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# SURVEY AND CONTROL OF POSTHARVEST FUNGAL ROT PATHOGENS OF IRISH POTATO (*Solanum tuberosum* L.) IN BIU LOCAL GOVERNMENT AREA, BORNO STATE

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***Abstract:*** **A survey for the incidence of post-harvest rot of fungal pathogens of Irish Potato was carried out in four locations within Biu Local Government Area of Borno State,the sample size of 80 tubers / market (320) was taken and the average percentage incidence of rot was 9.06%, the highest rot was in Mirnga Market (13.25%) while the lowest was in Hospital Market (6.25%). Four fungal pathogens. were associated with this rot (*Aspergillus flavus, Thielavia terricola, Rhizopus stolonifer and Scopulariopsis brevicaulis*) the most frequent occurring was Rhizopus stolonifer while the least frequently occurring was *Aspergillus flavus. Thielavia terricola* was most pathogenic with rot lesion size of 14.11mm. In vitro test with garlic oil, neem seed oil and *tridax* leaf ash showed that the test substances were significantly effective (p=0.0001). *Tridax* leaf ash had the highest inhibition of 18.50 while neem seed oil and garlic oil had the least inhibition rate of 20.16 and 24.18 respectively. In vivo test with garlic oil, neem seed oil and *tridax* leaf ash at p=0.0001showed that the test substances were significantly(0.0001) effective. *Tridax* leaf ash had the highest inhibition of 3.13. while Garlic oil and neem seed oil had the least inhibition rate of 4.02 and 4.19 respectively. It is therefore suggested that more research should be conducted on the ash with garlic oil and neem seed oil and their effects on the same pathogens or similar ones.**

***Keywords:*** **fungal pathogens, Irish Potato.**

## 1. INTRODUTION

Potato *(Solanum tuberosum)* is a dicotyledonous plant belonging to the *Solanaceae* family (Dutta, 2005). It is cultivated in temperate and subtropical regions across the world (Vaughan and Geissler, 2009). It is the most important vegetable crop of the world, ranking number one among all vegetables both in production and consumption (Abbas *et al.*, 2011). It has become an integral part of much of the world cuisine and is the fourth largest yielding crop in the world after wheat, rice and maize (FAOSTAT, 2008).

It contains higher crude protein than any other root and tuber crop because of the low colorie and high vitamin C content and can be regarded as an important nutritive food (Burton, 1966). Potato is an important source of dietary fiber and in Great Britain contributes 15% of the intake of food (Burton, 1966). Vitamin C is the main vitamin in potatoes (Burton, 1966). Fresh tubers have vitamin content in the range of 15-24mg per 100g fresh weight (Abbas *et al.*,2011). Potato is also used medicinally (Abbas *et al.*,2011). People take raw potato juice for stomach disorders and water retention (edema)(Abbas *et al.*,2011). A purified protein powder made from potato is mixed with water and used to control appetite for weight loss (Abbas *et al.*,2011). Some people put raw potato directly on the affected area for arthritis, infections, boils, burns, and sore eyes (Vaughan and Geissler, 2009). Apart from being used as food for humans and domestic animals, it is now used in the food industry as, for example, thickeners and binders of soups and sauces, in the textile industry, as adhesives, and for the manufacturing of paper and boards (Martin and Garry, 1994). Potatoes in storage are vulnerable to molds that feed on the stored tubers, quickly turning them rotten (Martin and Garry, 1994).

**Statement of the Problem:**

Potato is an important crop which provide for food to millions of people especially in developing countries (Arora and Paul.2004) and provides a balanced source of starch, vitamins and minerals to many communities in the global village (Rowe, 1993) it contributes to reducing worldwide food shortages (Han *et al.*, 2005) According to Selazar (1982) the crop is adapted to a cool moist climate, and grows in the high altitudinal ecosystem of Sub-Saharan Africa where rainfall is well distributed for 3-4months.

