EVALUATION OF AQUEOUS NEEM (*Azardirachta indica*) LEAF SOLUTION FOR THE CONTROL OF BACTERIAL SOFT ROT OF POTATO TUBERS (*Solanum tuberosum*)

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ABSTRACT

The experiment was set up in a completely randomized design with four replications in the pathology laboratory of the Department of Crop Protection, University of Maiduguri, Maiduguri to evaluate the effect of aqueous extract of neem leaf solution on the control of soft rot disease of potato (Erwinia carotovorum ssp. Carotovorum).The treatments were prepared in four concentrations; 10%, 25%, 50%, 100% of the neem extract and distilled water as control. The result showed that the lowest incidence (p<0.05) and severity of soft rot were obtained on tubers treated with the concentrated neem leaf solution while the control (0%) had the highest disease incidence and severity. Hence, aqueous Azardirachta indica leaf solution was found to be effective in reducing bacterial soft rot disease of Solanum tuberosum. Further studies are required to determine the actual active ingredient(s) responsible for the fungicidal effect of Azardirachta indica.

Keywords: *Azardirachta indica*, Concentrations, *Erwinia carotovorum*, *Solanum tuberosum*

INTRODUCTION

Potato, *Solanum tuberosum* (L) belongs to the family solanaceae, is indigenous to South America. It was introduced to Britain probably before the sixteen century and about 80% of the World production is in Western Europe, U.S.S.R and Poland (Nash, 1978). In Nigeria, the cultivation of potatoes started in the early 20th century but up till now its cultivation is still on a very limited scale. The factors responsible for this slow development include unsuitable climatic conditions, diseases and insect pests. The crop is grown mostly on Jos Plateau particularly in Mangu Local Government area. It is also grown on a small scale by Chad Basin and Rural Development Authority at Baga, Borno State (Abdullahi, 1999). Bacterial can invade potato tubers causing soft rot under poor storage conditions. In particular, the presence of water films over tubers and temperature above 25^{0}c creates condition favourable for decay not only by *Erwinia* strain but also by certain anaerobic bacteria such as *Pectolytic clostridia* (Hampson, 1980). The implements used during harvest cause a lot of damage to potato tubers and these injuries expose the tuber to attack by pathogens such as fungi and bacteria causing both soft rot and dry rot diseases. (Lund and Nicholls, 1970). Potato tuber soft rot caused by *Erwinia carotovora ssp. carotovora* is a major disease occurring wherever potatoes are grown. It can cause substantial losses in transit and storage, particularly in the warm regions where temperatures are high and there are no facilities available for cold storage. Though data on the losses caused by the disease in Nigeria is not documented, losses could be as high as 40% or more due to the lack of good storage

facilities. The storage of tubers in sacks and unventilated stores at market sites further increased tuber losses due to the soft rot infection. Several disease control methods such as hot water treatment (Shirsat *et al*., 1991) and air-drying of tubers (Bartz and Kelman, 1985b) have been tried with varying scale of success. Chemical control of the disease has not been successful even in the developed countries. Trials with sodium hypochlorite, chlorine dioxide and 5-nitro-8-hydroxyquinoline eliminated the bacteria mostly on tuber surfaces (Harris, 1979). The use of products of plant origin is much safer but these products are applied mostly in insect pest management than control of microorganisms. However, use of plant extracts such as ginger rhizomes, garlic bulb and aloevera have been used in controlling fungal pathogens (Amadioha, 1999; Obagwu *et al*., 1997; Ahmed and Beg, 2001). Neem tree derivatives have been used as pest control in rural areas of developing countries (Ganguli, 2002). All parts of the neem tree have medicinal properties and are used for many different medical preparations. Ganguli (2002) reported that neem is used for the treatment of scabies mite and also, it is effective in treating infestation of lice in humans. Ibrahim *et al*., (1987) reported that neem tree extract treatment have favourable effect on sprouting in stored yam, as it was able to suppress sprouting for 5 months in stored yam tubers (*Dioscorea rotundata*).
This paper reports the efficacy of neem leaf aqeous solution in reducing potato tuber soft rot caused by *Erwinia carotovora ssp. carotovora.*

Objectives of the Study

The broad objective of this study is evaluation of aqeous neem(Azardirachta indica) leaf solution for the control of bacterial soft rot of potato tubers (Solanum tuberosum).The specific objectives are to:
(i) evaluate the effect of neem aqueous leaf solution on the control of potato tuber soft rot disease induced by *Erwinia spp*.
(ii) determine the concentration of the solutions that best control the disease.

