Original article

Evaluation of Ethiopian plant extracts, *Acacia seyal* and *Withania somnifera*, to control green mould and ensure quality maintenance of citrus (*Citrus sinensis* L.)

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Evaluation of Ethiopian plant extracts, *Acacia seyal* and *Withania somnifera*, to control green mould and ensure quality maintenance of citrus (*Citrus sinensis* L.).

Abstract — Introduction. Green mould, *Penicillium digitatum* (Pers.: Fr.) Sacc., causes economically important postharvest disease in citrus. **Materials and methods**. Ethiopian plant extracts of *Acacia seyal* (Del. Var. *Seyal*) and *Withania somnifera* (L.) Dual were used to test the control of green mould in wound-inoculated fruit, stored for 21 d at 7 °C and at > 85% RH. The chemical compositions of the two extracts were determined using high-performance chromatography. Thereafter, freshly harvested (naturally infected) fruit were subjected to different postharvest treatments and stored for 50 d to investigate the effects of the two plant extracts on fruit quality parameters. Treatments included (pre-wax + leaf extracts), (wax + leaf extracts incorporated into wax, Citrosol A®), (leaf extract alone), (fruit washed in chlorinated water at 5.25%), (untreated fruit) and (commercially treated fruit). **Results**. Extracts of *A. seyal* and *W. somnifera* reduced the incidence of green mould by 56.1% and 50%, respectively, in wound-inoculated fruit. *A. seyal* extract contained a high concentration of gallic acid (60.3 $mg \cdot mL^{-1}$) whilst *W. somnifera* contained low concentrations of caffeic acid (8.7 $mg \cdot mL^{-1}$), salicylic acid (6.3 $mg \cdot mL^{-1}$) and 3,4 dihydroxy benzoic acid (3.8 $mg \cdot mL^{-1}$). Green mould was absent in naturally infected fruit subjected to (pre-wax + leaf extracts), (wax mixed with leaf extracts) and (leaf extracts) treatments. (Pre-wax + leaf extracts) and (wax mixed with leaf extract) treatments significantly reduced weight loss; retained firmness and colour; and they maintained eating qualities and a maturity index (SSC/TA) similar to commercial treatment. **Conclusion**. Both extracts of *A. seyal* and *W. somnifera* showed potential to be used as an alternative in combined applications with wax application under low temperature storage to replace synthetic fungicides, to ultimately control green mould and retain overall fruit quality.

South Africa / *Citrus sinensis* / fruits / quality / disease control / moulds / *Penicillium digitatum* / biological control / plant extracts / *Acacia seyal* / *Withania somnifera*

Évaluation d'extraits de plantes éthiopiennes, *Acacia seyal* et *Withania somnifera*, pour contrôler la moisissure verte et maintenir la qualité des agrumes (*Citrus sinensis* L.).

Résumé — Introduction. La moisissure verte, due à *Penicillium digitatum* (Pers.: Fr.) Sacc., est la maladie après récolte des agrumes la plus importante économiquement. **Matériel et méthodes**. Les extraits de deux plantes éthiopiennes, *Acacia seyal* (Del. Var. *Seyal*) et *Withania somnifera* (L.) Dual, ont été utilisés pour étudier leur effet sur le développement de la moisissure verte sur fruits inoculés par blessure, puis stockés pendant 21 jours, à 7 °C et HR > 85 %. La composition chimique des deux extraits a été analysée à l'aide d'un chromatographe liquide haute performance. Par ailleurs, des fruits naturellement infectés, récemment récoltés, ont été soumis à différents traitements après récolte et stockés pendant 50 jours pour étudier l'effet de ces deux extraits végétaux sur certains paramètres de qualité du fruit. Les traitements ont consisté en (un pré-enrobage de cire, puis application des extraits de feuille), (un enrobage avec les extraits de feuille incorporés à la cire, Citrosol A®), (une application des extraits de feuille sans cire), (un nettoyage des fruits avec de l'eau chlorée à 5.25 %), (un traitement des fruits effectué dans les conditions commerciales) et (l'absence de traitement des fruits). **Résultats**. L'utilisation d'extraits d'*A. seyal* et de *W. somnifera* a réduit l'incidence de la moisissure verte de 56.1 % et 50 %, respectivement, sur les fruits inoculés par blessure. Les extraits d'*A. seyal* ont révélé une forte concentration en acide gallique (60,3 $mg \cdot mL^{-1}$), tandis que *W. somnifera* a montré de basses concentrations en acide caféique (8,7 $mg \cdot mL^{-1}$), acide salicylique (6,3 $mg \cdot mL^{-1}$) et acide 3.4 benzoïque (3,8 $mg \cdot mL^{-1}$). Il n'y a pas eu de moisissure verte sur les fruits naturellement infectés soumis aux traitements (pré-enrobage de cire, puis application d'extraits de feuille), (enrobage avec extraits de feuille incorporés à la cire) et (application d'extraits de feuille sans cire). Les traitements (pré-enrobage de cire, puis application d'extraits de feuille) et (enrobage avec extraits de feuille incorporés à la cire) ont réduit de manière significative la perte de poids ; la fermeté et la couleur des fruits ont été maintenues ; leurs qualités gustatives et leur index de maturité ont été comparables aux caractéristiques des fruits ayant subi le traitement commercial. **Conclusion**. Les extraits des deux plantes, *A. seyal* et *W. somnifera*, pourraient être utilisés en combinaison avec de la cire, en conditions de stockage à basse température, pour remplacer les fongicides synthétiques afin de contrôler la moisissure verte des agrumes tout en maintenant la qualité globale du fruit.

