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EFFICACY OF SOME PLANT EXTRACTS ON THE CONTROL OF POTATO TUBER SOFT ROT CAUSED BY *ERWINIA CAROTOVORA* SSP. *CAROTOVORA*

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Abstract: The efficacy of some plant extracts on the control of potato tuber soft rot caused by *Erwinia carotovora* ssp. *carotovora* was evaluated in the laboratory conditions. Neem leaf and seed aqueous extracts significantly reduced the incidence and severity of tuber soft rot, while ironweed and Siamese cassia aqueous leaf extracts gave moderate control of the disease. Tuber treatment with river redgum aqueous leaf extract did not control tuber soft rot.

Key words: plant extracts, potato tuber soft rot, *Erwinia carotovora* ssp. *carotovora,* control

INTRODUCTION

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 methods of the disease control 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. Trails 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, extracts of ginger rhizomes, garlic bulb and aloe vera have been used in controlling fungal pathogens (Amadioha 1999; Obagwu et al. 1997; Ahmed and Beg 2001).

Neem products have been used extensively in insect pest management (Stoll 1998). Some fungicidal activity of that product has also been reported (Stoll 1998) and bactericidal properties as well (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 of effective and cheap method of controlling the disease in storage became essential.

This paper reports the efficacy of some plant extracts in reducing potato tuber soft rot caused by *Erwinia carotovora* ssp. *carotovora.*

MATERIALS AND METHODS

The experiments were conducted in the Plant Pathology laboratory of Department of Crop Protection, University of Maiduguri, Nigeria.

Potato tuber source

The local farmers and retailers from Jos area, Nigeria provided the fresh mature tubers of potato variety Nicola in July and August 2005. Apparently healthy and uniformly sized tubers (about 3800 tubers) were selected for the experiments. The selected tubers were washed under running tap water and stored in the laboratory conditions prior to the onset of the experiments.

Inoculum preparation

The *Erwinia carotovora* ssp *carotovora* (Ecc) isolates were obtained from potato tubers showing soft rot symptoms using the semi-selective medium as described by Bdliya and Langerfeld (2005a). Only pink or red colonies in deep cavities were subcultured into pure cultures and used as inoculum for inoculating the tubers. The bacterium cultures were preserved on nutrient agar (NA) slants at 4°C till required. Prior to the inoculation of tubers, the bacteria were grown on NA plates for 48 h at 27 ± 2°C. The plate cultures were suspended in sterile distilled water and the bacterial concentration adjusted to 10^9cells/ml by measuring the optical density (OD) at 650 nm using the Spectrophotometer (Lomb and Bausch USA).

Preparation of plant extract

The aqueous leaf extracts of neem (*Azadirachta indica* L.), Siamese cassia (*Senna siamea*), ironweed (*Vernonia galamensis*) and river redgum (*Eucalyptus camaldulensis*) were prepared by macerating 1 kg of fresh leaves of the particular plant species in 1 liter of sterile tap water using a blender and filtering through sieve (about 1mm) into clean plastic containers Tubers were submerged in 5 l of each extract. The extracts were prepared at most one hour before the onset of the experiments and agitated vigorously before application. The aqueous neem seed extracts were prepared as described by Stoll (1998). Fresh ripe seeds were collected and the flesh removed, and next the seeds were washed under running tap water. Then the seeds were sun dried, shelled and ground into powder. The powder was then tied in sterile coarse cloth and submerged in water at the rate of 200 g/litre of water overnight or at least 12 hours. The neem seed extracts were used immediately after removal of the immersed powder.

Tuber inoculation

Prior to inoculation, the tubers were surface sterilized in 10.0% sodium hypochlorite solution for three minutes followed by rinsing in five changes of sterile tap water. Tubers were allowed to dry at room temperature (about 30°C). After drying, the tubers were divided into two groups. The tubers in one group were wounded by punching 5 holes about 5 mm deep using 2 mm thick nails while the tubers in the other group stayed unwounded. Next the tubers were artificially inoculated by submerging in the bacterial suspension for ten minutes. After the inoculation the tubers were allowed to dry at room temperature for thirty minutes prior to the treatment with the plant extracts.