Irish potato *(Solanum tuberosum* L.) tuber is a nutritious but highly perishable crop that is subject to high fungal spoilage and wastage due to non-availability of appropriate post-harvest storage techniques (Thomas and Rosemary 2005). Post-harvest rot has been important in nearly every growing country of the world (Wikipedia 2013). The problem posed by post-harvest rot causes reduction of the quality of potatoes (Wikipedia 2013). Seed-borne diseases are controlled by seed treatment practices; seed treatment is the oldest in plant protection (Nelson *et al.*, 1993). The origins of seed treatment can be traced to the 18$^{th}$ century with brine for the control of cereal smuts (Neegard, 1988). The modern era of seed treatments began with the introduction of organo-mercury fungicides in which were widely used for several decades (McGee, 1995).

**Specific Objectives of the Research:**

I. To determine the incidence of the disease.

II. To isolate and identify the fungi associated with the rots.

III. To determine the severity of the pathogen.

IV. To carry out control trials using neem seed oil, garlic oil and tridax leaf ash.

***Fungi*:**

Thomas and Rosemary (2005) identified the following fungi; Fusarium species (potato rot), Scierotinia sclerotiornm (white mould), Helminthospnrium solani and Collctotrichum spp. (skin blemishes), Phytophthora infestans,(late blight), Phvtophthora crytltroseptic (pink rot). Botrytis cinerea (grey mould), Phoma exiguo var foveate: (gangrene), Synchvtrium elidibiotichuml (potato wart), Streptomyces scabiest, (common scab) while fungi responsible for rot included Alternaria solani, Fusarium species, Rhiotonia, Stemphylium species and Botrvtis Species). Other species of Fusarium reported to cause dry rot of potatoes include; F.avenaceum,and F.culmorum (Secor and Salas,2001;Peters et al.,2008a;b). A recent research showed that four species are responsible for almost 95% of the dry rot found the Great Britain (Peters et al.,2008a). F.equiseti, F.sambucimum, F.oxysporum, F. solani, F.culmorum, F.lateritium, and F.vertillioides were identified by Bita et al.(2003) as pathogens responsible for dry rot in Iran. Also Akinleye et al. (2003) reported Fusarium solani,. Fusarium oxysporium, Aspergillus niger and penicillium spp to cause post-harvest rot of potato in Nigeria.

***Control:***

In-order to ensure steady supply of the crop for any purpose, disease control measures have to be employed to curtail disease spread and effects.

***Plant or Botanical extracts with fungal properties:***

Considerable research activity has occurred in the Asian-Pacific region on the potentials for plant extracts to control soil-borne fungi. The oils of cassia and clove inhibit the growth of established soil-borne infection of *Aspergillus flavus,Curvularia pallescens* and *Chaetomium indicum* in maize (Chatterjee, 1990). Neem seed and garlic extracts contain Azadiractin and allicin respectively which are believed to have antifungal properties which were used in the treatment of *Alternaria padwickii* in rice seed (Shetty, *et al.*, 1989. In addition, since high glycaemia increases yeast infections risk and components in garlic drop glycaemia, therapy of garlic provides an extra advantage in the treatment of yeast infections (Ayaz and Alpsoy, 2007). Ann *et al*. (2009) demonstrated that a combination of Amphotericin B (AmB), which is the gold standard of antifungal treatment for the most severe invasive mycoses, with allicin proved to be a promising strategy for the therapy of disseminated candidiasis. The researchers, Ann *et al*. (2009) observed that allicin, an allyl sulphur compound from garlic, has shown to significantly enhance the effect of AmB against *Candida albicans* and *Aspergillus fumigatus in vitro* and *in vivo*, although allicin did not exert a fungicidal effect.

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## 2. MATERIALS AND METHODS

**Study Area:**

The study was conducted in the laboratory of Plant science Department, Modibbo Adama University of Technology, Yola, in 2018 where the isolation, identification and control was carried out.

