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Full Length Research Paper

Post-harvest fruit decay-inducing pathogen in medicinally important *Cucumis* species indigenous to South Africa

R. R. Mphahlele, P. W. Mashela* and K. M. Pofu

School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, Republic of South Africa.

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Crude extracts of wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) fruits have been used extensively in animal, human and plant health in marginal communities of South Africa. However, collected fresh fruits of *Cucumis* species have high rate of post-harvest decay, without information on disease-inducing causal agents. A study was carried out to isolate, identify, perform pathogenicity tests and develop possible tactics to manage the rate of decays. Spores of isolated fungus from harvested fruits were repeatedly cultured on potato dextrose agar. Based on purity and morphological features, the pathogen was isolated, identified and confirmed through pathogenicity tests as *Penicillium simplicissimum*. The rate of decay was higher in *C. africanus* than in *C. myriocarpus* fruits. Overall, proliferation of *P. simplicissimum* as shown by purity of cultures, suppressed the growth of tactical contaminants, suggesting the existence of antimicrobial-excreting properties in this pathogen. Dipping fruits in 12.5 µg benomyl 50% WP/L tapwater prevented post-harvest decay for over 60 days. In conclusion, association of *P. simplicissimum* with post-harvest fruit decay in *Cucumis* species promoted the potential of long-term storage for use of fresh fruit in animal and plant health.

Key words: *Cucumis africanus*, *Cucumis myriocarpus*, pathogenicity test, *Penicillium simplicissimum,* traditional medicine.

INTRODUCTION

Traditional medicine is an integral part of African heritage (Onwuanibe, 1979), with limited research support on post-harvest technologies, particularly where fresh materials are involved. Wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) - all indigenous to South Africa (Kristkova et al., 2003), produce fruits which contain unlimited pharmacological properties (Chen et al., 2005; Hardman et al., 1996). Extracts of fruits from the two *Cucumis* spp. are widely used in treatment of liver damage, weakening of immune system, lumps, jaundice, acute chronic viral hepatitis, chronic viral hepatitis, hepatocirrhosis, persistent dyspepsia, epilepsy due to wind-phlegm, gonorrhoea, boils, infertility, inflammation and intestinal roundworm infection (Agil et al., 1999; Balkema-Boomstra et al., 2003; Blaskovich et al., 2003; Dinah et al., 2001; Duncan et al., 1996; Guha and Sen, 1975; Hardman et al., 1996; Jayaprakasam et al., 2003; Oh et al., 2002; Peters et al., 2003; Peters et al., 1999; Yesilada et al., 1988). Also, extracts from fruits of these two *Cucumis* spp. have anti-cancer properties at high concentrations, but are toxic to healthy cells, while at low concentrations, they stimulate healthy cells to be cancerous (Jayaprakasam et al., 2003).

In *C. africanus*, the major potent chemical is cucurbitacin B ($C_{32}H_{48}O_8$), which is insoluble in water, whereas cucurbitacin A in *C. myriocarpus* fruits comprising two potent chemicals, *viz.* cucumin ($C_{27}H_{40}O_9$)

*Corresponding author. E-mail: phatu.mashela@ul.ac.za.

and leptodermin ($C_{27}H_{38}O_8$), is soluble in water (Chen et al., 2005; Rimington, 1938). After noting anthelmintic properties of these materials from literature on intestinal roundworm (*Ascaris suum*), Mashela (2002) developed the ground leaching technology (GLT) system, which uses dry crude extracts of *C. africanus* and *C. myriocarpus* fruits in suppression of root-knot nematodes (*Meloidogyne* spp.). In GLT system, small quantities (0.20 to 0.70 t/ha) of ground materials are applied in ca. 5-cm-deep holes on cardinal points of stems at transplanting, with potent chemicals being leached out through irrigation water as opposed to the usual microbial activities (Mashela et al., 2011). The efficacy of crude extracts of *C. myriocarpus* fruits on nematode suppression in tomato production was comparable to that of synthetic nematicides, *viz*. aldicarb and fenamiphos (Mashela et al., 2008). Various studies demonstrated that these two *Cucumis* spp. were highly resistant to *Meloidogyne* spp. (Mashela et al., 2011). Also, using observations that at low concentration, extracts from fruits of these two *Cucumis* spp. stimulated healthy cells to be cancerous (Jayaprakasam et al., 2003; Van Wyk et al., 2002), Mafeo et al. (2011) used a curve-fitting allelochemical response data (CARD) computerised model (Liu et al., 2003) to establish suitable dosages of crude extracts of *C. myriocarpus* fruit for use as a pre-emergent bio-nematicide.