Tuber treatment with plant extracts

The artificially inoculated tubers were treated with the plant extracts in two ways: Submerging the artificially inoculated tubers in the plant extracts for the period of 1, 6, 12, and 24 hours and spraying the inoculated tubers with the plant extracts to run-off (approximately at the rate of 100 ml per 100 tubers) using a hand sprayer. The control consisted of artificially inoculated tubers submerged in sterile distilled water for 1, 6, 12, and 24 hours or sprayed with sterile distilled water. All the tubers were allowed to dry before incubation.

Experimental setup

After the treatment with the plant extracts, the tubers were placed in surface sterilized plastic containers (about 30 x 40 x 10 cm) with lids at the rate of thirty tubers per container, replicated four times for each treatment (method of extract application – tubers submerged or sprayed with extract, and exposure time). Moist tissue paper was placed at the bottom of each container to maintain high humidity. The control treatments were setup in the same manner. The containers were arranged in a completely randomized design on shelves in an incubator and incubated for three days at 27°C. The experiments were evaluated based on the incidence and severity of the tuber soft rot. The tuber soft rot severity was assessed on a scale of 0–5 as described by Bdliya and Langerfeld (2005b)

where:

0 = no symptom of rot
1 = 1–15% of tuber rotten
2 = 16–30% of tuber rotten
3 = 31–45% of tuber rotten
4 = 46–60% of tuber rotten
5 ≥ 61% of tuber rotten.

The severity was then computed using the formula

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

where: S = severity of tuber rot (%);
$\sum n$ = summation of individual ratings;
N = total number of potato tubers assessed and
5 = highest score on the severity scale.

Incubation of tubers sprayed with the neem products (leaf and seed extracts) under dump condition

This experiment was set up to ascertain the efficacy of neem products after it was found to be more effective than the other plant extracts in controlling the tuber soft rot. Fifty artificially inoculated tubers sprayed with the neem leaf or seed aqueous extracts percontainer, and replicated four times were arranged in a completely randomized design on clean benches in the laboratory and incubated at room temperature (30±2°C). The experiments were evaluated at weekly intervals for three weeks. The inside of the containers was maintained dump by placing at the bottom two layers of moist tissue paper. Evaluation was based on the incidence and severity of the tuber soft rot. The soft rot severity was computed as described above. The control consisted of artificially inoculated tubers sprayed with sterile distilled water and incubated as described earlier.

Incubation of tubers sprayed with the neem products for 30 days

One hundred artificially inoculated tubers were sprayed with the aqueous neem leaf or seed aqueous extracts and placed in surface sterilized plastic crates with lids (about 35 x 45 x 35 cm), replicated four times, arranged in a completely randomized design on clean benches in the laboratory and incubated at room temperature (about 30°C) for 30 days. The same number of inoculated tubers sprayed with sterile distilled water served as the control. Evaluation of the experiment was also based on the disease incidence and severity.

All the potato tubers used in this study were surface sterilized in 10% sodium hypochlorite solution for three minutes followed by rinsing in five changes of sterile tap water. Also all the experiments were repeated three times.

Data analysis

The collected data were subjected to the analysis of variance and the mean compared either using least significant difference (LSD) or plotting standard error of means at 5% level of probability as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The effect of submerging the tubers in the plant extracts under different exposure times on the incidence of the tuber soft rot is shown in Figure 1. The disease incidence was significantly lower following the treatment with the neem leaf or seed aqueous extracts regardless of tuber condition and exposure time to the extracts. This was followed by the treatment with Siamese cassia leaf extract. The treatment with river redgum leaf extract gave significantly higher disease incidence than the other extracts. The submergence of the tubers in the extracts for 1 or 6 hours gave significantly lower tuber soft rot incidence than the submergence for 12 or 24 hours. The disease severity followed similar trend to the incidence of tuber soft rot. Submerging the tubers in the neem leaf or seed extracts gave significantly lower tuber soft rot severity than the other extracts (Fig. 2). The highest tuber soft rot severity was also recorded in the tubers treated with river redgum leaf extract, which was not significantly different from the control. Similarly, submergence of the tubers for fewer hours in the extract (1 or 6 hours) gave significantly lower tuber soft rot severity than submergence for 12 or 24