**Site for Survey and Sampling Collection:**

The survey and collection of potatoes was carried out in Biu Main Market, Shopping Complex, Hospital Market and Mirnga Market, in Biu Local Government Area of Born state in the year 2015. The diseased potatoes was collected from each location where the types of rot were identified as either soft or dry rot,

Eighty potatoes samples was purchased / collected randomly in each of the four markets mentioned. The samples collected from the four markets were kept in sterile polythene bags and labeled then taken to the laboratory for further studies. Three hundred and twenty potatoes constituted the sample size.

***Incidence of potato tuber rot assessment:***

This was estimated by identifying the total diseased tubers from the Three hundred and twenty tubers purchased minus the diseased tubers this was expressed as percentage incidence as follows:

Taking A= number of potatoes purchased.

B= number of diseased potatoes.

C=% incidence of diseased tubers.

$$\frac{A-B}{A} \times 100 = C$$

***Media Preparation and Sterilization:***

The media employed for the studies was similar to the one of Smith and Onions, (1994). Industrially prepared potato dextrose agar (PDA) was used. Twenty grams was dissolved in 1 liter conical flask and volume was made up to one liter using sterile distilled water homogenized in an autoclave for 15mins at labs Pressure.

The media after preparation was sterilized by autoclaving the flasks with the media in an autoclave for 15mins at 101 lbs pressure and allowed to cool. The Petri dishes used were sterilized in an oven at 160°C for 6 hours, by using sterilization can. The needle and cork borer used for inoculation was sterilized by flaming and after which they were cooled by dipping them into a methylated spirit. The inoculation of the organisms was carried out in an inoculating chamber. The table in the inoculation chamber was wiped with 95% ethanol and the UV light was put on to sterilize the inoculating chamber for 30mins.

**Isolation and Identification of Causal Organisms:**

Five millimeter square piece of the lesion on the potato was removed using scissors, and then surface sterilized using Mercuric chloride solution for thirty seconds in a Petri dish to remove surface contaminants. Subsequently the sectioned piece surface was dried between two sterile filter papers. This was aseptically transferred to the solidified sterile potato dextrose agar (PDA) in a 9cm diameter Petri-dish using flamed and cooled forceps.

The plates was incubated at $25^0$C in the inoculation chamber, for 72 hours and observed for any growth. The resulting single spore colonies after series of sub-culturing was transferred to fresh potato dextrose agar (PDA). Stock cultures are usually stored in McCartney bottles at 40°C The organism isolated was stained with lactophenol cotton blue viewed under the microscope and subsequently identified, by comparing the morphological characteristics of the organisms observed under the light microscope with the structures in Alexopoulus and Mins (1966).

**Pathogenicity Test:**

Healthy lesion free potato tubers (Solanum tuberosum ) were used in this experiment. Tubers appearing healthy and uniform in weight (I00-120g) were selected and washed in sterile water to remove excess soil, and surface sterilized in Mercuric chloride solution for thirty seconds and rinsed in three changes of sterile distilled water and then air dried (Liu

and Kushalappa, 2002). The tubers were wounded with a sterile cork borer with a diameter of 4mm to a depth of 20mm and borer tissue was removed. (Choiseul et al., 2007; Peters et al., 2008,). A sterile needle was used to section the pathogen culture of each from the plate (2mm)and inoculate into the tuber where the wound was sealed with sterile vesper prepared from wax and Vaseline. The control was set up in the same manner except that 2mls of sterile distilled water was used instead of the inocula. All the potato tubers wounded were wrapped in black polythene bags (Manici and Cerate, 1991).

Tubers inoculated were placed in desiccators and incubated at $30^0$C the setup was four isolates replicated three times in a completely randomized design. All these were carried out under aseptic conditions. Regular observations were made while making a regular measurement using millimeter ruler and re-isolation of pathogenic organism was done for comparison with the original isolates. Records/ruler of lesion size in millimeters measurements were made for a period of fifteen days. Measurements were subjected to analysis of variance while lesions were compared to the original lesion observed on the collected samples.

