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Occurrence of nutrients and plant hormones (cytokinins and IAA) in the water fern *Salvinia molesta* during growth and composting

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Abstract

The aquatic fern *Salvinia molesta* is classified as an invasive, noxious weed in southern Africa. It has rapid growth and forms a thick mat in slow moving water bodies. It is hypothesized that *S. molesta* is a suitable candidate to promote as an organic fertilizer. The aims of this study were to determine the mineral content as well as the cytokinin and auxin content in *S. molesta*. The release of these compounds into the culture medium and upon decomposition of the air-dried plant matter was also determined. Minerals detected in the fresh plant material were $N > K > P > Ca > Mg > Fe > Na > Cu > Zn$ while Cd, Pb and Ni were below the level of detection. The mineral concentrations changed after 14 days decomposition with there being an increase in Ca, Mg and Fe and a decrease in K. Sixteen isoprenoid cytokinins were detected in the plant material (total concentration 81.62 pmol g^{-1} DW) while only four cytokinins occurred at a greatly reduced concentration in the culture medium (4.31 pmol 500 ml^{-1} medium). The material that had been composted for 14 days showed a 17% reduction in cytokinin concentration (67.96 pmol g^{-1} DW) compared to the plant material. Run-off, termed leachate, collected during the composting experiment also had high amounts of cytokinin-like activity measured using the soybean callus bioassay. This activity peaked on day 7 of the composting experiment, indicating a rapid release of cytokinin-like compounds. IAA occurred in much higher concentrations than the cytokinins in the plant material (393.6 pmol g^{-1} DW) while very little IAA was detected in the culture medium (30.8 pmol g^{-1} DW). IAA concentrations were much lower in the composted material (148.1 pmol g^{-1} DW) with a 62% loss being recorded compared to the plant material. No auxin-like activity was measured using the mungbean rooting bioassay in the leachate collected at the start of the composting experiment. However, the leachate collected on days 7 and 14 had biological activity indicating that auxin-like compounds were released from *S. molesta* upon decomposition.
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1. Introduction

Owing to high energy costs, increased fertilizer costs and increasing concern over the adverse effects of long term use of agro-chemicals on soil productivity and environmental quality, there is now considerable interest in the use of renewable organic fertilizers. Biofertilizers have the advantages of being an inexpensive, renewable resource that is non-polluting, providing a source of nutrients, vitamins and growth substances. They also improve soil structure and fertility by increasing the organic matter content and improving the water holding capacity of the soil (Wagner, 1997).

The nitrogen-fixing water fern *Azolla* has been successfully used as a biofertilizer in China and other Asian countries as well as in South America and Africa for many years. *Azolla* is able to fix atmospheric nitrogen due to the symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena azollae* found in the dorsal leaf cavity of *Azolla* (see refs. in Lejeune et al., 1999). Beneficial effects of *Azolla* used as a biofertilizer in rice paddies and other dry-land crops such as wheat and vegetable crops include increased crop productivity, improved soil porosity and higher organic matter content (Lumpkin and Plucknett, 1980; Wagner, 1997). The beneficial effects are not caused by elevated nitrogen levels alone but also by the release of other nutrients, hormones, vitamins and other growth-promoting substances (Pedurand and Reynaud, 1987; Wagner, 1997).

Plant growth regulators play an important role in plant growth and development and can produce many desirable effects in crops. For example, the use of Seaweed Concentrates where the active ingredients have been identified as cytokinins and auxins (Stirk et al., 2004), is a well-established practice in agriculture. Their application influences many physiological processes and results in an improved root system, increased growth and yield, increased nutrient absorption and mobilization, increased resistance to stress and disease and retardation of senescence (Crouch and van Staden, 1994). Thus, if plants selected for use as biofertilizers contained nutrients as well as hormones, specifically cytokinins and auxins, they would have additional beneficial effects on the crops.