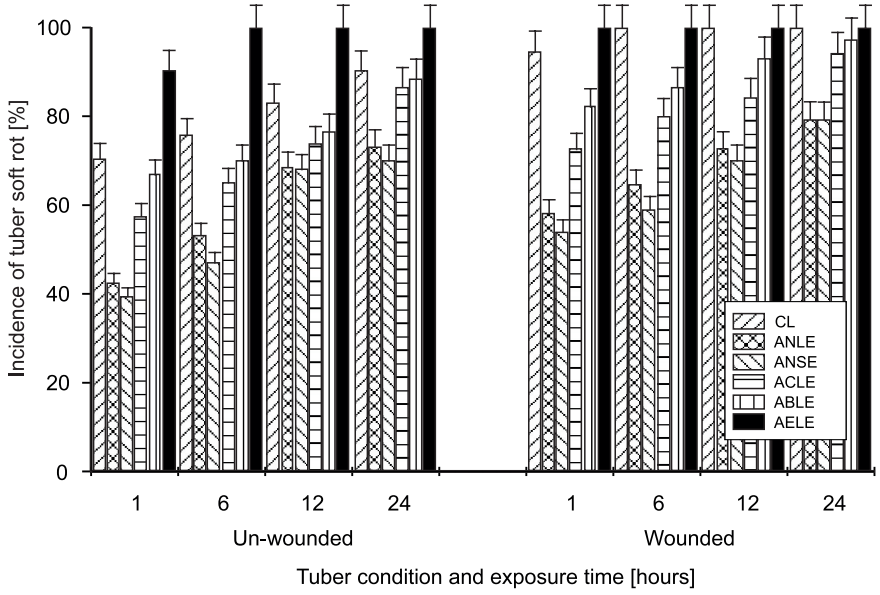

Fig. 1. Effect of some plant extracts on the incidence of potato tuber soft rot disease. Tubers submerged in plant extracts for 1, 6, 12 and 24 hours followed by incubation for three days at 27°C CL = Control, ANLE = Aqueous neem leaf extract, ANSE = Aqueous neem seed extract, ACLE = Aqueous cassia leaf extract, ABLE = Aqueous Bitter leaf, leaf extract, AELE = Aqueous Eucalyptus leaf extract

