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Effect of smoke-water and a smoke-isolated butenolide on the growth and genotoxicity of commercial onion

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ABSTRACT

Smoke-water and a biologically active butenolide compound (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) derived from burning plant material, show stimulating effects on a number of agricultural and horticultural crops. In these trials, onion (*Allium cepa* L.) plants were treated (drenched) with either a 1:500 (v/v) smoke-water solution or a butenolide solution of 10^{-10} M under greenhouse conditions. Onion plants supplied with smoke-water and butenolide solution exhibited a significantly greater number of leaves, increased leaf length, and a higher fresh and dry leaf weight than untreated plants at 175 days after seed sowing (DASS) (third harvest). In addition, smoke-water and butenolide-treated onion plants exhibited a significantly higher bulb diameter and bulb weight than untreated plants, when these plants were harvested at 175 DASS. Overall, smoke-water was more effective than butenolide and achieved the highest harvest index. Genotoxicity was not detected in the bulbs of onion when they were treated with either smoke-water or butenolide.

1. Introduction

Plant-derived smoke shows stimulatory effects on seed germination as well as on the growth of plants. With the isolation of an active butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) compound (Flematti et al., 2004; Van Staden et al., 2004) – a potent germination stimulant from smoke, the use of smoke in various fields has become an important tool. Smoke and butenolide have the potential to be used in horticulture, agriculture and weed control (Light and Van Staden, 2004; Light et al., 2009).

A number of studies have reported a positive influence of smoke treatments on either seed germination or seedling growth of several crop plants. The crop plants which show response to smoke (in the form of aerosol-smoke, smoke-water or butenolide solution) are celery (Thomas and Van Staden, 1995), lettuce (Drewes et al., 1995), mung bean (Taylor and Van Staden, 1996), red rice (Doherty and Cohn, 2000), indigenous maize (Modi, 2002, 2004), carrot, parsley and leek (Merritt et al., 2005), commercial maize (Sparg et al., 2006), commercial bean (Van Staden et al., 2006), indigenous rice (Kulkarni et al., 2006), bush tomato (Ahmed et al., 2006), okra (Kulkarni et al., 2007), commercial tomato (Kulkarni et al., 2008) and tef (Ghebrehiwot et al., 2008). Increase in the yield of bush tomato was possibly due to the stimulating effect of smoke on seed germination (Ahmed et al., 2006). However, when a commercial variety of tomato was tested with smoke-water, it showed an increase in the number of marketable fruits (Kulkarni et al., 2008). The findings of all the above studies indicate that smoke can be used to improve growth and yield of crops.

According to the United Nations Food and Agricultural Organization (FAO), after tomato onion (*Allium cepa* L.) is one of the most important vegetable crops cultivated around the world. The size of the bulb is considered an essential quality aspect of onions (De Visser and Van den Berg, 1998). Much research has been conducted to improve the yield and bulb size of onion (Pelter et al., 2004; Aisha et al., 2007; Amin et al., 2007; Kumar et al., 2007). Even though it is generally accepted that burning fields improves soil fertility and crop yield (Meland and Boubel, 1966; Altieri, 1993), and despite smoke having a positive impact on a number of

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important crops, no studies have been reported on the influence of smoke or its constituents on the growth of onions. This study was therefore conducted to determine the effects of smoke solutions on growth and bulb size of commercial onion. Smoke is a complex chemical mixture with potentially hazardous substances, such as polycyclic aromatic compounds (PAHs), which are of health concern (Maga, 1988; Verschaeve et al., 2006). Hence, genotoxicity tests were also performed on treated and untreated bulbs of onion.

2. Materials and methods

2.1. Seed supplier

Onion seeds (*A. cepa* L. var Texas Grano 502 PRR) were purchased from McDonalds Seeds, Pietermaritzburg, South Africa.

2.2. Preparation of smoke-water and butenolide solution

Smoke-extract was prepared by bubbling smoke from smouldering *Themeda triandra* Forssk. grass leaf material through 500 mL water for 45 min (Baxter et al., 1994). One milliliter of this extract was added to 500 mL of distilled water to make smoke-water with concentration of 1:500 (v/v). The butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) was isolated from plant-derived smoke by the method of Van Staden et al. (2004) and based on the preliminary results a concentration of 10^{-10} M was chosen for this experiment.