Although *Cucumis* spp. are perennial (Mashela et al., 2011), the harvest period is short, resulting in large quantities of fresh fruits being collected from the wild for medicinal uses. The material is used in fresh and occasionally in dried form. Despite widespread uses of fruits from the two *Cucumis* spp., the pathogen inducing fruit decays is not well documented and therefore, post-harvest control tactics could hardly be instituted. Also, certain pathogens produce toxins as by-products, which can be lethal to animals and human beings, for instance, the mycotoxins (Marasas and Van Rensburg, 1979). Consequently, potential health hazards exist when using these materials medicinally. Therefore, the objective of this study was to isolate, identify, perform pathogenicity tests and develop potential tactics for managing the rate of decay in fruits of *C. africanus* and *C. myriocarpus*.

MATERIALS AND METHODS

The experiment was conducted in the VLIR Nematology Laboratory, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Ripe fruits of *C. africanus* and *C. myriocarpus* were collected locally from the wild. Fruits were separately washed with distilled water and stored in the growth chamber (Model LTIM 70) in batches of two at 25°C and 80% relative humidity (RH) to induce the post-harvest fruit decay.

Isolation

The growing medium was prepared by mixing 10 g potato dextrose agar (PDA) in 250 ml distilled water and pasteurised at 121°C and 1.03×10^2 kPa for 20 min, with pH adjusted to 6.5 to 7.0 using adjuvants after autoclaving (Ali et al., 2006). After cooling, the medium was poured into sterile Petri dishes. Partially decayed fruits of *C. africanus* and *C. myriocarpus* were individually surface-sterilised using 1.0% sodium hypochlorite (NaOCl) solution, with small decayed pieces severed using sterile scalpel and placed separately on solidified plates of PDA. The scalpel was intermittently dipped in 10% NaOCl solution. Cultures were incubated in the growth chamber at 25°C and 80% RH fro m the first to the fifth generations to allow for verification of purity in fungal accessions.

Identification

Isolates were delivered to the Biosystematics Division of the Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI) for expert identification to the species level. Isolates from *C. africanus* and *C. myriocarpus* were code-named by ARC-PPRI Cuit and Cont, respectively. Repeated culturing on PDA was done to ascertain whether morphological characteristics of the isolates would be stable over three generations.

Pathogenicity tests

Plants of *Cucumis* spp. were raised in the greenhouse to produce relatively uncontaminated fruits. After harvest, individual fruit were placed in small plastic bags to reduce contact-to-contact contamination and taken to the laboratory for pathogenicity tests. Separate tests for *Cucumis* spp. were conducted in the growth chamber under conditions described previously in a 2 × 4 factorial experiment (Gomez and Gomez, 1984). The first main factor comprised two inoculation sites, while the second comprised (i) control, (ii) sterilisation with NaOCl, (iii) inoculation with isolates and (iv) NaOCl + isolate. Generally, NaOCl alone and NaOCl + isolate treatments were achieved by first placing whole fruits in 500 ml bottles, containing 1% NaOCl solution and mechanically shaken for 30 min, with untreated control and isolate alone being shaken in distilled water. Fruits were rinsed in distilled water to remove excess solution and wiped off by wrapping individual fruits in tissue paper and squeezing slightly. A 5-mm-diameter cork borer was used to drill holes, with either one or two inoculation sites, which were inoculated with and without spores from their respective isolates. Individual treatments were placed in 22 × 12 mm transparent untied plastic bags to ensure that there was no direct contact from the fruits, and arranged on rails of the growth chamber (25°C, 80% RH) in a randomised complete block design, with five replications. Sixteen days after initiating the treatments, an arbitrary developed decay scale (0 = not rotten, 1 = rotten around inoculation site, 3 = rotten half of the fruit, 6 = rotten whole fruit) was used to rank the treatment effects.