Afrique du Sud / *Citrus sinensis* / fruits / qualité / contrôle de maladies / moisissure / *Penicillium digitatum* / lutte biologique / extrait d'origine végétale / *Acacia seyal* / *Withania somnifera*

1. Introduction

Sweet orange citrus (*Citrus sinensis* L.) production is expanding in African countries, including Ethiopia, mainly to cater for the nutritional needs of local consumers. Green mould, *Penicillium digitatum* (Pers.: Fr.) Sacc., causes the most economically important postharvest disease in citrus. Wounds present on fruit surfaces are a result of improper harvesting and subsequent handling practices provide a primary infection site for the pathogen [1]. This wound-obligate pathogen has a short disease cycle (3–7 d at 25 °C) and produces billions of conidia that are efficiently dispersed via air currents [2]. Currently, green mould is primarily controlled by synthetic fungicides such as imazalil, sodium orthophenyl phenate and thiabendazole.

The adverse effects of synthetic chemical residues on human health and the environment have led to intensified worldwide research efforts to develop alternative control strategies. In addition, fungicides are too expensive for many developing countries on the African continent. Alternative methods such as the use of biocontrol formulations using *Candida oleophila* [3] or *Pseudomonas syringae* [4] and Generally Regarded as Safe (GRAS) compounds, such as sodium bicarbonate dip treatments [5] and application of plant extracts, have already been extensively researched for the control of green mould rot during storage. The majority of subsistence farmers in Ethiopia are dependent on locally available disease control measures and plant extracts easily obtained and commonly used in traditional medicinal practices in Ethiopia [6, 7]. Under *in vitro* conditions, *Acacia seyal* (family Fabaceae) and *Withania somnifera* (family Solanaceae) leaf extracts exhibited a broad-spectrum antimicrobial activity against food-borne and plant pathogens [8]. Application of *Aloe vera* gel on wounded grapefruit reportedly reduced green mould decay by 75%, 6 d after inoculation with *P. digitatum* [9]. The essential oil from cumin (*Cuminum cyminum* L.) has also been reported to protect citrus fruit from *P. digitatum* [10].

The aims of our study were, firstly, to evaluate the efficacy of plant extracts obtained from two indigenous Ethiopian plants, *A. seyal* and *W. somnifera*, on the control of green mould rot and, secondly, to evaluate the quality maintenance of sweet orange under semi-commercial conditions for growers on the African continent, including South African small-scale and emerging growers.

2. Materials and methods

2.1. Leaf extract preparation

Leaf material of two plant species, *i.e., Acacia seyal* and *Withania somnifera*, were collected from Methahara and Hursso, Ethiopia, and air-dried. Undamaged leaves were powdered in a blender (Russell Hobbs) and stored at 18 °C in amber bottles until further use. Dried plant samples were transported to the Plant Pathology Laboratories, University of Pretoria, South Africa, using an import permit. One gram of the dried plant powder was suspended in 20 mL (w/v) methanol solvent mixture [(methanol / acetone / water) (7:7:1)] followed by three successive extractions. The combined supernatants were concentrated and the methanol + acetone were removed from the extract by means of vacuum distillation at 30–35 °C using a Büchi rotary evaporator. The remaining aqueous solution is referred to as the crude extract. A volume of distilled water equal to that of the original extraction solvent system was added to the crude extract to prepare the final stock solution. The suspension was filter-sterilised into sterilised Shott bottles using 0.45-µm pore size sterile filters (Sartorius, Germany) and stored at (4 ± 1) °C until further use.

The chemical composition of the extract was determined using high-performance liquid chromatography (HPLC) (Varian) equipment equipped with a Luna 5-µm 3u C18 reverse-phase column [(250 × 4.60) mm]. Acetonitrile and water (pH 2.6, acidified with phosphoric acid, H_3PO_4) were used as eluents with a gradient programme from 7% acetonitrile / water at 0 min to 20% at 20 min, increasing to 23% at 28 min, 27% at 40 min, 29% at 45 min, 33% at 47 min and

80% at 50 min. An aliquot of 20 µL of each sample [(CE (= crude extract), 20×; FA (free acid), 10×; Gly (glycoside), 10× and EB (ester bound), 10× diluted] was injected and chromatogrammed at a flow rate of 1 $mL \cdot min^{-1}$. Reference standards for gallic acid, caffeic acid, ferulic acid, syringic acid, quercetin, umbelliferone, naringin, hydroxyl benzoic acid, 3,4 dihydroxy benzoic, sinapic acid, vanillic acid, p- coumaric acid, salicylic acid, scopoletin, catechin, kaempferol, chlorogenic acid, luteolin and fisetin were obtained from Sigma Aldrich Pty. Ltd. (Johannesburg, South Africa). Peak area measurements were used to quantify the components.

