium rolfsii,	13.40
	Aspergillus niger	10.90
NNLE	*Botryodiplodia theobromae,*	16.50
	Sclerotium rolfsii,	12.60
	Aspergillus niger	10.60
UELE	*Botryodiplodia theobromae,*	20.90
	Aspergillus niger	14.30
	Sclerotium rolfsii	11.10
NELE	*Botryodiplodia theobromae*	19.00
	Sclerotium rolfsii	12.30
	Aspergillus niger	10.20
USLE	*Botryodiplodia theobromae*	18.30
	Sclerotium rolfsii	12.20
	Aspergillus niger	10.30
NSLE	*Botryodiplodia theobromae*	16.00
	Sclerotium rolfsii	10.00
	Aspergillus niger	9.20
URI	*Botryodiplodia theobromae*	15.40
	Aspergillus niger	5.30
	Sclerotium rolfsii	2.40
NRI	*Botryodiplodia theobromae*	13.90
	Aspergillus niger	4.90
	Sclerotium rolfsii	1.90
UZLE	*Botryodiplodia theobromae,*	38.50
	Sclerotium rolfsii,	26.60
	Aspergillus niger,	21.00
	Rhizopus stolonifer,	18.20
	Fusarium solani,	15.50
	Penicillium digitalium,	14.01
	Trichoderana viride	12.40
NZLE	*Botryodiplodia theobromae*	32.60
	Sclerotium rolfsii	23.50
	Aspergillus niger	19.00
	Rhizopus stolonifer	15.00
	Fusarium solani	17.20
	Penicillium digitalium	13.00
	Trichoderma viride	10.40

UNLE = Ugwuta neem leaf extract, NNLE = Nachi neem leaf extract, UELE = Ugwuta eucalyptus leaf extract, NELE = Nachi eucalyptus leaf extract, USLE = Ugwuta siam leaf extract, NSLE = Nachi siam leaf extract, URI = Ugwuta ridomil, NRI = Nachi ridomil, UZLE = Ugwuta zero leaf extract, NZLE = Nachi zero leaf extract..

niger, Fussarium solani, Rhizopus stolonifer, Penicillium digitalium and Trichoderma viride. The percentage occurrence varied with different fungi associated with the rotten taro cultivars cormels samples. *Botryodiplodia theobromace* showed the highest percentage occurrence of 38.50 % in Ugwuta cultivar with control treatment, while *Sclerotium rolfsii* showed the least percentage occurrence of 1.90% in Nachi cultivar with Ridomil gold treatment in the entire taro cultivars cormel samples (Table1).

DISCUSSION

Pre-harvest agronomic practices are key determinants of proper growth and development of taro cultivars and if well adopted will effectively promote post-harvest myco rot pathogens management. Post-harvest myco-rot organisms are very troublesome pathogens during storage globally, but the current increasing adoption of botanical pesticides in phytopathogenic disease management are effective for their control.

The myco-rot organisms responsible for post-harvest rot of taro cultivar cormels in this study were *Botryodiplodia theobromae, Sclerotium rolfsii, Aspergillus niger, Rhizopus stolonifer, Fusarium solani, Penicillium digitalium,* and *Ttrichoderma viride.* These were commonly isolated from rotten taro cormels (Okigbo *et al.,* 2009; Anukworji *et al*., 2012). These organisms have been reported to cause extensive rots of taro cormels in storage (Anukworji *et al*., 2012; Okigbo *et al.,* 2009; Nwachukwu and Osuji, 2008); Ugwuanyi and Obeta, 1996; Eze and Maduewesi, 1990). The isolation of more than one organism from a particular treated taro cormels confirms the possibility of multiple infections whose cumulative complex effect may cause rapid rotting of root and tuber crops which was in tandem with reports of Sangoyomi (2009) on yam. *Botryodiplodia theobromae* occurred in all treated taro cormel samples with also the highest percentage occurrence in all the respective taro treated cormel samples. This showed that *Botryodiplodia theobromae* was the most virulent organism among the isolated pathogens with 38.50% percentage of occurrence, while *Sclerotium rolfsii* showed the least percentage occurrence of 1.90%. Okigbo and Nnadiri, (2017) reported the highest virulence of *Botryodiplodia theobroamce* in the stored taro cormels. In most cases, fungi gain entry into taro cultivars cormels through natural openings and wounds, created by plant parasitic nematodes, field fungi or during harvesting, packing, transportation, handling and marketing. However, Okigbo and Nmeka, (2005) reported that roots and tuber crops at the time of harvest may already be infested by soil-borne pathogens derived from foliage disease, roots/mother corms/cormels. Arya (2010) has reported that pre-harvest treatment is the best strategy against soil-borne myco-inducing organisms as some pathogens start from field to storage.