The water fern *Salvinia molesta* (Salviniales), commonly called the "kariba weed" is a free-floating aquatic plant which forms large expanses of thick mats in slow moving water bodies. It is a sterile hybrid introduced from South America and reproduces only by vegetative fragmentation (Henderson and Cilliers, 2002). The run-off of nutrients from fertilizers used in agriculture and the slowing of rivers by dams and weirs have created ideal conditions for its growth (Hill and Cilliers, 1999) and it is now classed as an invasive, noxious weed in southern Africa (Schelpe and Anthony, 1986; Burrows, 1990). It causes numerous problems such as reduced quality of drinking water, an increase in siltation of rivers and dams, reduced water surface for recreational use, clogging of irrigation systems and reduced water flow in irrigation canals. The thick mats also provide an ideal breeding place for carriers of malaria and bilharzia (Hill and Cilliers, 1999; Henderson and Cilliers, 2002).

It is hypothesized that *S. molesta* is a good candidate to promote as an organic fertilizer and growth biostimulant as it grows quickly on natural water bodies, is available throughout the year and requires little processing. It can be easily harvested by mechanical means (Mansor, 1996). The harvesting of *S. molesta* would also contribute towards its control in natural water systems. Thus the aims of this study were to quantify the mineral content as well as the cytokinin and auxin concentrations in *S. molesta* and to determine if these compounds are released upon decomposition.

2. Material and methods

2.1. Plant material and experimental design

S. molesta D.S. Mitchell was collected from a pond in the Botanical Gardens of the University of KwaZulu-Natal Pietermaritzburg and transferred to a bucket filled with 10 l tap water supplemented with Chemicult, a commercial fertilizer containing macro- and micro-nutrients used in hydroponic systems. The fern grew vigorously, providing a stock of young, healthy and clean plants for the experiment. Young *S. molesta* plants were transferred into a large plastic container filled with 70 l water supplemented with 140 g Chemicult. Due to transpiration and evaporation, it was necessary to replenish the medium with 15 l water and Chemicult mixture on days 5 and 21. The average minimum and maximum temperatures in the green house where *S. molesta* was grown during the experiment were 19 °C and 28 °C, respectively. *S. molesta* was harvested on day 31. A portion of this material was freeze-dried and ground into powder. The culture medium was also collected as 5 l units, filtered with Whatman No. 1 filter paper and dried down under vacuum at 35 °C.

The remaining plant material was air-dried (2 kg) and placed in a wooden bin (8×10^{-2} m^3) in a green house for the composting experiment. Water (1 l) was immediately poured over the plant material and the run-off collected in a tray placed beneath the wooden bin. This was referred to as the leachate. The average temperature of the compost, monitored by placing three thermometers in the decomposing plant material, was 25 °C. On days 7 and 14, 1 l water was poured over the compost and leachate collected in the tray. The leachate was filtered with Whatman No. 1 filter paper and dried down under vacuum at 35 °C. The experiment was terminated on day 14. The remains of the decomposed *S. molesta* were freeze-dried and ground into powder.

2.2. Quantification of mineral elements

Plant and composted material were analysed for their mineral content. Samples for nitrogen analysis were mineralized using sulphuric acid and a selenium catalyse (Kjeltabs) in a mineralization apparatus (Tecator Digestion System, Tecator, Sweden). Nitrogen estimation was performed in a Kjeltec Distilling Unit (System 1002) according to the manufacturer's procedure. All other mineral elements were analysed in 500 mg samples which were mineralized in a mixture of 3 ml nitric acid and 2 ml hydrogen peroxide using a Microwave Digestion Unit (Plazmatronika, Poland; Parosa and Reszke, 1995). Phosphorus was determined

by VIS spectrophotometry (DR 2000, HACH, USA) and all other elements by atomic absorption spectroscopy (Avanta Σ, GBC, Australia) according to the parameters listed in Table 1. Unspecific absorption was corrected using deuterium lamps (Antanasopoulos, 1996).