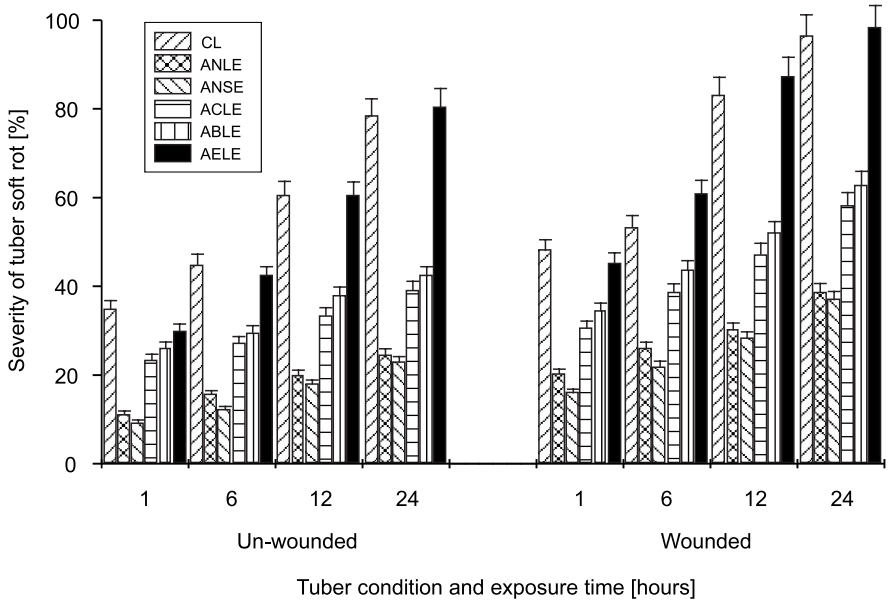

Fig. 2. Effect of some plant extracts on the severity of potato tuber soft rot disease. Tubers submerged in plant extracts for 1, 6, 12 and 24 hours followed by incubation for three days at 27°C CL = Control, ANLE = Aqueous neem leaf extract, ANSE = Aqueous neem seed extract, ACLE = Aqueous cassia leaf extract, ABLE = Aqueous Bitter leaf, leaf extract, AELE = Aqueous Eucalyptus leaf extract

hours. Generally, the incidence and severity of tuber soft rot was significantly lower in the unwounded than wounded tubers regardless of the method of application of extract and exposure time.

Figure 3 shows the incidence and severity of tuber soft rot on unwounded inoculated tubers sprayed with the plant extracts. The incidence and severity of tuber soft rot were significantly lower on the tubers sprayed with the neem leaf or seed aqueous extracts than the other extracts. The incidence and severity of tuber soft rot were higher in tubers sprayed with river redgum leaf extract. Similarly, the incidence and severity of tuber soft rot were significantly lower on the wounded tubers sprayed with the neem leaf or seed aqueous extracts compared to treatment with the other extracts (Fig. 4). The tubers sprayed with river redgum leaf extract also gave the highest incidence and severity of soft rot. The incubation of inoculated tubers sprayed with the neem products under dump condition for three weeks is shown in Figure 5. The result showed a significant reduction in the incidence and severity of tuber soft rot on tubers sprayed with the neem leaf or seed aqueous extracts compared to the control at the weekly intervals of assessment. Also spraying artificially inoculated tubers with the neem leaf or seed extracts showed a significant reduction in tuber soft rot compared to the control even after storing the tubers for 30 days at the room temperature (Fig. 6). All the experiments showed that treatment with the neem leaf or seed extracts significantly reduced the incidence and severity of tuber soft rot regardless of the mode of application (immersion or spraying with extract). Also wounding tubers increased the incidence and severity of the tuber soft rot.

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 repellants properties. Neem seed oil and aqueous extract has both insecticidal and repellant properties and has 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 our study, the neem leaf and seed aqueous extracts have also shown some antimicrobial properties by reducing significantly the incidence and severity of the potato tuber soft rot compared to the other extracts. 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. Siamese cassia and ironweed extracts that also reduced the incidence and severity of the tuber soft rot might have some antimicrobial properties. 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).

The results of this study showed that the neem leaf and seed 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 the soft rot caused by *E. carotovora* spp. *carotovora*.