LITERATURE REVIEW

Potatoes are commonly attacked by pathogens such as fungi, bacteria and viruses in the field, transit and storage. Potato tuber soft rot disease is caused by *Erwinia carotovora, ssp. Carotovora* and *Erwinia chrysanthami* because of the high temperature (Perombelon and Lowe, 1975).Losses in storage solely depend on the storage conditions and therefore losses can be limited by maintaining favourable conditions in the store (Bdilya 1995). It was observed that rottening takes place very rapidly in warm, humid conditions than in cooler environments.Thomas et al., (1979), reported that the major factors limiting the storage of potato tubers in the tropical region is bacterial soft rot and it is favoured by the high temperatures in storage. In a study with several Indian cultivars, they obtained losses of about 30- 70% in storage due to the presence of *Erwinia carotovora* during 2-4 months storage period at 27-32^{o}c. Lenticels, wound inflicted on tubers during harvesting, handling and transportation provide an avenue for the entry of rot causing pathogens before reaching the store (Perombelon 1973). Gusev,(1980) reported that high Nitrogen fertilizer (N_{120} P_{60} K_{60}) during growing period favour rotting of stored tubers while tubers with high phosphorus and potassium (N_{60} P_{120} K_{60} N_{60} P_{60} K_{120}) decreased infection.

Lipson (1967), Lund and Nicholis (1970), and Cromarty and Easton (1973) noticed that damp storage conditions resulting from high relative humidity and high temperature encourage tuber deterioration caused by soft rot bacteria. Similar results have been reported by Perombelon and Lowe (1975). The lowest incidence and severity of bacterial soft rot disease was recorded when potato tubers were treated with 20% sodium hypochlorite solution (Abdullanhi, 1999). Neem products have been used extensively in insect pest management (Stoll, 1998). Some fungicidal and bactericidal activity of that product has also been reported (Stoll, 1998), (Emechebe and Alabi 1997). The lack of appropriate storage facilities in Nigeria has led to high losses of potato tubers in transit and storage thus the search for effective and cheap method of controlling the disease in storage became essential.

MATERIALS AND METHODS

The Study Area

The experiment was set up in a completely randomize design with four replications in the pathology laboratory of the Department of Crop Protection University of Maiduguri, Maiduguri, North eastern part of Nigeria.

Sampling Technique and Sample Size

Potato tubers showing soft rot symptoms were procured from two markets (Gomboru and Monday market) in Maiduguri metropolitan. The selected rotten tubers were kept in a clean, labelled polyethylene bags for 2-3 days in the laboratory to promote further rottening. The rotten tubers were crushed with hands in distilled water to prepare inoculum suspension. The suspension was allowed to settle for about 1hour and the supernatant solution was filtered through a Whatman No1 filter, and the residue discarded. About two hundred (200) apparently health tubers were obtained from the same market. The tubers were washed with running tap water and allowed to dry at room temperature. The tubers were then inoculated by submerging them in the inoculums suspension for 24 hours, 12 hours and 30 minutes respectively for the three (3) trials.

Fresh neem leaves were collected within the university compound using cutlass. The leaflet were detached from the branches to obtain 1kg required for the extract. The leaves crushed in Phillip electric blender for 10 minutes by adding 200ml of the required 1000ml of distilled water. The paste formed poured into plastic container and the remaining 800ml added, stirred with clean glass rod, covered with aluminium foil to prevent evaporation. This was left on the laboratory bench for 24hours. After 24 hours, the solution was filtered through a What Man No.1 filter paper and the supernatant solution diluted into different concentrations of T_1, T_2, T_3, T_4 respectively.