2.3. Analysis for cytokinin-like activity

The plant (2 reps × 4.5 g DW) and composted material (2 reps × 4.5 g DW) were extracted with 100 ml 80% ethanol for 24 h at 10 °C. The extracts were briefly sonnicated and then filtered with Whatman No. 1 filter paper, rinsed with a further 100 ml 80% ethanol and dried down under vacuum at 35 °C. The plant extracts, compost extracts, culture medium (2 reps × 5 l culture medium) and the compost leachates collected on days 0, 7 and 14 (1 rep × 250 ml) were partially purified using the method described by Dobrev and Kamínek (2002) where each sample was redissolved in 3 ml 80% ethanol and passed through an activated Sep-Pak® Plus C_{18} (SPE) cartridge. The eluent was collected and dried under vacuum. The samples were redissolved in 5 ml 1 M formic acid and loaded onto an activated Oasis® MCX (150 mg/6 cc) cartridge. The cytokinin-like compounds were eluted from the cartridge using 5 ml 0.35 M NH_4OH in water followed by 5 ml 0.35 M NH_4OH in 60% methanol. These two fractions were combined and dried down. The extracts were resuspended in 2.5 ml 80% ethanol and strip loaded onto Whatman No. 1 chromatography paper and developed in a descending manner in isopropanol:ammonia:water (10:1:1, v/v/v) until the solvent front had moved approximately 30 cm. After drying at 60 °C for 24 h, the chromatograms were divided into 10 equal R_f zones and placed in individual flasks. These were assayed for cytokinin-like activity using the soybean callus bioassay (Miller, 1965).

Modified Millers solidified media (excluding the kinetin) was added to the flasks containing the cut-up chromatograms. Three pieces of approximately 1 mg 3–4-week-old soybean callus (*Glycine max* cv acme) were placed in each flask under sterile conditions. The callus grew for 28 days in continuous low light conditions (1 μmol m^{-2} s^{-1}) at 24 °C after which the callus was weighed. A triplicate set of kinetin standards was included where kinetin was added to the medium at various concentrations (1, 5, 10 and 50 μg l^{-1}) as well as a control in which no kinetin was added to the medium.

2.4. Analysis for auxin-like activity

The dried, powdered *S. molesta* plant material (2 reps × 4.5 g DW) and the composted material (2 reps × 4.5 g DW) were extracted with 80% ethanol as described in Section 2.2. Auxin-like activity was determined using the mungbean rooting assay described by Crouch and van Staden (1991). The plant extracts, compost extracts, culture medium (2 reps × 5 l culture medium) and compost leachates collected on days 0, 7 and 14 (1 rep × 250 ml) were each resuspended in 100 ml distilled water. These were further diluted with distilled water as follows: 1:1, 1:4, 1:9, 1:19, 1:49 and 1:99 to give four replicates of 20 ml for each dilution. An IBA dilution series (10^{-3} to 10^{-7} M) was prepared as a standard and distilled water was used as a control. Five 10-day-old mungbean (*Vigno mungo* L.) cuttings were placed in each vial for 6 h in a growth chamber at 25 °C and light intensity of 160 μmol m^{-2} s^{-1}. The mungbeans were then rinsed in distilled water and placed in clean vials containing water and grown at 25 °C in a 16/8 h light/dark regime with a light intensity of 160 μmol m^{-2} s^{-1}. After 10 days, the number of roots was recorded.

2.5. Cytokinin and IAA identification and quantification

Cytokinins and IAA in the plant material, composted material and the culture medium were analysed using a modified protocol described by Novák et al. (2003). Freeze-dried samples were extracted in ice-cold 70% ethanol (v/v) and deuterium labelled standards added, each at 5 pmol per sample to check recovery during purification and to validate determination. The cytokinin standards were [^{2}H$_5$]tZ, [^{2}H$_5$]tZR, [^{2}H$_5$]tZ9G, [^{2}H$_5$]tZR5MP, [^{2}H$_3$]DHZ, [^{2}H$_3$]DHZR, [^{2}H$_3$]DHZ9G, [^{2}H$_3$]DHZR5MP [^{2}H$_6$]iP, [^{2}H$_6$]iPR, [^{2}H$_6$]iP9G, [^{2}H$_6$]iPR5MP, [^{2}H$_5$]tZOG, [^{2}H$_5$]tZROG, [^{2}H$_3$]DHZOG, [^{2}H$_3$]DZROG (Olchemim Ltd., Czech Republic). The topolins were analysed using internal deuterium standards for BA ([^{2}H$_7$]BA, BAR ([^{2}H$_7$]BAR), [^{2}H$_7$]BA9G, and [^{2}H$_7$]BAR5MP) as no labelled standards were available for the topolins. Thus their values might have an error load which originates from insufficient internal standardization. Also included was an auxin standard of 50 000 dpm 3[5(*n*)-^{3}H]IAA (0.55–1.11 TBq/mmol) per sample.