The study on the inhibitory properties of these botanicals on fungal growth occurrence showed that botanical extract posed some inhibitory components which caused a significant reduction in the percentage occurrence of the pathogens. This showed that fungi toxic compounds were present in Neem, *Azadirachta indica*, Siam weed, *Chromolaena odorata* and *Eucalyptus spp* since they were able to reduce the fungal isolates, as well as the percentage occurrence of isolated pathogens. This was in consonance with the earlier reports of several researchers, though on different fungal pathogens - Udo *et al.* (2001) on the inhibition of growth and sporulation of fungal pathogens on *Ipomea batata* and *Dioscorae spp* with garlic extract; Okigbo and Nmeka (2005) on the use of *Xylopia aethiopia* and *Zingiber officinale* against *Fusarium oxysporium* of yam tuber rot. *A. niger* and *A. flavus;* Amienyo and Ataga (2007) on use of *Zingiber officiale*, *Annona muricata Garcinia cola*, *Alchorniea cordifolia*, *Allium sativum* against fungal wet rot on sweet potato; Ebele (2011) on use of *Carica papya, Chromoleana odorata* and *Acalypha ciliate* against pawpaw fungal fruit rot and; Tijjani *et al.* (2013) on use of *Azadirachta indica* and *Moringa oleifera* against wet rot disease on mechanically injured sweet potato.

The result also showed that the effectiveness of the applied botanical extracts (Neem, Siam weed and *Eucalyptus spp*) depends on the phytochemical properties and taro cultivar genotypes. The presence of phytochemical materials has been reported to exhibit resistance to plants against bacteria, fungi and pests (Srinivasan *et al.,* 2001). Both botanicals and taro cultivars have been reported to contain all the promising phytochemicals; flavonoids, alkaloids, tannin, phenol, saponin, phytates, oxalate, which are anti-microbial agents (Okwu and Joshia, 2006).

This study has also shown the potential of botanicals (Neem, Siam weed, *Eucalyptus spp*) extracts on the management of taro rots from field to storage. The Siam weed extract was more effective than other botanicals. This may be attributed to some bioactive components that may have anti-fungal and anti-parasitic nematode properties. Agaba *et al.* (2015) have reported that siam weed extract has both fungicidal and nematicidal properties which may help to reduce field damage, as well as reduction in post-harvest rot organisms leading to a low percentage occurrence of the isolated organisms in taro cultivars cormels treated with siam weed (*Chromolaena odorata*).

The cultivar genotypes variabilities play a key role in the post-harvest rots and disease damage in general. Ugwuta cultivar was the most susceptible to myco organisms in all the respective treated taro cultivars cormels. Omeje *et al.* (2015) reported that Ugwuta is highly susceptible to rot pathogens, while Nachi cultivar is more resistant to rot organisms.

The commercial fungicide showed a very significant effect in reducing the percentage occurrence of the isolated fungi, meanwhile, *Sclerotium rolfsii* and *Aspergillus niger* showed the least percentage occurrence with Ridomil gold in all respective Ridomil gold treated taro cormel samples. However, the result of this study has gone a long way in providing better alternatives to the over dependence on imported/commercial synthetic fungicides. The use of botanicals in reducing rot causing organisms could reduce over reliance and cost of production on one source of agro-chemical to the farmers, which have been reported to cause long-term harmful consequences on environmental safety, humans and wildlife, as well as food poisoning. The prospects of relatively cheaper means of reducing rot organisms could then be safer and brighter, particularly for peasant farmers across the globe and Nigeria in particular.

The study revealed that extracts of botanicals; Neem and Siam weed, could be an alternative to synthetic fungicides in reducing taro cultivars cormels rot from the field to storage and where not available, *Eucalyptus spp* which are locally available, less expensive and environmentally friendly could be an alternative. The challenges of post-harvest rots from taro field production to storage are really clear and require urgent management at pre-harvest stage in order to salvage taro, the third-ranked root and tuber crops in Nigeria and the most essential diet in southeastern Nigeria for further declining crops.

Conclusion and Recommendation

The study showed that the selected botanicals have a promising fungitoxic potential in reducing post-harvest rot organisms of stored taro cultivars cormels. The botanical extracts of siam weed, *Chromolaena odorata* and Neem, *Azadiratchta indica* could be alternatives to synthetic fungicides in reducing post-harvest rot organisms from field to storage environment and where not available *Eucalyptus spp* leaf extracts could be alternative natural fungicides that are less expensive, locally available, non-food poisoning, anti-nutrients reducing agents and environmentally safe. Thus, pre-field sprays of botanical extracts are highly recommended to farmers as an alternative natural fungicide to the common health danger of synthetic commercial fungicides.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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