2.2. Effect of *A. seyal* and *W. somnifera* extracts on the development of green mould rot infection

Citrus, cv. Valencia, were collected from the J.M. du Toit citrus pack-house (Tzaneen, Limpopo Province, South Africa). Fruit were disinfected with 1% NaOCl for 2 min and air-dried for 5 min prior to wounding. The fruit were wounded on the side (one wound per fruit) and wounds were treated with each plant extract (30 µL) separately. Thereafter, the treated fruit were left for 12 h before inoculating with 30 µL spore suspension (3×10^4 spores per wound).

A spore suspension was prepared from a 10-day-old *Penicillium digitatum* culture by dislodging spores gently with a sterile glass rod. Thereafter, the suspension was filtered through four layers of cheesecloth in order to remove the mycelia. The concentration of the spores was determined using a haemocytometer and adjusted to 10^6 $spores \cdot mL^{-1}$. The following treatments were included in this study: (1) fruit wounded + extract of *A. seyal* + *P. digitatum*; (2) fruit wounded + extract of *W. somnifera* + *P. digitatum*; (3) untreated (unwashed), unwounded; (4) fruit wounded only; (5) fruit wounded + *P. digitatum*.

After treatments, the treated fruit were kept for 21 d in commercial cartons at 7 °C and at more than 85% RH. Evaluation of fruit for disease development was performed weekly and percentage disease incidence was recorded. Each treatment in this study consisted of three replicate boxes each containing 20 fruits and the study was done in duplicate during the growing season.

2.3. Effect of *A. seyal* and *W. somnifera* extracts on freshly harvested fruit

For this study, a total of 1 800 fruits were randomly selected. In each treatment application, fruit were dipped in treatment solutions for 2 min and air-dried for 10 min. Fruit were subjected to one of the following postharvest dip treatments (*table I*).

For [pre-wax + leaf extract] treatments and combined application [wax + leaf extract treatments], fruit were dipped in 500 mL leaf extract stock solution, in 4 L Citrosol-A® wax and 5 L plant extract-water solution [1 L leaf extract (5%) and 4 L water], respectively. The commercial treatment included was based on the South African citrus industry application and includes: dipping fruit in chlorine water (sodium hypochlorite, 250 $\mu L \cdot L^{-1}$) for 2 min; Sporekill (12% didecyl dimethyl ammonium chloride, 900–1400 $\mu L \cdot L^{-1}$) [Hygrotech (Pty) Ltd., Johannesburg, South Africa] spray application (30 s); Quattro kill (N, N didecyl-N, N-dimethyl ammonium chloride, 1300 $\mu L \cdot L^{-1}$) [Hyper Agrochemicals (Pty) Ltd., Johannesburg, South Africa] at 45 °C for 5 min; imazalil (1350 $\mu L \cdot L^{-1}$) (Sanachem, Johannesburg, South Africa) spray application (30 s); air-drying for 2 min; wax with Citrosol-A® (100 000 $\mu L \cdot L^{-1}$) (Brenntag, Germany) for 2 min and air-drying.

Fruit were dipped in postharvest treatment suspensions for 2 min and, thereafter, air-dried for 10 min and stored at 7 °C and 80–90% RH for 50 d. Each postharvest dip treatment consisted of a set of 44 fruits in commercial cartons (three replicate boxes). Next, the fruit were evaluated for overall quality maintenance and organoleptic parameters. This experiment was performed in duplicate with five replicate boxes (50 fruits per box) per treatment.

Table I.
Postharvest treatments used to test control of green mould in wound-inoculated citrus fruit, stored for 21 d at 7 °C and at more than 85% RH (South Africa).

Treatment considered	Description of the treatment considered
Pre-wax + plant extract	• *A. seyal* leaf extract application followed by air-drying and wax with Citrosol-A® (water-based wax formulation in polyethylene wax), Brenntag, Germany) • *W. somnifera* leaf extract application followed by air-drying and wax with Citrosol-A®
Combined application: Wax + Plant extracts	• Combined treatment of *A. seyal* leaf extract incorporated into Citrosol-A® • Combined treatment of *W. somnifera* leaf extract incorporated into Citrosol-A®
Leaf extract (alone)	• Treatment with *A. seyal* leaf extract alone • Treatment with *W. somnifera* leaf extract alone
Control	• Untreated control • Dipped in chlorinated water • Commercial packing line treatment

2.4. Fruit quality parameters

Postharvest fruit quality was assessed for incidence of green mould rot and browning related to phytotoxicity.

Fruit subjected to all treatments (each replicate box) described were weighed before and after storage at 7 °C to assess percentage weight loss.

Rind colour was measured for 20 fruits from each replicate box using a Minolta chromameter (model CR-300; Osaka, Japan), expressing CIELAB Commission internationale de l'éclairage (CIE) colour space (with the L^*, a^*, b^* coefficients). A colour index (CI) was calculated: [CI = 1000 a / ($L \times b$)] [11]. According to the CI, negative and positive values indicate greenish and reddish tonalities, respectively. Within the positive range, less intense yellow (orangish) and more intense red colours (orangish red) are shown by a higher value of CI.