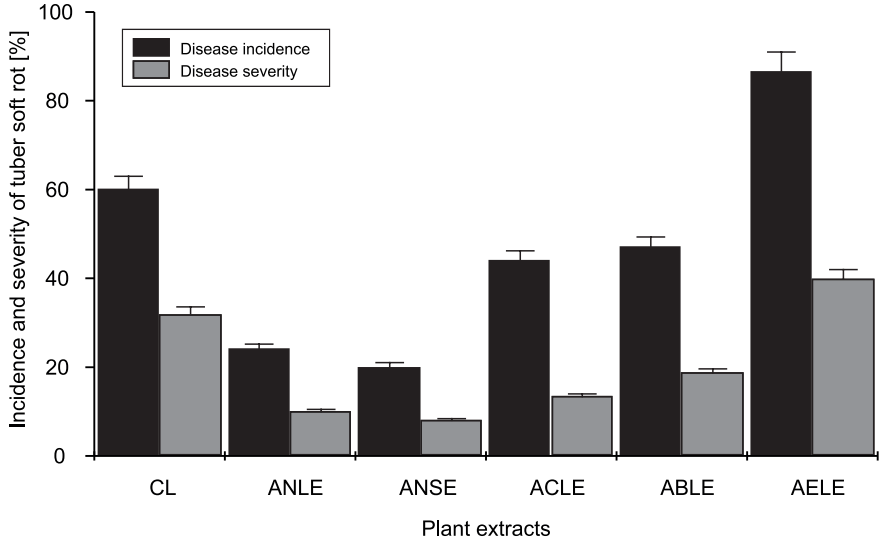

Fig. 3. Effect of some plant extracts on the incidence and severity of potato tuber soft rot disease. Test on unwounded tubers sprayed with the extracts and incubated at 27°C for three days CL = Control, ANLE = Aqueous neem leaf extract, ANSE = Aqueous neem seed extract, ACLE = Aqueous cassia leaf extract, ABLE = Aqueous Bitter leaf, leaf extract, AELE = Aqueous Eucalyptus leaf extract

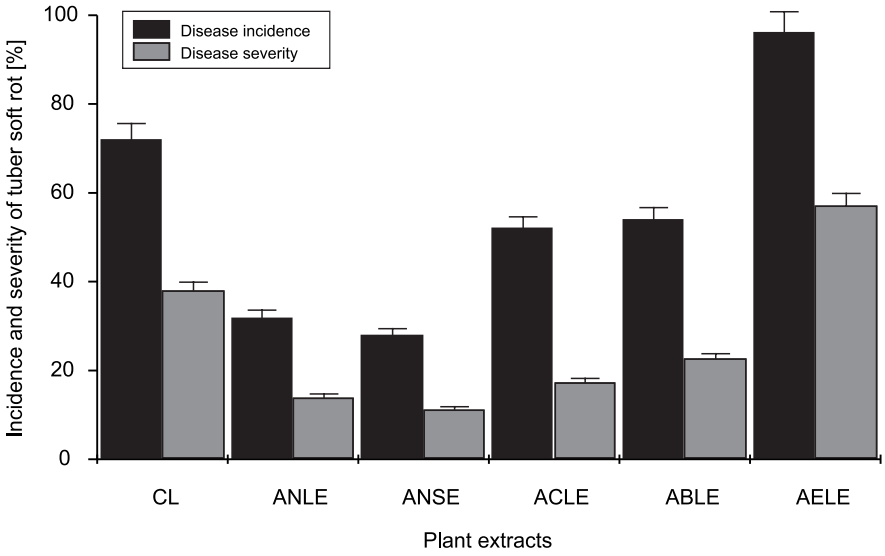

Fig. 4. Effect of some plant extracts on the incidence and severity of potato tuber soft rot disease. Test on wounded tubers sprayed with the extracts and incubated at 27°C for three days CL = Control, ANLE = Aqueous neem leaf extract, ANSE = Aqueous neem seed extract, ACLE = Aqueous cassia leaf extract, ABLE = Aqueous Bitter leaf, leaf extract, AELE = Aqueous Eucalyptus leaf extract