2.3. Experimental design and greenhouse conditions

The experiment was conducted in the summer of 2007. The design of the experiment was fully randomized with 30 replicates for each treatment. On 23 March, five seeds of onion were sown at a depth of 1.5 cm in each plastic pot (500 mL), containing a soil mixture of compost and garden soil (1:1, v/v). Untreated (control) pots were irrigated with water three times weekly until the pots were drenched. The treated pots were drenched with smoke-water or butenolide solution twice per week and irrigated once weekly with water. Thirty days after sowing, one healthy seedling was retained in each pot and the rest uprooted and removed. The greenhouse temperature during the experiment was 20–23 °C with an average midday photosynthetic photon flux density of 400–485 μmol m^{-2} s^{-1}. Sixty days after sowing, 10 g of powder of the commercial fertilizer Kompel (Chemicult Products Pty. Ltd., Camps Bay, South Africa) was dissolved in 5 L of tap water and plants irrigated with 500 mL of this liquid. The plants were harvested at 121, 144 and 175 days after seed sowing (DASS) and various growth parameters were recorded. The dry weight of leaves was recorded after drying them at 70 °C for 7 days. The average absolute growth rate (AGR) (Clipson, 1994) and harvest index (HI) (Henderson et al., 2000; Prasad et al., 2006) were calculated. Subsequently, the onion bulbs of the last harvest (175 DASS) were investigated for genotoxicity. At the 175 DASS harvest, four samples from each exposure group were used in the Ames test and three in the micronucleus test.

2.4. Genotoxicity tests

Onion (175 DASS) juice was prepared in a blender and immediately frozen at −24 °C. The juice was thawed just before use and filtered through a Millipore Sterivex (0.22 μm) filter unit to obtain a sterile test substance. Usually 2 or 3 filter units were necessary to filter a complete sample (up to over 30 mL juice per onion). All juices were previously coded and codes were not known by the investigator (=blind testing). The code was broken only after all experiments were completed.

2.4.1. Mutagenicity and antimutagenicity testing

2.4.1.1. Ames test. The bacterial Ames assay was conducted in the absence and presence of a direct acting mutagen. The purpose was to test whether treated onions had acquired mutagenic properties due to the treatment, and whether eventual antimutagenic properties of onions were altered compared to untreated onions. This was based on the fact that vegetables often have antimutagenic properties. For example, organically cultivated vegetables have higher antimutagenic properties than conventionally cultivated vegetables (Ren et al., 2001).

Experiments were performed using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 and TA1538. Positive controls (100 μL $plate^{-1}$) were 4-NQO (4-nitroquinoline-oxide) in TA98, TA100 and TA1538 (0.2 μg mL^{-1}) or TA102 (10 μg mL^{-1}), 9-AA (9-aminoacridine) in TA1537 (0.5 mg mL^{-1}) and SA (sodium azide) in TA1535 (0.05 mg mL^{-1}). These concentrations were based on the results of preliminary experiments. From these experiments it was also decided to use 100 μL juices together with 100 μL of the mutagen for antimutagenicity testing. Initially, both the pre-incubation and plate incorporation tests were performed and as no differences were found in the results, it was decided to perform the plate incorporation test only. There were 3 replications of Petri dishes per exposure and a positive control as mentioned above was always incorporated together with a solvent control.

The Ames assay was performed according to standard methods (Maron and Ames, 1983). In brief, bacteria from overnight His^--*S. typhimurium* cultures were incubated on a minimal growth medium with a small amount of histidine and biotin so that only His^+-revertants could form visible colonies after 48 h cultivation time at 37 °C. Bacteria were incubated in the presence of onion juice or onion juice + mutagen. Negative (unexposed) or positive control cultures (=with the appropriate mutagen) were also made. A background layer of cultures was inspected for signs of toxicity, but no toxicity was evident in any of the experiments.

Antimutagenicity was evaluated according to the following formula of Ong et al. (1986),

$$\%\text{inhibition} = \frac{[1 - T]}{M} \times 100$$

where T is the number of revertants per plate in the presence of the mutagen (for example, 4-NQO and onion juice as a test sample) and M is the number of revertants per plate for the mutagen alone. An extract is considered highly antimutagenic when the inhibition (INH) exceeds 40%. For moderate antimutagenic properties INH should be between 25 and 40%, whereas a low or no antimutagenic property is considered when INH is below 25%. A negative percentage INH is indicative of a co-mutagenic action rather than antimutagenicity. For TA98 and TA100 up to four independent experiments were conducted and two independent experiments were performed for the other strains.

2.4.1.2. The micronucleus test. The genotoxic effects of treated or untreated onion juices were also tested in the micronucleus test that was performed in C3A cells. These are a human liver cell line derived from a hepatoblastoma, a tumor of early childhood (Kelly, 1994). C3A cells have been shown to maintain a wide array of metabolic functions characteristic of the human liver. The micronucleus test allows the visualization of structural and numerical chromosome aberrations by the presence of micronuclei in binucleated, cytochalasin B blocked telophase cells (Fenech and Morley, 1985).

Cell cultures were incubated in a humified atmosphere with 5% CO_2. They were exposed to the test substances after cultivation for 24 h. Following another 24 h, cells were washed and recultured in