Skin browning, caused by phytotoxic damage due to plant extract dip treatments adopted in this study, was assessed after cold storage visually as 0 = no browning; 1 = 10% of fruit surface brown; 2 = 25%; 3 = 50%; 4 = 75%; 5 = entire fruit surface brown.

Total Soluble Solids (TSS): TSS was determined from 20 fruits using fruit juice and a hand-held refractometer (Atago, Tokyo, Japan). Results were expressed as percentages of TSS. Titratable acidity (TA) was also determined by titrating 10 mL of the sample filtrate against 0.1 M NaOH with phenolphthalein as indicator. Acidity was expressed as percentage citric acid equivalent. The maturity index (MI) was determined as the [SSC / TA] ratio.

2.5. Sensory evaluation

For sensory evaluation, fruit samples removed from cold storage (from different treatments) were held at room temperature (25 °C) for 6 h. A set of 10 fruits per treatment was placed on white plates and immediately presented to a trained taste panel of 14 panellists familiar with the quality and sensory parameters of citrus fruit. Each sample was identified by a random three-digit code. The order of presentation of the samples on the plates was randomised for each panellist. The evaluation was conducted under sensory laboratory conditions. Distilled water was used to cleanse the palate between samples. The following attributes were selected for characterisation of citrus fruit: colour of the skin, juiciness, taste, development of off-flavours and off-odours, and overall acceptance. The panellists assessed all the samples and at the same time gave ratings for the above-mentioned parameters according to a 9-point hedonic scale, where 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely.

2.6. Statistical analysis

The experiments were performed in a completely randomised design. Analysis of variance was used to test for differences between treatments. Treatment means were separated using Fisher's protected least significant difference (LSD) at the 5% level of significance.

Multivariate Canonical Variate Analysis (CVA) is a useful statistical tool to identify differences between groups of individuals (or treatments) and to improve the understanding of the relationships between the variables measured within those groups. The CVA maximises the variation between the groups of individuals whilst minimising variation within the groups of original variables. The CVA summarises in one or two analyses the information contained in the different independent variables. This method of multivariate analysis can be used for multiple comparisons between different postharvest treatments, and to determine to what extent the variation observed in the results was accounted for by each parameter studied [12]. In our study, the panellists evaluated the sensory parameters of citrus fruit for different postharvest treatments adopted during the experiment.

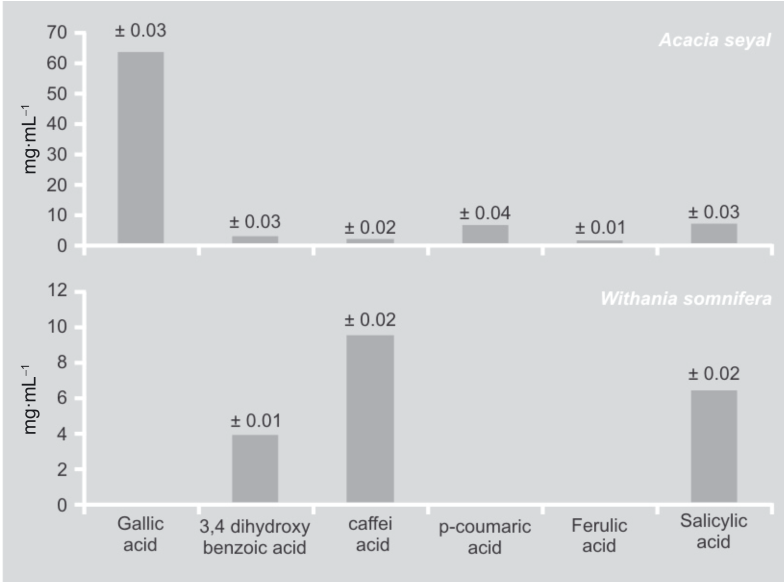


Figure 1.
Quantification of different phenolic compounds present in *Acacia seyal* or *Withania somnifera* using high-performance liquid chromatography. Each value is the mean ± standard deviation (n = 5).

3. Results

The major compound identified in the leaf extract of *A. seyal* was gallic acid (62.46 $mg \cdot mL^{-1}$). However, salicylic acid (6.23 $mg \cdot mL^{-1}$), p- coumaric acid, caffeic acid, 3,4 dihydroxy benzoic acid (2.2 $mg \cdot mL^{-1}$) and ferulic acid (0.91 $mg \cdot mL^{-1}$) were also observed in the HPLC chromatogram (*figure 1*). In *W. somnifera* leaf extract, the major compounds were caffeic acid (9.54 $mg \cdot mL^{-1}$) salicylic acid (6. 42 $mg \cdot mL^{-1}$) and 3,4-dihydroxy benzoic acid (3.81 $mg \cdot mL^{-1}$) (*figure1*).

In vivo wound treatments with *W. somnifera* extract and *A. seyal* reduced green mould incidence by 56.1% and 50%, respectively, compared with the control fruit inoculated with *P. digitatum* during 21 d in cold storage (*figure 2*).

