RESISTANCE OF *ANACARDIUM OCCIDENTALE* TO DECAY FOLLOWING ULTRAVIOLET RADIATION

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SUMMARY

Many host plants produce defense substances against fungal pathogens. These chemicals are at times induced in harvested crops by physical agents employed in plant disease control. The pseudo-apple of whole, unripe cashew fruits inoculated with *Gilbertella persicaria* were exposed to ultraviolet - B (UV-B) radiation, and when reduction in decay was noticed thereafter, the peels were investigated for antifungal substances. Ten major compounds were detected from the peels by GC-MS, three of which had been previously reported to exhibit antifungal activities. The three were 2, 4-Di-tert-butylphenol (24DTBP); (Z)-3-pentadec-8-en-1-yl) phenol and 2-Methyl-Z, Z-3, 13-octadecadienol. Some fractions of the peel extract also inhibited fungal spore germination *in vitro* revealing the mode of action of the compounds. The results suggest that unripe host cashew fruits actively contributed to decay suppression with antifungals production in addition to the disinfecting action of UV-B.

Keywords: Disease, defense, fungus, ultraviolet - light, antifungals.

CASHEW (*Anacardium occidentale* L.) is affected by many diseases although attention has been concentrated mainly on field infections which affect the yield of the whole crop (2, 9, 12, 15). For economic reasons, attention on postharvest diseases has been on the nut (10). The fleshy part of the fruit (pseudo-apple) is affected by a number of fungi after harvest but *G. persicaria* is particularly devastating on the pseudo-apple growing rapidly and causing complete decay within a day of harvest (1). Ultraviolet light of B and C spectra have been applied on

various fruits for postharvest decay control with varying degrees of success (3, 16). On strawberries, UV-C controlled decay caused by *Botrytis cinerea* and prolonged shelf-life by 4 – 5 days. On citrus fruits, UV light was effective in reducing the development of postharvest diseases. UV light is effective partly as a result of its germicidal action against microorganism thereby acting as 'disinfectant' on commodities exposed to it. It is also effective because it is able to induce resistance of fruits and vegetables to postharvest storage rots. It elicits the production of flavonoids, phytoalexins and other defense substances against pathogen attack, injury and abiotic stress (22). The mode of action of these substances could be antifungal, antibacterial or antioxidant, inhibiting either germination or growth of the pathogen in the tissues of the host. Earlier study revealed that UV-B reduced *Gilbertella* rot on Cashew pseudo-apples (1). This work is therefore an attempt to investigate the involvement of phytochemicals in the disease resistance observed with the use of UV-B.

MATERIALS AND METHODS

Preparation of fruits for chromatography

Whole unripe cashew fruits were surface disinfected, inoculated on the shoulder with the spores of *Gilbertella persicaria* by lacerating with a needle dipped in the spore suspension, then exposed to UV-B separately for 1-5 minutes in triplicates before or after inoculation and then stored at 28^0 C. Those whose pseudo-apples showed no decay or with only the first sign of infection on the third day of storage were those exposed for 2 minutes pre- and post- inoculation and were used for the resistance studies three days after treatment. Their peels were excised, air dried for 48 hours and pulverized. The powder (10 g) was soaked in 100ml of 70% ethanol for 72 hours before filtering through sterile cotton wool and evaporating to dryness using a rotary evaporator (Labo Rota 300 Resona).

The concentrated extract was loaded unto chromatographic column packed with silica gel (silicon dioxide 60 x 120 mesh) as stationary phase. It was eluted with methanol: ethanol: petroleum ether: water (8:8:1:3). Fractions were collected from the column in 5ml portions until the column ran dry.

Spore germination studies

Each fraction collected from the column was separately tested for ability to inhibit fungal spore germination using the spores of *G. persicaria* as test organism. This was

done by combining a drop of fungal spore suspension with that of the chromatographic fraction on a microscope slide and incubating for 24 hours. The slide was observed under the microscope for germ tube appearance indicative of germination thereafter, using x40 objective.

The fractions which completely inhibited spore germination were pooled together and subjected to gas chromatography – mass spectrometry (GC-MS) in order to separate and identify the components.

RESULTS

The fungal spore germination inhibiting fractions from unripe cashew peels revealed ten major compounds by GC-MS (Fig. 1). The compounds were five esters, two phenolic substances, two acids and one alcohol. The most abundant substance was a phenol (Z)–3–(pentadec–8–en–1–yl) phenol (Fig. 2) detected at 29.512 minutes which constituted 75.534%. The other phenolic compound was the volatile 2, 4-Di-tert-butylphenol (Fig. 3) detected at 13.346 minutes. 24DTBP constituted only 1.70% of the fractions.

The alcohol 2–Methyl–Z, Z–3, 13–octadecadienol (Fig.4) detected at 30.043 minutes constituted 0.061% and was the least abundant.

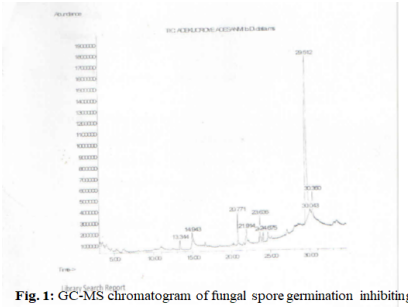

Fig. 1: GC-MS chromatogram of fungal spore germination inhibiting fractions.