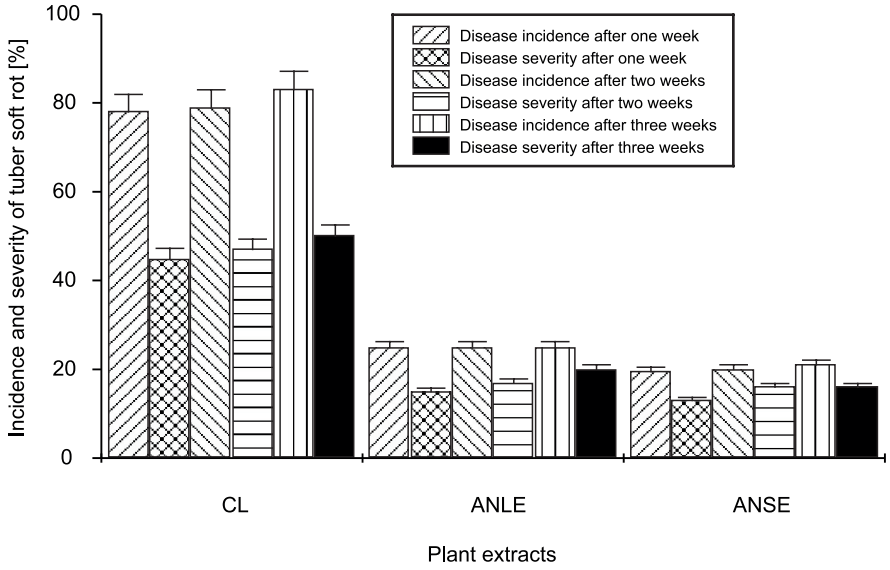

Fig. 5. Effect of neem aqueous leaf and seed extracts on the incidence and severity of potato tuber soft rot disease. Tubers sprayed with the extract and incubated under dump condition at about 27°C and evaluated after one, two and three weeks

ANLE = Aqueous neem leaf extract, ANSE = Aqueous neem seed extract

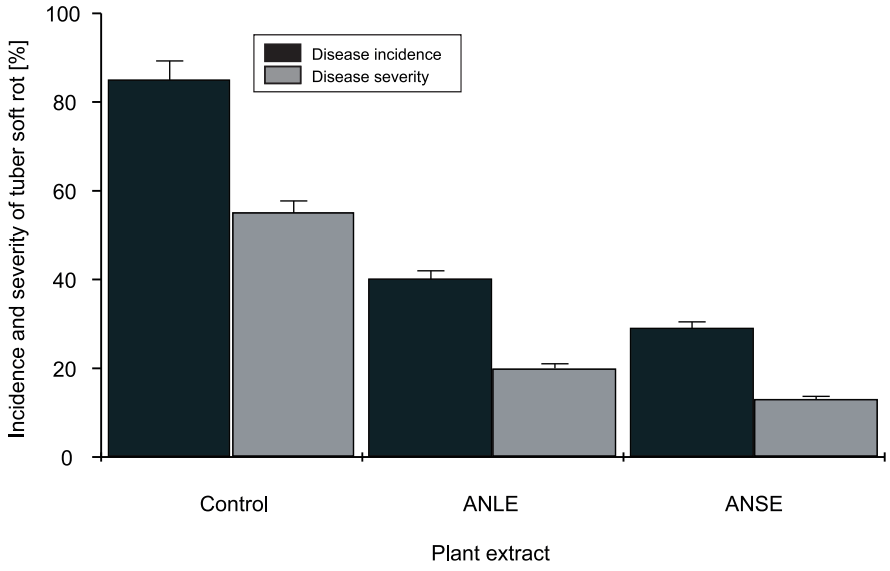

Fig. 6. Effect of neem aqueous leaf and seed extracts on the control of potato tuber soft rot disease. Tubers sprayed with the extracts and stored for 30 days at room temperature (about 29±2°C).

ANLE = Aqueous neem leaf extract, ANSE = Aqueous neem seed extract

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REFERENCES

Ahmed I. Beg A. 2001. Antimicrobial phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacul. Studies 7: 113–123.

Amadioha A.C. 1999. Evaluation of some plant extracts against *Colletotrichum lindermuthianum*. Arch. Phytopathol. Plant Protection 32: 141–149.

Bartz J.A., Kelman A. 1984. Bacterial soft rot potential in washed potato tubers in relation to temperature of tubers and water during simulated commercial handling practices. Am. Potato J. 61: 485–493.

Bartz J.A., Kelman A. 1985a. Infiltration of lenticels of potato tubers by Erwinia carotovora subsp. carotovora under hydrostatic pressure in relation to bacterial soft rot. Plant Dis. 69: 69–74.

Bartz J.A., Kelman A. 1985b. Effect of air-drying on soft rot potential of potato tubers inoculated by immersion in suspension of *Erwinia carotovora.* Plant Dis. 69: 128–131.