Fruit subjected to postharvest treatments with plant extracts of *A. seyal* and *W. somnifera* and wax treatments as pre-wax or incorporated into the wax showed higher fruit firmness than commercially treated fruit (*table II*). The observations showed that fruit subjected to wax and leaf extract treatments (pre-wax and incorporated into wax) and the commercial treatment had significantly ($P < 0.05$) less weight loss than untreated fruit or those washed in chlorinated water (*table II*). It is evident from these observations that the plant extracts combined with wax application helped to prevent moisture loss from fruit, thereby preventing weight loss and loss of fruit firmness. The rind CI (reddish-orange) increased significantly ($P < 0.05$) during storage for untreated control and for fruit dipped in chlorinated water (*figure 3*). However, fruit subjected to pre-wax and plant extracts or combined application of wax and plant extract, or the commercial treatment, showed CI (yellowish-orange) closer to the degreened fruit. Fruit dipped in plant extract alone showed a more reddish-orange colour in the rind but less than the untreated control fruit or fruit dipped in chlorinated water. This observation indicates that there is an absence of phytotoxic skin browning in fruit subjected to leaf extract treatments. Significantly ($P < 0.05$)

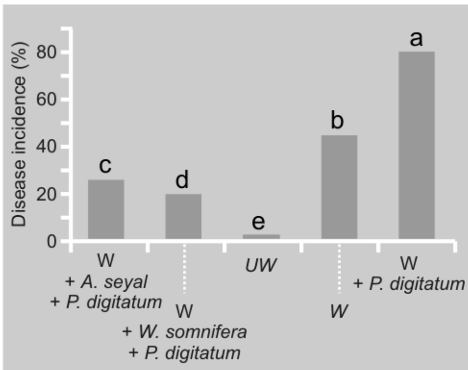


Figure 2.
Effect of antifungal activity of plant extracts of *Acacia seyal* and *Withania somnifera in vivo*. Means in each bar with the same letter are not significantly different at $P < 0.05$ by Fisher's protected least significant difference test.

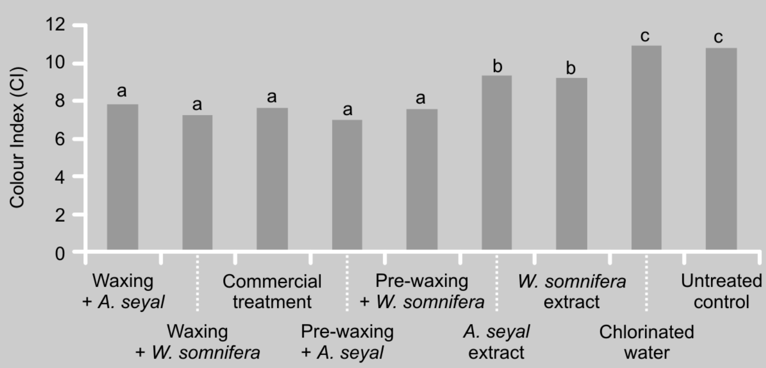
Table II.
Effect of plant extracts, *Acacia seyal* (*A. seyal* Del. Var Seyal) and *Withania somnifera* (*W. somnifera* L. Dunal), on the control of green mould rot incidence and overall quality retention of sweet orange under simulated semi-commercial conditions (– 7 °C at 85% RH) for 50 days.

Postharvest treatments	*Penicillium* decay incidence (%)	Weight loss (%)	Maturity index[a]
Pre-wax + plant extract treatment			
Pre-wax application of *A. seyal* extract	0.00 c	0.01 a ± 0.0	9.34 a ± 0.45
Pre-wax application of *W. somnifera* extract	0.00 c	0.01 a ± 0.0	9.36 a ± 0.34
Combined treatments: wax + plant extract			
A. seyal extract + wax mix	0.00 c	0.00 a ± 0.0	9.32 a ± 0.56
W. somnifera extract + wax mix	0.00 c	0.01 a ± 0.0	9.34 a ± 0.37
Plant extracts			
A. seyal extract	0.00 c	0.58 b ± 0.0	10.14 b ± 0.62
W. somnifera extract	0.00 c	0.56 b ± 0.0	10.30 b ± 0.52
Control			
Untreated	40.80 a ± 1.30	0.98 c ± 0.0	10.85 b ± 0.66
Dipped in chlorinated water	9.00 b ± 2.40	0.95 c ± 0.0	10.71 b ± 0.58
Commercial packing line treatment	0.00 c	0.01 a ± 0.0	9.35 a ± 0.72

[a] Maturity index calculated from [SSC / TA], with SSC: soluble sugar concentration; TA: titratable acidity.

Means in each column followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected least significant difference test.

Figure 3.
Effect of postharvest dip treatments with *Acacia seyal* and *Withania somnifera* on rind colour index. Means in each bar with the same letter are not significantly different at $P < 0.05$ by Fisher's protected least significant difference test. Waxing is done with Citrosol-A® (Brenntag, Germany).



higher maturity indexes (MI) were observed in untreated fruit or fruit treated with chlorinated water. However, an increase in MI was noted in fruit dipped in plant extracts and stored at low temperature.

Mean separation analyses of sensory parameters showed significant ($P < 0.05$) differences between different postharvest treatments with plant extracts alone, or with the combination of treatments, or with pre-wax treatments and plant extracts (data not presented). In further analyses, CVA, used to evaluate the differences between the six sensory parameters and to show the relative contribution of each variable to the sensory quality on citrus fruit with respect to the different postharvest treatments, was effective for data assessment.