Sugar content and pH

After pathogenicity tests, pulp of *C. africanus* and *C. myriocarpus* fruits from untreated controls was removed and centrifuged at 1 500 rpm to separate seeds from juice. Percentage Brix (sugar content) of juice was determined using a hand-held refractometer (Labotec), while pH was measured using a pH meter (Labotec), in order to compare the two variables in fruits of the two *Cucumis* species.

Control of identified pathogen

Fruits from the wild were rinsed in water to remove soil particles, with excess water being removed by pressing in tissue paper. Fruits

Table 1. Pathogens associated with decay in *Cucumis africanus* and *Cucumis myriocarpus* fruits as identified at the Biosystematics Division of the Plant Protection Research Institute of Agricultural Research Council.

Cucumis	Code A[x]	Code B[y]	Identification
C. africanus	Cuit	M-48/377	*Penicillium simplicissimum* (Oudem.) Thom.
C. myriocarpus	Cont	M-48/378	*Penicillium simplicissimum* (Oudem.) Thom.

[x] = code for the sender, University of Limpopo; y = code determiner (Ms M. Truter), at the Biosystematics Division of the Plant Protection Research Institute of Agricultural Research Council.

Table 2. Analysis of variance for responses of *Cucumis africanus* and *C. myriocarpus* fruit sterilized with and without sodium hypochlorite (NaOCl) when inoculated with *Penicillium simplicissimum* in one and two holes (n = 20).

Source of variation	df	*Cucumis africanus*		*Cucumis myriocarpus*	
		SS	Percentage	SS	Percentage
Replication	4	11.15	3.9[ns]	4.61ns	4[ns]
Treatment (T)	3	214.78	67.0***	95.43	73***
Hole (H)	1	0.03	0.10[ns]	3.62	3[ns]
T × H	3	20.68	7.0[ns]	2.61	2[ns]
Error	28	71.08	22.0	22.93	18[ns]
TOTAL	39	317.78	100	129.20	100

Values with *** are significant at P ≤ 0.01; while those with ns are not significant at P ≤ 0.05.

were dipped into 0, 1.25, 2.5, 3.75, 5.00, 6.25 and 7.50 mg benomyl (methyl-l-butylcarbamoyl-2-benzimidazole) 50% WP/L tap water. Fruits were put in sterilised (5% NaOCl solution) 15-cm-diameter plastic pots in groups of 10 in a growth chamber (25°C and 80% RH), with treatments arranged in a completely randomised design, with four replications. At 60 days after initiating the treatments, fruits were ranked for decay as described in the pathogenicity tests.

Data analysis

Data were subjected to analysis of variance using SAS software (SAS Institute, Cary, NC). Fruit decay data were transformed using $\log_{10}(1 + x)$ prior to analysis in order to homogenise the variances (Gomez and Gomez, 1984), but untransformed data were reported. Sources of variation were partitioned to estimate the percentage contribution of individual sources to total treatment variation in fruit decay. Mean treatment separation was achieved through Fisher's least significant difference test. Only data where treatments were significant at the 5% level of probability were discussed, unless otherwise stated.

RESULTS

Isolation and identification

Four to seven days after incubation of infected tissues from decayed fruits, green to dark green mycelia with greyish coloured spores proliferated on PDA. Repeated culturing demonstrated that the fungus retained its morphological characteristics from the first to the fifth generation. Repeated culturing at ARC-PPRI also confirmed the stability and purity of the morphological characteristics observed in the VLIR Nematology Laboratory. The pathogen associated with post-harvest fruit decay of both *Cucumis* spp. was identified as *Penicillium simplicissimum* (Oudem.) Thom (Table 1).