**Control of Fungal Pathogens:**

The food poison method of Nene and Thalpiyal. (2000) was used to assess the efficacy of the oils and ash in controlling pathogens of Irish potato the *in vitro* test was carried out as follows. The fungicidal properties of each of the treatment ash of tridax and neem with garlic oils respectively was tested on the mycelial growth of the isolated pathogens by growing it on PDA amended with different concentrations of botanical oils and quantities of aqueous ash. In the in vivo control trial, a sterile needle was used to cut the pathogen from the plate and inoculate into the tuber. Similarly ruler measurements of lesion sizes of the incubated potatoes were taken.

***In-vitro control:***

*In-vitro* control was carried out using food poisoning method of Nene and Thalpiyal (2000). The potato dextrose agar (PDA) was amended with 2mls, 3mls and 5mls of the botanical oils and aqueous solution of tidax leaf ash after which the pathogen was introduced and allowed to grow / incubated for seven days under observation and record. Growth inhibition of the mycelial at different concentration of the oils and quantities of ash were determined by measuring the diameter of the mycelial growth using millimeter ruler.

***In-vivo control:***

The method of Nene and Thalpiyal (2000) was used where healthy fresh potato tubers were purchased from the Market in Biu Main Market and conveyed to the laboratory of plant science department in sterile polythene bag. The tubers were washed with sterile distilled water and deeped in mercuric chloride for thirty seconds and rinsed in three sets of distilled water. The tubers were wounded with a sterile cork borer (flamed and cooled) with a diameter of 4mm to a depth of 20mm and borer tissue was removed. Volume of 2mls, 3mls and 5mls of the botanical oil and aqueous ash was mixed with the media before inoculating the pathogen. Similar set was made to serve as control using sterile distilled water. A sterile needle was used to section 2mm of the pathogen from the plate and inoculated into the tuber. The wound was sealed with sterile vesper prepared from wax and vaseline. The control was set up in the same manner except that 2mls of sterile distilled water was used instead of the inocula. Rot development due different concentrations of the oils and quantities of ash was determined by measuring the diameter of the rot lesion using millimeter rule. All were done in replicates of three and incubated at room temperature for seven days.

***Preparation of neem seed extract:***

Dried neem (*Azadirictin indica*) seeds collected and the shells cracked using pestle and mortar to separate the shell from the kernel. The kernels were grounded into paste using mortar and pestle. 50gm of the paste was weighed and 250mls of water added stirred for ten minutes and allowed to stand for 24 hours before filtering with muslin cloth (Amuchi, R. T, 1999).

***Preparation of garlic oil:***

Garlic (*Allium sativum*) where collected and weighed up to 50gm. The shell is separated from the bulb manually and the bulb grounded into paste using mortar and pestle and paste mixed with 250mls of water stirred for 10 minutes and filtered after 24 hours using muslin cloth (Amuchi, R. T, 1999).

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***Preparation of Tridax extract from leaves:***

The leaves of *Tridax procumbens* was obtained and dried under shade to maintain the composition of the leaves. The leaves was weighed up to 100gm and was flamed to ash .

**Experimental Design and Statistical Analysis:**

Completely randomized design of three treatments which include neem seed oil, garlic oil and tridax leaf ash was done. All the data was analyzed using analysis of variance (ANOVA) according to Gomez (1984). Significant means was separated using least significance difference (LSD) at p=0.0001 according to Ogbulu *et al*. (2009). The statistical package used was Statistical Analysis System (SAS).

## 3. RESULTS AND DISCUSSION

**Disease incidence:**

The study was carried out between 18th July and 20th September, 2015. Dry and soft rots were encountered as shown on Table 1 and four organisms were responsible for the rots: *Aspergillus flavus*, *Thielavia terricola*, *Rhizopus stolonifer* and *Scoupulariopsis brevicaulis*. Pathogenicity test on these isolates proved them to be post-harvest rot fungal pathogens of potatoes in the four markets in Biu Local Government, viz: Main Market, Shopping Complex, Hospital market and Mirnga Market.