T_1 = (100%) undiluted extract
T_2= (50%) 50% extract added to 50% of water
T_3= (25%) 25% extract added to distilled water of 75%
T_4= (10%) 10% extract added to distilled water of 90%
Control= distilled water alone- no extract

The treatments were four concentrations of the aqueous neem solution and control. The concentrations used were 0% (control), 10%, 25%, 50% and 100% (undiluted) as standard. Each treatment consisted of 10 tubers replicated four times. The tubers that were inoculated by puncturing and submerging in inoculums were submerged in extract solution for 12hours, 6hours and 30 minutes and incubated in clean and sterilized basins. Moist tissue paper was placed at the bottom of each basin to maintain high relative humidity in the basin. The experiment was evaluated on daily basis for three days.

Methods of Data Collection

Evaluation was based on incidence and severity of soft rot disease development. Incidence was base on number of tubers infected in each replication and expressed as a percentage of the total number of tubers replication. Severity was based on visual observation of the total area of tuber infected or covered with rot lesion. Percentage area of tuber covered with rot lesion was based on a scale of 0-5.

0 = no infection
1 = 1-20%of tuber covered with rot lesions
2 = 21-40% of tuber covered with rot lesions
3 = 41-60% of tuber covered with rot lesions
4 = 61-80% of tuber covered with rot lesions
5 = 81-100% of tuber covered with rot lesions
The severity of rot was then computed using the formula

$$AS = \frac{\sum n}{N \times 5} \times 100$$

Where $\sum n$ = summation of individual severity rating.
N = Total number of tubers observe
5 = Highest score on the severity scale

Methods of Data Analysis

All the data collected were subjected to analysis of variance (ANOVA) using SAS (SAS, 2003). Treatment means were separated using Duncan multiple Range Test (DMRT).

RESULTS

In Table 1, there was significant difference among the treatments for the incidence of soft rot disease at 10% aqueous neem solution (T_4) having the lowest incidence. But no significant difference among the treatments for the severity of the disease, although the lowest incidence was recorded at 100% aqueous neem solution (T_1) in 2008.Similarly,in 2009 there was significant difference among the treatment with T_4 (10% aqueous neem solution) having the lowest incidence of soft rot disease. However, significant difference was observed in the severity of disease in 2009 with T_1 (100% aqueous neem solution) having the lowest severity of soft rot for potato tubers punctured and submerged in inoculums and aqueous neem solution for 12hours. Table 2 showed significant difference among the treatments with T_1 (100% aqueous neem solution) having the lowest incidence of soft rot disease. So also, there was significant difference ($p<0.05$) in the severity of soft rot in 2008. In 2009, significant difference was recorded for both incidence and severity of soft rot with T_1 being the lowest incidence and severity of the disease for potato tubers submerged in inoculums and aqueous solution for six (6) hours. There were clear significant difference in 2008 and 2009 for both incidence and severity of soft rot disease. The lowest incidence and severity were recorded in I_1 in both years for potato tubers submerged in inoculums and aqueous neem solution for thirty (30) minutes.

DISCUSSIONS

The soft rot disease incidence tends to decrease with highest strength of aqueous neem solution concentration. There was a sharp drop in the percentage incidence when treated with 100% concentrated aqueous neem solution. The incidence of soft rot also decreased as time of submersion in inoculation decreased, while the severity of soft rot decreased as the concentration of aqueous neem solution increased. Decreased in the severity of soft rot of tuber, was synonymous with reduction in the time of inoculation. Hence, the tubers submerged for 12 hours as shown in Table 1, which shows highest severity of disease compared to the tubers in Table 2 and 3 that were submerged for six hours and thirty minutes respectively. Temperature as a factor which influences the condition for preservation of potato quality might have played an important role in increasing the rate of rotting of tubers. Although, incubation of tubers was done under room temperature, increasing the rotting of tubers occurred due to the hot weather and high atmospheric temperature. Thomas *et al.*, (1979) observed that pathogenicity of these bacteria increased with increased in storage temperature up to 37^0c and above. The botanical pesticides have found much usage in insect pest management (Stoll, 1998) than the control of microorganisms. This might be partly due to the mode of action of some of the plant products which exhibit repellant properties. Neem seed oil and aqueous extract have both insecticidal and repellant properties and have been used in the control of storage pests (Maina and Lale 2004, 2005). Garlic and neem products have also shown some antimicrobial properties and have been used in the control of fungal pathogens (Stoll 1998; Obagwu *et al.*, 1997). In this study, the neem leaf aqueous solution have also shown some antimicrobial properties by reducing significantly the incidence and severity of the potato tuber soft rot. Probably neem plant contains some compounds, which have bactericidal effects on the bacterial inoculum on the tubers. Emechebe (1996) also reported that foliar application of the aqueous neem seed extract controlled bacterial blight of cowpea in Nigeria, further buttressing the bactericidal properties of neem products. Submerging the tubers in the plant extracts more than 12 hours increased the soft rot incidence and severity, probably, due to reduced tuber resistance as a result of increased anaerobiosis as reported by other authors (Bartz and Kelman 1984, 1985b; Cromarty and Eastern 1973; Bdliya 1995).