Pathogenicity tests

Treatment effects in pathogenicity tests were highly significant (P ≤ 0.01) for fruit decay, contributing 67 and 73% to the total treatment variation in fruit decay of *C. africanus* and *C. myriocarpus*, respectively (Table 2). The number of infection sites had no effect on fruit decay. In *C. africanus*, inoculation alone and NaOCl + inoculation treatments had the highest rate of fruit decay, which was different from the untreated control, while NaOCl alone had the least rate of fruit decay (Table 3). In *C. myriocarpus*, NaOCl alone and inoculation alone had the lowest rate of fruit decay, while NaOCl + inoculation had the highest rate when compared with untreated control. Compared with *C. myriocarpus* fruit, *C. africanus* fruit had the highest rate of fruit decay (Table 4). Also, juice of *C. africanus* fruit had lower pH and sugar content than that of *C. myriocarpus* fruit.

Control of identified pathogen

Sixty days after the treatment, 85% control fruits were completely rotten, while the other 15% was rotten only on sides which were in contact with rotten fruits (data not

Table 3. Responses of *Cucumis africanus* and *C. myriocarpus* fruits sterilized with and without sodium hypochlorite (NaOCl) when inoculated with *Penicillium simplicissimum* (n = 20).

Treatment	*C. africanus*	*C. myriocarpus*
Untreated control	1.3[b]	0.60[b]
NaOCl alone	0.9[c]	0.03[c]
Inoculation alone	3.8[a]	0.10[c]
NaOCl + inoculation	3.7[a]	1.90[a]

Values followed by different letters are different (P ≤ 0.05) according to Fisher's least significant difference test.

Table 4. Fruit decay, juice pH and sugar content of *Cucumis africanus* and *C. myriocarpus* fruits when inoculated with *Penicillium simplicissimum* (n = 10).

Species	Decayed fruit	Juice pH	Sugar content (% Brix)
Cucumis africanus	3.43[a]	4.90[b]	4.84[b]
Cucumis myriocarpus	1.65[b]	5.92[a]	5.67[a]
Standard error	0.51	0.25	0.34

Values followed by different letters are significantly different (P ≤ 0.05) according to t-test.

shown). In contrast, fruits treated with benomyl solutions remained intact.

DISCUSSION

Various tests conducted in this study confirmed that *P. simplicissimum* was a causal agent of post-harvest decay in both *C. africanus* and *C. myriocarpus* fruits. *Penicillium* spp., along with *Trichoderma* spp. and *Sporidesmium* spp., are associated with suppressive soils (Agrios, 2005). The two *Cucumis* spp. have stolons, with fruits being in direct contact with soil surface under foliage (Mashela et al., 2011) and therefore, predisposing fruits to infection from soil-borne pathogens. Previously, *P. simplicissimum* was isolated and identified from rotten fruits of apples (*Malus domestica*), citrus (Smoot et al., 1971), grapes (*Vitis vinifera*) and most fruiting vegetables (Ali et al., 2006; Domsch et al., 1980; Sage et al., 2004), all of which have fruits that are aerially-borne on twigs. However, it is commonly accepted that soil splashes during irrigation or rainfall are associated with contamination of fruits with soil-borne pathogens. Approximately 150 species had been isolated and identified in the genus *Penicillium* (Pitt, 1979), which resemble each other in colour, branching mycelium growth characteristics and rot symptoms, with minor distinguishing features for each species (Tournas, 2005). Consequently, expert advice is almost always necessary for identifying the genus to the species level.

In *C. africanus* and *C. myriocarpus*, *P. simplicissimum*-infected fruits were hardly contaminated with other microorganisms as observed on various field-collected decaying fruits where *Penicillium* rot was involved. The Kingdom Fungi is known to excrete substances which increase pH of the growth medium (Gadd, 1993), while *P. simplicissimum* is an acid-loving fungus (Franz et al., 1993). Mild changes in temperature, sugar content and pH level of fruit juices had been identified as important environmental conditions in bacterial and fungal infections (Reuveni et al., 2004). Sugar content is indirectly proportional to pH of juice in fruits (Sage et al., 2004). Generally, most fungal pathogens prefer fruits with juice that contains alkaline pH values, while the opposite is true for bacterial pathogens (De Roever, 1999). However, *P. simplicissimum* is an exception to this general rule. *P. simplicissimum* excretes citric acids, which reduce pH of the growth medium in order to exclude growth and development of alkaline-loving fungal species (Franz et al., 1993; Schinner and Burgstaller, 1989).