Rot incidence was of universal occurrence in all the four markets sampled with an average of 9.06%. Mirnga Market had the highest incidence of 13.75%, followed by Biu Main Market with 8.75% while Shopping Complex Market had 7.5%, with the least in Hospital Market of 6.25%.

***Isolation and identification:***

The following fungi were isolated and identified as post-harvest rot fungal pathogens of Irish potatoes: *Aspergillus flavus*, *Thielavia terricola*, *Rhizopus stolonifer* and *Scoupulariopsis brevicaulis*.

**Table 1: Disease incidence**

| Market | Incidence of rot ( % ) |
|---|---|
| Biu Main Market | 8.75 |
| Shopping Complex Market | 7.5 |
| Hospital Market | 6.25 |
| Mirnga Market | 13.75 |
| Average | 9.06 |

***Frequency of the fungal pathogens per Markets sampled:***

Out of the four fungal pathogens sampled, *R.stolonifer* had the highest level of occurrence with the average of 5 pathogens per sample location, while *A. flavus* had the least average level of occurrence of 1 organism per sample location. *T.terricola* and *S.brevecaulis* had 1.5and 3 pathogens per sample location respectively (Table2).

**Table 2: Frequency of the fungal pathogens per Markets sampled**

| | Frequency | | | |
|---|---|---|---|---|
| Market | *A. flavus* | *T. terricola* | *S. brevicaulis* | *R. stolonifer* |
| Biu Main Market | - | 1 | 2 | 5 |
| Shopping Complex Market | 1 | 1 | 5 | 5 |
| Hospital Market | - | 2 | 1 | 4 |
| Mirnga Market | 3 | 2 | 4 | 6 |
| Total | 4 | 6 | 12 | 20 |
| Average | 1 | 1.5 | 3 | 5 |

***Pathogen severity:***

Severity of the individual fungal pathogens was determined. The severity of fungal isolates responsible for Irish Potatoes rots varied significantly at p= 0.0001. Table3 shows *T.terricola* has the highest mean diameter rot (14.11mm) and *A. flavus* has the least (8.97mm). However there was no significant difference between *R.stolonifer* and *S.brevecaulis.*

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**Table 3: Severity of fungal Pathogen of Irish Potato Postharvest rot in Biu Local Government Area of Borno State**

| Fungal species | Lesion Size (mm) |
|---|---|
| *Aspergillus flavus* | 8.97 mm |
| *Thielavia terricola* | 14.11mm |
| *Rhizopus stolonifer* | 10.82 mm |
| *Scoupulariopsis brevicaulis* | 9.96 mm |

**Effects of the Garlic Oil, Neem Seed Oil and *Tridax* Leaf Ash on Pathogens *In-vitro:***

All the extracts had a significant difference with the control at P=0.0001. There was a reduced growth in means of the three treatments compared to the control (Table 4). There was also a significant difference between *Tridax* leaf ash and garlic and neem seed oil. The latter two performed lower than the ash but there was no variation between oils of garlic and neem seed.

**Table 4: Effects of the Garlic oil, Neem seed oil and Tridax leaf ash on pathogens *In-vitro***

| Extracts | Growth of colony (mm) |
|---|---|
| Garlic oil | $24.18^{b}$ |
| Neem seed oil | $20.16^{b}$ |
| Tridax leaf ash | $18.50^{a}$ |
| Control | $34.70^{c}$ |

Means followed by the same letters are not significantly different (P=0.0001) according to Duncan Multiple Range Test.

***Interaction Effects of the Garlic oil, Neem seed oil and Tridax Leaf Ash on Pathogens In-vitro.***

There was a significant difference on the interactive effects of the three treatments on the pathogens (P=0.0001) for the in vitro control trial. The best control of growth of the culture was by tridax leaf ash on *T. terricola* (12.67), those of neem seed oil on *T. terricola* (14.31) and *A. flavus* (18.16) and garlic oil on *S. brevecaulis (*17.79) as shown in Table 5. The least interaction effects was observed in tridax leaf ash on *S. brevecaulis* (20.10) and garlic oil on *T. terricola* (20.05), *A. flavus* (26.69) and *R. stolonifer* (32.18).

