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Post-harvest fruit decay-inducing pathogen in medicinally important Cucumis species indigenous to 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 anticancer 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})
and leptodermin (C_{27}H_{38}O_{6}), 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° 5 3'10"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. 1.03 × 10^5 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 from 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 Cult 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 was 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. afric anus 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

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
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.

<table>
<thead>
<tr>
<th><em>Cucumis</em></th>
<th>Code A</th>
<th>Code B</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. africanus</em></td>
<td>Cuit M-48/377</td>
<td></td>
<td><em>Penicillium simplicissimum</em> (Oudem.) Thom.</td>
</tr>
<tr>
<td><em>C. myriocarpus</em></td>
<td>Cont M-48/378</td>
<td></td>
<td><em>Penicillium simplicissimum</em> (Oudem.) Thom.</td>
</tr>
</tbody>
</table>

* = 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).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th><em>Cucumis africanus</em></th>
<th></th>
<th></th>
<th><em>Cucumis myriocarpus</em></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SS</td>
<td>Percentage</td>
<td></td>
<td>SS</td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>4</td>
<td>11.15</td>
<td>3.9&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.61ns</td>
<td>4&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>3</td>
<td>214.78</td>
<td>67.0***</td>
<td>95.43</td>
<td>73***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hole (H)</td>
<td>1</td>
<td>0.03</td>
<td>0.1&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>3.62</td>
<td>3&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T × H</td>
<td>3</td>
<td>20.68</td>
<td>7.0&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>2.61</td>
<td>2&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>28</td>
<td>71.08</td>
<td>22.0</td>
<td>22.93</td>
<td>18&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>317.78</td>
<td>100</td>
<td>129.20</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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 available).
However, it is commonly accepted that soil splashes all of which have fruits that are aerially-borne on twigs. Approximately 150 species had been isolated and identified in the genus *Penicillium* (Pitt, 1979), which 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).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>C. africanus</em></th>
<th><em>C. myriocarpus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>1.3b</td>
<td>0.60f</td>
</tr>
<tr>
<td>NaOCl alone</td>
<td>0.9c</td>
<td>0.03f</td>
</tr>
<tr>
<td>Inoculation alone</td>
<td>3.8a</td>
<td>0.10f</td>
</tr>
<tr>
<td>NaOCl + inoculation</td>
<td>3.7a</td>
<td>1.90f</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Decayed fruit</th>
<th>Juice pH</th>
<th>Sugar content (% Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cucumis africanus</em></td>
<td>3.43a</td>
<td>4.90b</td>
<td>4.84b</td>
</tr>
<tr>
<td><em>Cucumis myriocarpus</em></td>
<td>1.65b</td>
<td>5.92a</td>
<td>5.67a</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.51</td>
<td>0.25</td>
<td>0.34</td>
</tr>
</tbody>
</table>

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

**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 (Mashele 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 (*Citrus sinensis*), grapes (*Vitis vinifera*) and most fruiting vegetables (Ali et al., 2006; Domshch 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).

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In another incident, post-flowering inoculation of dead tomato flowers with conidia of *Penicillium* spp. completely suppressed subsequent infection of developing...
Fruits by Botrytis cinerea (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. africana 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. africana and C. myriocarpus containing P. simplicissimum are used in soil and plant health.

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REFERENCES