Additionally, *P. simplicissimum* excretes an antibiotic, *viz*. 4-allyl-2-azetidinone (B-143), which suppresses other forms of acid-loving microbial activities, but does not have auto-suppressive capabilities (Kobayashi et al., 1997). Also, Kobayashi et al. (1997) demonstrated that purified B-143 did not have antifungal activity on proliferation of *Fusarium* spp. in commercial cucumber (*Cucumis sativus*) cultivars. Probably, B-143 is active when combined with citric acids, as shown that the purified material suppressed growth of *Fusarium* spp. when combined with benomyl (Kobayashi et al., 1997). *Penicillium* spp. are renowned for excreting antibiotics, which are substances produced by one microorganism and toxic to another microorganism (Agrios, 2005).

In another incident, post-flowering inoculation of dead tomato flowers with conidia of *Penicillium* spp. completely suppressed subsequent infection of developing

fruits by *Botrytis cinera* (Agrios, 2005).

Excretion of citric acid and B-143 is unique to *P. simplicissimum* (Kobayashi et al., 1997). However, when crude extracts of *Cucumis* fruits were used as a bio-nematicide in tomato cultivation, the materials had no effect on soil pH, although there was exhibition of fertiliser effect on plant growth (Mashela, 2002; Mashela et al., 2008; Mashela et al., 2011). The role of the two excreta in suppression of soil-borne diseases has not been documented, except that *Penicillium* spp. are part of a group of fungal species which are associated with suppressive soils (Agrios, 2005). Additionally, since cucurbitacin A from *C. myriocarpus* fruit is soluble in water, allowing fruit of this plant species to rot prior to application in GLT may have an added advantage producing crude extracts that have suppressive effects on soil-borne pathogens.

The higher rate of post-harvest decay in *C. africanus* fruit when compared with *C. myriocarpus* fruit was probably due to the lower pH of juice in the former, which improved growth of *P. simplicissimum* as an acid-loving fungus. Although the conditions become suitable for bacterial growth, bacterial contamination under the microscope was negligible, probably due to the suppressive nature of antibiotics produced by *P. simplicissimum*. In most post-harvest decay, fruit bruising had been identified as one of the major factors in post-harvest handling that contributed to fruit decays (De Rover, 1999). Generally, *Penicillium* spp. enter tissues through wounds (Agrios, 2005). Lack of effect of the number of holes on fruits suggested that fruit bruising had a negligible effect in infection of *P. simplicissimum* in fruits of *Cucumis* spp.

Effects of various concentrations of benomyl solutions did not differ on prevention of fruit decay of these two *Cucumis* spp. In this study, we recommend to use the lowest concentration of 1.25 mg/L tap water for fruit storage, with a waiting period of 2 days as specified on label instructions. Treatment of fruits is particularly important, especially for the cases where the material is used in traditional medicines, since *Penicillium* spp. produce mycotoxins, such as patulin (Agrios, 2005), which may contaminate infusions and fruit under GLT systems.

Patulin had been reported to cause edema and bleeding in lungs and brains, damage to kidneys, paralysis of minor nerves and also stimulate cells to be cancerous in humans and animals (Marasas and Van Rensburg, 1979). Incidentally, patulin is highly toxic to lower and higher organisms, including most domestic animals (Marasas and Van Rensburg, 1979). Using fruit from *Cucumis* spp. may, due to contamination by *Penicillium* spp., invariably constitute a serious health hazard. Thus, dipping the fruit in benomyl solutions would mitigate against the described potential hazard. However, when dried crude extracts are intended for use in suppression of plant-parasitic nematodes, we recommend that fruits should not be dipped.

Conclusion

Post-harvest fruit decay in *Cucumis* spp. was induced by *P. simplicissimum*, which excretes citric acids and antibiotics, which act synergistically to eliminate contamination in the growing medium. Findings in this study open various research avenues in determining the extent of contamination in medicinal infusions and influence of soil-borne diseases when fermented crude extracts of *C. africanus* and *C. myriocarpus* containing *P. simplicissimum* are used in soil and plant health.

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