CONCLUSION

The neem leaf aqueous extracts significantly reduced the incidence and severity of the tuber soft rot and could therefore be used to reduce losses due to the disease in storage. Spraying of tubers requires less volume of extracts and is much easier to apply thus could be easily adopted by farmers. However, more research is needed to determine the degree of penetration of the extracts into the lenticels so as to ascertain the efficacy of the extract in controlling latent infection which is the predominant mode of contamination of potato tubers by this pathogen.

RECOMMENDATIONS

[illegible] therefore neem leaf aqueous extract can be used in reducing the incidence and [illegible] of potato soft rot. Further studies are required to determine the actual active [illegible] that responsible for the fungicidal effect of neem (*Azadirachta indica)*

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Appendix

Table 1: Effect of different concentrations aqueous neem solution on the incidence and severity of potato tuber soft rot disease in 2008 and 2009. Potato tubers punctured and submerged in inoculum and submerged in aqueous neem solution for twelve (12) hours.

Treatment Aqueous Neem Solution (%)	Incidence (%) 2008	Severity (%) 2008	Incidence (%) 2009	Severity (%) 2009
Control	95.0a	70.5	96.5a	70.0a
100	100.0a	65.5	100.0a	50.0a
50	97.5a	72.5	80.5a	60.0a
25	100.0a	70.0	92.5a	70.0a
10	75.0b	68.0	72.5a	68.0a
SE±	4.23	6.30	4.5	6.8
Significance	ns	Ns	ns	ns

Means followed by the same letter within the column are not significantly different.* Significant at 0.05 probability ns – Not significant. Source: - (SAS, 2003)

Table 2: Effect of different concentration of aqueous neem solution on the incidence and severity of potato tuber soft rot disease in 2008 and 2009. Potato tubers submerged in inoculums for six (6) hours and submerged in aqueous neem solution for six (6) hours.

Treatment Aqueous Neem Solution (%)	Incidence (%) 2008	Severity (%) 2008	Incidence (%) 2009	Severity (%) 2009
Control	97.0a	358.5a	98.0a	41.5a
100	58.0b	16.5b	45.0b	17.5b
50	70.0b	23.0b	68.0b	18.5b
25	52.0b	19.0b	51.0b	20.1b
10	70.0b	259.0b	52.0b	25.0b
SE±	3.6	8.52	4.1	5.5
Significance	ns	ns	ns	ns

Means followed by the same letter within the column are not significantly different.* Significant at 0.05 probability ns – Not significant. Source: - (SAS, 2003)

Table 3: Effect of different concentration of aqueous neem solution on the incidence and severity of soft rot disease in 2008 and 2009 Potato tubers submerged in inoculums for thirty (30) minutes and submerged in aqueous neem solution for thirty (30) minutes.

Aqueous Neem Solution (%)	Incidence (%) 2008	Severity (%) 2008	Incidence (%) 2009	Severity (%) 2009
Control	92.5a	31.5a	95.1a	36.5a
100	58.0b	16.5b	50.1b	14.1b
50	72.5b	23.0b	66.0b	20.1b
25	45.0b	26.0b	72.5b	24.5b
10	60.0b	15.0b	66.5b	26.1b
SE±	4.21	4.90	4.6	5.1
Significance	ns	ns	ns	ns

Means followed by the same letter within the column are not significantly different.* Significant at 0.05 probability ns – Not significant. Source: - (SAS, 2003)