Bdliya B.S. 1995. Studies on the detection and identification of soft rot causing *Erwinia* species (*Erwinia carotovora* ssp *atroseptica* (van Hall) Dye, *Erwinia carotovora* ssp *carotovora* (Jones) Bergey *et al*. and *Erwinia chrysanthemi* Burkholder *et al*.) on potato tubers. Ph.D. Thesis, University of Göttingen, Germany, 148 pp.

Bdliya B.S., Langerfeld E. 2005a. A semi-selective medium for detection, isolation and enumeration of *Erwinia carotovora* ssp. *carotovora* from plant materials and soil. Trop. Sci. 45: 90–96.

Bdliya B.S., Langerfeld E. 2005b. Soft rot and Blackleg [Erwinia carotovora ssp. atroseptica (Van Hall) Dye] of potato as affected by inoculum density and variety. Nigerian J. Plant Protection 22: 65–75.

Cromarty R.W., Easton G.D. 1973. The incidence of decay and factors affecting bacterial soft rot of potatoes. Am. Potato J. 50: 398–407.

Emechebe A.M. 1996. Evaluation of aqueous extracts of parts of some plants and black local soap solution for the control of cowpea diseases at Samaru. Cropping scheme meeting: Report on legumes and oil seeds research programme, 26th February – 1st March, Samaru, Zaria, Nigeria, 1996, 79 pp.

Emechebe A.M., Alabi O. 1997. Evaluation of aqueous extracts of parts of some plants for the control of cowpea diseases at Samaru. Samaru Cropping Scheme Meeting, Samaru, Zaria, Nigeria: Report on Legumes and Oil seeds Research Programme, 77 pp.

Gomez K.A., Gomez A.A. 1984. Statistical procedure for Agricultural Research, 2nd ed., John Wiley and Sons, 680 pp.

Harris R.I. 1979. Chemical control of bacterial soft-rot of wounded potato tubers. Potato Res. 22: 245–249.

Maina Y.T., Lale N.E.S. 2004. Integrated management of *Callosobruchus maculatus* (F) infesting cowpea seeds in storage using varietal resistance, application of neem (*Azadirachta indica* A. Juss) seed oil and solar heat. Int. J. Agric. Biol. 6: 440–446.

Maina Y.T., Lale N.E.S. 2005. Influence of duration of storage of insecticidal plant oil and oil treated seeds on the efficacy of neem seed oil in the control of *Callosobruchus maculatus* (F) infesting stored cowpeas. Nigerian J. Entomol. 22: 54–63.

Obagwu J. Emechebe A.M., Adeoti A.A. 1997. Effects of extracts of garlic (*Allium sativum*) bulb and neem (*Azadirachta indica*) seed on the mycelia growth and sporulation of *Colletotrichum capsici*. J. Agric. Technology 5: 51–55.

Shirsat S.G., Paul T., Nair P.M. 1991. Evaluation of treatment with hot water, chemicals and ventilated containers to reduce microbial spoilage in irradiated potatoes. Potato Res. 34: 227–231.

Stoll G. 1998. Natural Crop Protection in the Tropics. AGRECOL, Margraf Verlag, Weikersheim, Germany, 188 pp.

POLISH SUMMARY

SKUTECZNOŚĆ EKSTRAKTÓW ROŚLINNYCH W ZWALCZANIU MOKREJ ZGNILIZNY BULW ZIEMNIAKA WYWOŁYWANEJ PRZEZ *ERWINIA CAROTOVORA* SSP. *CAROTOVORA*

Badanie skuteczności ekstraktów z czterech gatunków roślin w zwalczaniu mokrej zgnilizny bulw ziemniaka prowadzono w warunkach laboratoryjnych. Wodne ekstrakty z liści i nasion *Azadirachta indica* istotnie ograniczały występowanie i nasilenie mokrej zgnilizny bulw, natomiast wodne ekstrakty z liści *Vernonia galamensis* i *Senna siamea* ograniczały chorobę w stopniu umiarkowanym. Ekstrakt wodny z liści *Eucalyptus camaldulensis* nie zwalczał mokrej zgnilizny bulw ziemniaka.