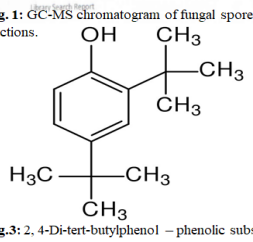

Fig.3: 2, 4-Di-tert-butylphenol – phenolic substance

Fig.2: (Z)-3-(pentadec-8-en-1-yl) phenol- an alkyl phenol

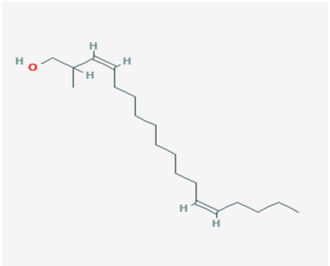

Fig.4: 2–Methyl–Z, Z–3, 13–octadecadienol – an alcohol

The esters detected were Diethyl phthalate with retention time of 14.897 minutes, Hexadecanoic acid methyl ester with retention time of 20.768 minutes, 11-Octadecanoic acid methyl ester at 23.634 minutes, methyl stearate at 24.058 minutes and Bis (2-ethylhexyl) phthalate at 30.352 minutes. The acids were Octadecanoic acid with retention time of 21.912 minutes and Palmitoleic acid at 24.676 minutes.

Other compounds detected include heptadecanoic acid, 14-methyl, methyl ester; ethyl oleate; 14-pentadecenoic acid; 4-methylphenol, isopropyl ether; 3-((4Z, 7Z)-heptadeca-4,7-dien-1-yl) phenol; and (Z)-3-(heptadec-10-en-1-yl) phenol (Fig. 1).

DISCUSSION

Many of the detected compounds are components of essential oils of plants. The 2, 4-Di-tert-butylphenol compound was reported to exhibit antifungal and antioxidant activities. Its fungicidal activity was reported against *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium chrysogenum in vitro* while a supplemented fraction of 24DTBP also prevented growth of the fungi on wheat grains (21). Although 24DTBP constituted only 1.70% of the fractions, its previously reported activities against fungi suggests that it was active in inhibiting spore germination leading to the reduced decay observed on unripe cashew exposed to UV-B for 2 minutes in the present study. Increase in phenolic compounds involvement in mango fruit resistance to anthracnose has been reported (4). This further supports the role of phenolics in host resistance mechanisms in cashew peels as has been reported in other plants (11, 20). The alcohol presently detected was also reported to be included among the compounds which inhibited growth of Gram-positive bacteria (18). These literature reports on the two groups of compounds confirm their antimicrobial activities, hence their possible role in the observed inhibition of fungal spore germination. The acids in the peel extract could have also contributed to inhibition of spore germination as reported for *in vitro* studies of p-coumaric acid on six fungal pathogens (7). Ascorbic acid was also indirectly implicated in plant defense (17). The presence of these compounds in the peels of inoculated unripe cashew exposed to UV-B, show that the host produced the chemicals to defend itself against invasion by pathogen. Phenolics are constitutive defense compounds in many plants (14). Some compounds particularly phytoalexins are also

produced by host in response to infection or exposure to abiotic stress including UV light. Such compounds are responsible for induced resistance. Any of the phenolic compounds or alcohol detected in the present study might be constitutive or induced. Although some esters and low concentrations of acids have been reported directly or indirectly associated with host defense against pathogen (6, 12), none of those presently detected have been reported implicated in such action.

The reduction in decay observed on unripe cashew was likely due to the direct effect of UV-B on spores of *G. persicaria* by inactivating them and also on the inhibitory action of some of the identified compounds on spore germination resulting in host protection. Two antifungal alkyl phenol compounds named 5–(12 –cis heptadecenyl) resorcinol and 5–pentadecenyl resorcinol from the peel of mango also a member of the Anacardiaceae, were also found acting as preformed agent defending the fruit against *Alternaria alternata* (5, 8). It was noted from a previous report that two antifungal compounds named cis, cis–1–acetoxy–2–hydroxy–4–oxo– heneicosa–12–15–diene, and 1–acetoxy–2, 4–dihydroxy–n–heptadeca–16–ene inhibited spore germination of *Colletotrichum gloeosporioides* infecting avocado (20).

The high concentration of (Z)–3–(pentadec–8–en–1–yl) phenol in the peel of unripe cashew is indicative of its activity since high levels of defense substances in plants is associated with their roles in reducing or preventing pathogenesis. High levels of the two alkyl phenols were reported in unripe mango peels that showed latency to *A. alternata* which caused black spot, but their concentrations decreased as the fruit ripened and disease developed (5).

The presence of these compounds in the peels of pseudo-apples with reduced decay coupled with the observed inhibition of spore germination in this work has shown the advantage of applying physical agent such as ultraviolet light on cashew fruits to control postharvest decay on a limited scale since rot was not completely prevented. This is particularly attractive because of the need to reduce the use of chemical protectants on fruits and vegetables consumed whole, recognizing the potential injurious effects of such chemical applications on consumer health. Further investigations may however be necessary to actually determine the production status of the detected antifungals in order to fully elucidate their roles in host defense.

Any of the compounds detected in the present study might be constitutive or induced.

CONCLUSION

Our results show that Cashew pseudo-apples produce antifungal substances probably constitutively or by induction as a result of combination of infection and exposure to UV-B. These compounds serve as protectants against fungal rot induced by inoculation with *G. persicaria* giving insight to the inate ability of the fruit to resist fungal colonization of its tissues.

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