**Table 5: Interactive Effects of the Garlic oil, Neem Seed Oil and *Tridax* Leaf Ash on Pathogens *In-vitro***

| Fungal species | Garlic | Neem | Tridax |
|---|---|---|---|
| *Aspergillus flavus* | $26.69^{c}$ | $18.16^{a}$ | $20.13^{b}$ |
| *Thielavia terricola* | $20.05^{c}$ | $14.31^{a}$ | 12.67a |
| *Rhizopus stolonifer* | $32.18^{c}$ | $28.58^{b}$ | $21.10^{c}$ |
| *Scoupulariopsis brevicaulis* | $17.79^{a}$ | $19.57^{b}$ | $20.10^{c}$ |

**Effects of Garlic Oil, Neem Seed Oil and Tridax Leaf Ash on Pathogens *In-vivo:***

All the extracts had a significant difference with the control at P=0.0001. There was a reduced growth in means of the three treatments compared to the control (Table 6).

**Table 6: Effects of the Garlic oil, Neem seed oil and Tridax Leaf Ash on Pathogens *In-vivo***

| Extracts | Growth of colony (mm) |
|---|---|
| Garlic oil | $4.02^{b}$ |
| Neem seed oil | $4.19^{b}$ |
| Tridax leaf ash | $3.13^{a}$ |
| Control | $7.90^{c}$ |

Means followed by the same letters are not significantly different (P=0.0001) according to Duncan Multiple Range Test.

***Interaction effects of garlic oil, neem seed oil and Tridax leaf ash on pathogens in-vivo*:**

There was a significant difference on the interactive effects of the three treatments on the pathogens ( P=0.0001) for the *in vivo* control trial. The best control of growth of the culture was by tridax leaf ash on *A. flavus* (1.81) followed by neem seed oil on *A. flavus* (2.46) and those of tridax leaf ash on *S. brevecaulis*(2.69) *T. terricola* (2.83) as shown in Table 7. The least interaction effects were observed in garlic oil on *T. terricola and R. stolonifer* (4.95) and (5.00) respectively, also tridax leaf ash on *R. stolonifer (5.19)* and neem seed oil on *R. stolonifer* (6.86).

**Table 7: Interactive Effects of Garlic Oil, Neem Seed Oil and Tridax Leaf Ash on Pathogens *In-vivo***

| Fungal species | Garlic | Neem | Tridax |
|---|---|---|---|
| *Aspergillus flavus* | $3.10^{c}$ | $2.46^{b}$ | $1.81^{a}$ |
| *Thielavia terricola* | $4.95^{c}$ | 3.93b | $2.83^{a}$ |
| *Rhizopus stolonifer* | $5.00^{a}$ | $6.86^{c}$ | $5.19^{b}$ |
| *Scoupulariopsis brevicaulis* | $3.05^{b}$ | $3.52^{c}$ | $2.69^{a}$ |

Results of control trial of *Aspergillus flavus*, *Thielavia terricola*, *Rhizopus stolonifer* and *Scoupulariopsis brevicaulis* using garlic oil, neem seed oil and aqueous solution of *Tridax* leaf ash showed moderate control *in-vitro* and *in-vivo*. The analysis in-vitro trial showed that there is inhibition of colony growth of the four isolates. Incidence and severity of the isolates were significantly reduced in the *in-vivo* trial in all the four isolates after the control treatments. In view of the above garlic oil, neem seed oil and aqueous solution of *Tridax* leaf ash have the potential to replace the hazardous and environmental unfriendly fungicides.

Generally, most Nigerian farmers are peasants and the possibility to buy agricultural inputs is limited and besides control of the isolates becomes necessary especially *A. flavus* which contain aflotoxin which is dangerous to both human and animal. This together with, environmental pollution owing to the misuse of chemicals provide good atmosphere for carrying out research on natural disease control using plant oils and ash. This is strongly supported since the aim is to develop reliable disease control methods which are attractive and safe to farmers to use.

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