The CVA plot axis CA 1 accounts for 61% of the variance and the axis CA 2 for 19%, together accounting for nearly 80% of the total variance observed (*figure 4*).

For the CVA plot *x*-axis (CV 1, 61% of the variance), four sensory attributes (taste, flavour, overall acceptance and odour) mostly discriminated the treatments (pre-wax + plant extracts), (combined wax + plant extracts), and (commercial treatment) and the treatment (plant extracts) from the two treatments (untreated control) and (chlorinated water treatment). CV 1 was loaded negatively with taste ($r = -0.636$), flavour ($r = -0.899$), odour ($r = -0.769$) and overall acceptance ($r = -0.738$). For the *y*-axis (CV 2, 19% of the total variation), two parameters (skin colour and juiciness)

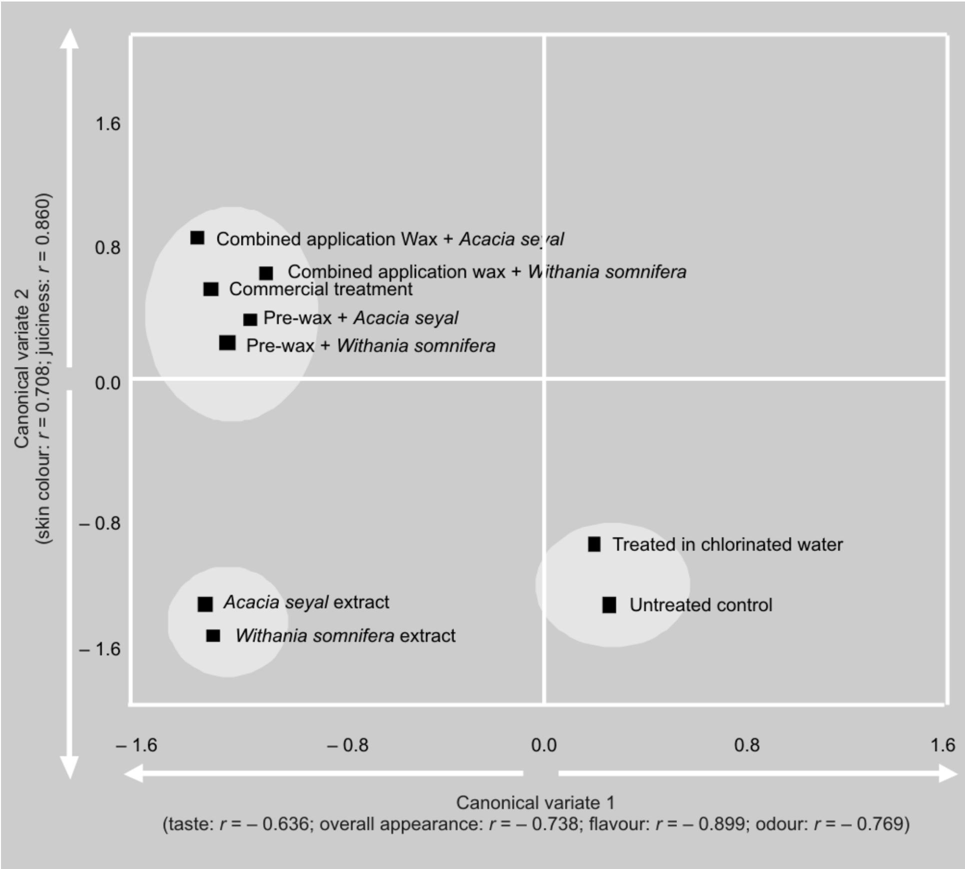


Figure 4.
Canonical Variate Analysis (CVA) achieved for assessing the effect of postharvest treatments adopted during the experiment on six sensory characters of citrus fruit. This two-dimensional representation of the characters accounted for nearly 90% of the total variation in the data.

mostly discriminated the three treatments (pre-wax + plant extracts), (combined wax + plant extracts), and (commercial treatment) from the treatment (leaf extract dip treatment, alone). This axis was loaded positively with fruit skin colour (r = 0.708) and juiciness (r = 0.86).

4. Discussion

Acacia seyal and *Withania somnifera* extracts are used in traditional healing of human ailments in Ethiopia [6, 7], and other African countries [7, 13].

The major compounds found in *W. somnifera* leaf extract were caffeic acid, salicylic acid and 3,4 dihydroxy benzoic acid. This observation confirms previous findings [14]. Furthermore, the majority of antimicrobial compounds in *W. somnifera* are withanolides and glycowithanolides [15].

The major compounds in *A. seyal* were reported as tannins referred to as bitter plant polyphenols [16]. Gallic acid (3,4,5-trihydroxy benzoic acid) is an organic acid found both free and as part of tannins. The mode of action of these two extracts was due to a significant increase in total soluble phenolics concentration at the wound sites [17]. The increase in soluble phenolics indicates the activity of the key enzyme phenylalanine ammonia-lyase (PAL) towards the synthesis of soluble phenolics that inhibits the progress of disease due to the induced defence mechanism in the fruit [18]. Furthermore, it was reported that the extracts showed a direct interaction with the pathogen by sticking its spores together at the infection site [17, 19]. The other mechanism, involved with direct reaction to the pathogen by adhesion, indicates the putative involvement in the physical and biochemical defense responses against the pathogen.

The non-target effect of the plant extracts on the orange fruit microflora showed a general trend of decrease in microbial diversity, whilst favouring surface colonisation by yeasts and bacteria.

Fruit subjected to commercial treatment and leaf extract and wax combinations (pre-wax or incorporated into the wax) maintained their maturity index (MI) by preventing the increase in SSC, by reducing the rate of respiration during storage. Wax coating creates a modified atmosphere around the fruit and results in an increase in CO_2 concentration within the coating, slowing down the rate of respiration [20]. The observed retention of the [SSC / TA] ratio in fruit subjected to combined application of leaf extract and wax or to the commercial treatment could probably be due to the direct effect of reduced rate of respiration. It is evident from this evaluation that pre-wax + application of plant extracts (*A. seyal* or *W. somnifera*) or combined application of wax with plant extracts (leaf extract incorporated into the wax) maintained the quality of the fruit at 7 °C equal to the commercial treatment. However, the combined application with leaf extract and wax (leaf extract incorporated into the wax) is beneficial and cost-effective. This is due to less volume of leaf extract used for the combined application than for the (pre-wax + leaf extract treatment). Furthermore, the incorporation of the leaf extract into the wax solution retained the total solids of the wax formulation at 13%. Wax coating containing 13% total solids was reported to retain the fruit qualities and prevented the build-up of CO_2 concentrations in the internal atmosphere of the fruit [21]. Build-up of CO_2 concentrations within the internal fruit atmosphere favours anaerobic conditions, thereby causing ethanol accumulation that ultimately results in off-flavour development in the edible portion [22].

5. Conclusion

Both extracts of *A. seyal* and *W. somnifera* showed potential to be used as an alternative in combined applications due to the availability of *A. seyal* and *W. somnifera* in the African region, with wax application under low temperature storage, to replace synthetic fungicides to ultimately control green mould and retain overall fruit quality. This application could be regarded as a novel postharvest treatment for citrus fruit destined for the domestic market.

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References

[1] Eckert J.W., Brown G.E., Evaluation of post-harvest treatments for citrus fruits, in: Hickey K.D., Methods for evaluating pesticides for control of plant pathogens, Am. Phytopathol. Soc. Press, St.-Paul, MN, USA, 1986, pp. 92–97.

[2] Snowdon A.L., Color atlas of post-harvest diseases and disorders of fruits and vegetables, Vol. 2., CRC Press, Boca Raton, Boston, USA, 1992.

[3] Droby S., Cohen L., Daus A., Weiss B., Horev B., Chalutz E., Katz H., Keren-Tzur H., Shachnai A., Commercial testing of aspire: a yeast preparation for the biological control of postharvest decay of citrus, Biol. Control 12 (1998) 97–107.

[4] Bull C.T., Wadsworth M.L., Sorensen K.N., Takemoto J.Y., Austin R.K., Smilanick J.L., Syringomycin E., Produced by biological control agents controls green mold on lemons, Biol. Control 12 (1998) 89–95.

[5] Similanick J.L., Mansour M.F, Margosan D., Mlikota Gabler F., Goodwine W.R., Influence of pH and $NaHCO_3$ on the effectiveness of imazalil to inhibit germination of spores of *Penicillium digitatum* and to control citrus green mold on citrus fruit, Plant Dis. 89 (2005) 640–648.

[6] Demissew S., Este debdabe: Ethiopian traditional medicine, Dep. Biol., Sci. Fac., Addis Ababa Univ., Ethiopia, 1989.

[7] Bekele T.A., Useful trees of Ethiopia, RSCU, SIDA, Nairobi, Kenya, 1993.

[8] Mekbib S.B., Identification of citrus (*Citrus sinensis*) postharvest pathogens from Ethiopia and their control, thesis, Univ. Pretoria, South Africa, 2007.

[9] Saks Y., Baraki-Golan R., *Aloe vera* gel activity against plant pathogenic fungi, Postharvest Biol. Technol. 6 (1995) 159–165.

[10] Yigit F., Ozcan M., Akgul A., Inhibitory effect of some spice essential oils on *Penicillium digitatum* causing postharvest rot in citrus, Grasasy Aceites 51 (2000) 237–240.

[11] Palou L., Marcilla A., Rojas-Argudo C., Alonso M., Jacas J.A., del Río M.A., Effects of x-ray irradiation and sodium carbonate treatments on postharvest *Penicillium* decay and quality attributes of clementine mandarins, Postharvest Biol. Technol. 46 (2007) 252–261.

[12] Sivakumar D., Korsten L., Influence of modified atmosphere packaging and postharvest treatments on quality retention of litchi 'Mauritius', Postharvest Biol. Technol. 41 (2006) 135–142.

[13] Duke J.A., Medicinal plants of the Bible, Trado-Medic Books, Owerri, N. Y., USA, 1983.

[14] Cowan M.M., Plant products as antimicrobial agents, Clin. Microbiol. Rev. 12 (1999) 564–582.

[15] Rahman A., Jamal S.A., Choudhary M.I., Asif A., Two withanolides from *Withania somnifera*, Phytochem. 30 (1991) 3824–3826.

[16] Mhinzi G.S., Intra-species variation of the properties of gum exudates from *Acacia senegal* var. *senegal* and *Acacia seyal* var. *fistula* from Tanzania, Bull. Chem. Soc. Ethiop. 17 (2003) 67–74.

[17] Mekbib S.B., Regnier T.J.C., Korsten L., Control of *Penicillium digitatum* on citrus fruit using two plant extracts and study of their mode of action, Phytoparasit. 35 (2007) 264–276.

[18] Cheng G.W., Breen P.J., Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit, J. Am. Soc. Hortic. Sci. 116 (1991) 865–869.

[19] Porat R., Vinokur V., Weiss B., Cohen L., Daus A., Goldschmidt E.E., Induction of resistance to *Penicillium digitatum* in grapefruit by β-aminobutyric acid, Eur. J. Plant Pathol. 109 (2003) 901–907.

[20] Ben-Yehoshua S., Fishman S., Fang D., Rodov V., New development in modified atmosphere packaging and surface coating for fruits, in: Chang M., Champ B.R., Highley E., Johnson G.I. (Eds.), Postharvest handling of tropical fruit, ACIAR, Canberra, Aust., 1994, pp. 250–260.

[21] Porat R., Weiss B., Cohen L., Daus A., Biton A., Effects of polyethylene wax content and composition on taste, quality and emission of off-flavour volatiles in 'Mor' mandarins, Postharvest Biol. Technol. 38 (2005) 262–268.

[22] Hagenmaier R.D., Shaw P.E., Gas permeability of fruit coating waxes, J. Am. Soc. Hortic. Sci. 117 (1992) 105–109.

Evaluación de extractos de plantas etíopes, *Acacia seyal* y *Withania somnifera,* para regular el moho verde y mantener la calidad de los cítricos (*Citrus sinensis* L.).

Resumen — Introducción. El moho verde causado por *Penicillium digitatum* (Pers.: Fr.) Sacc. es la enfermedad más importante económicamente después de cosecha de los cítricos. **Material y métodos**. Los extractos de dos plantas etíopes, *Acacia seyal* (Del. Var. *Seyal*) y *Withania somnifera* (L.) Dual, se emplearon para estudiar su efecto en el desarrollo del moho verde en los frutos inoculados por lesión, almacenados posteriormente durante 21 días, a 7 °C y HR > 85 %. Se analizó con la ayuda de un cromatógrafo líquido de alto rendimiento la composición química de ambos extractos. Acto seguido, los frutos naturalmente infectados, cosechados recientemente, se sometieron a diversos tratamientos tras su cosecha y se almacenaron durante 50 días para estudiar el efecto que ejercen estos dos extractos vegetales sobre ciertos parámetros cualitativos del fruto. Los tratamientos consistieron en (un pre-recubrimiento de cera, seguido de aplicación de extractos de hoja), (un recubrimiento con los extractos de hoja incorporados a la cera, Citrosol A®), (una aplicación de los extractos de hoja sin cera), (una limpieza de los frutos con agua clorada al 5.25 %), (un tratamiento de los frutos efectuado en las condiciones comerciales) y (ausencia de tratamiento de los frutos). **Resultados**. El uso de extractos de *A. seyal* y de *W. somnifera* redujeron la incidencia del moho verde del 56.1 % y el 50 %, respectivamente, en los frutos inoculados por lesión. Los extractos de *A. seyal* resultaron tener una fuerte concentración de ácido gálico (60,3 $mg \cdot mL^{-1}$), mientras que *W. somnifera* mostró bajas concentraciones de ácido caféico (8,7 $mg \cdot mL^{-1}$), acide salicílico (6,3 $mg \cdot mL^{-1}$) y ácido 3.4 benzoico (3,8 $mg \cdot mL^{-1}$). No hubo maho verde en los frutos infectados de forma natural sometidos a los tratamientos (pre-recubrimiento de cera, seguido de aplicación de extractos de hoja), (recubrimiento con extractos de hoja incorporados a la cera) y (aplicación de extractos de hoja sin cera). Los tratamientos (pre-recubrimiento de cera, seguido de aplicación de extractos de hoja) y (recubrimiento con extractos de hoja incorporados a la cera) redujeron de modo significante la pérdida de peso; la firmeza y el color de los frutos se mantuvieron; sus respectivas cualidades gustativas y su índice de madurez fue comparable a las características de los frutos sometidos a un tratamiento comercial. **Conclusión**. Los extractos de dos plantas, *A. seyal* y *W. somnifera*, podrían emplearse combinados con cera, en condiciones de almacenamiento a baja temperatura, para reemplazar los fungicidas sintéticos con el fin de regular el moho verde de los cítricos y mantener a la vez la calidad general del fruto.

Sudáfrica / *Citrus sinensis* / frutas / calidad / control de enfermedades / moho / *Penicillium digitatum* / control biológico / extractos vegetales / *Acacia seyal* / *Withania somnifera*