Table 1
Parameters used to measure mineral elements by atomic absorption spectroscopy in *Salvinia molesta* samples

Element	Wavelength (λ) (nm)	Lamp current (I) (mA)	Slit width (d) (nm)	Flame type
K	766.5	Emission	0.2	Air-acetylene
Na	589.0	Emission	0.2	Air-acetylene
Ca	239.9	10.0	0.5	Nitrous oxide-acetylene
Mg	202.6	3.0	1.0	Nitrous oxide-acetylene
Ni	232.0	4.0	0.2	Air-acetylene
Zn	213.9	5.0	0.5	Air-acetylene
Cu	324.7	3.0	0.5	Air-acetylene
Pb	217.0	5.0	1.0	Air-acetylene
Cd	228.8	3.0	0.5	Air-acetylene
Fe	372.0	7.0	0.2	Air-acetylene

After 3 h of extraction, the homogenate was centrifuged (15 000 × g, 4 °C) and the pellets re-extracted. The combined supernatants were concentrated to approximately 1.0 ml under vacuum at 35 °C, diluted to 20 ml with ammonium acetate buffer (40 mM, pH 6.5) and purified using a combined DEAE-Sephadex (1.0 cm × 5.0 cm)-octadecylsilica (0.5 cm × 1.5 cm) column and immunoaffinity chromatography based on generic cytokinin (Faiss et al., 1997) and auxin antibody, respectively. This resulted in three fractions containing (1) the free bases, ribosides and N-glucosides, (2) nucleotides and IAA; (3) O-glucosides. Before auxin immunoaffinity chromatography, half of fraction 2 was dissolved in 100 μl acidified methanol and methylated using excess of ethereal diazomethane, dried under a stream of nitrogen and dissolved in 500 μl of PBS (50 mM, pH 7.2). The methanolic eluate from all immunoaffinity chromatography columns were evaporated to dryness and dissolved in 75 μl of the mobile phase for HPLC analysis. Cytokinin O-glucosides and nucleotides were hydrolyzed to free bases and ribosides by action of β-glucosidase or alkaline phosphatase, respectively before subsequent HPLC–MS analysis (Novák et al., 2003).

The samples were analysed by HPLC (Waters Alliance 2690) linked to a Micromass ZMD 2000 single quadrupole mass spectrometer equipped with an electrospray interface [LC(+)ES-MS] and photodiode array detector (Waters PDA 996). Using a post column split of 1:1, the effluent was introduced into an electrospray source (source block temperature 100 °C, desolvation temperature 250 °C, capillary voltage +3.0 V, cone voltage 20 V) and PDA (scanning range 210–300 nm; with 1.2 nm resolution) and quantitative analysis of the different cytokinins was performed in selective ion recording mode. Quantification was performed by Masslynx software using a standard isotope dilution method. The ratio of endogenous cytokinin to appropriate labelled standard was determined and further used to quantify the level of endogenous compounds in the original extract, according to the known quantity of added internal standard (Novák et al., 2003). The final IAA analysis was done either by a specific competitive ELISA using anti-C1-IAA antibodies and IAA-alkaline phosphatase tracer or by fluorescence HPLC. The determination of the total amount was based on a calibration curve technique and internal standardization using [^{3}H]IAA.

3. Results

S. molesta grew rapidly, increasing from the initial 0.285 kg FW to 2.611 kg air-dried biomass after 31 days growth under the experimental conditions. Decomposition was also rapid with the plant biomass being reduced to approximately a fifth of its original volume within 7 days. The initial air-dried plant mass for composting was 2.00 kg and a final mass of 31.12 g DW was recorded 14 days later.

The mineral content of S. molesta and the composted material are shown in Fig. 1. Concentrations of Cd, Pb and Ni were below the level of detection (<1.5, 9.0 and 4.5 mg kg^{-1}, respectively). There was little change in the N and P content of the plant material after 14 days decomposition. However, there was
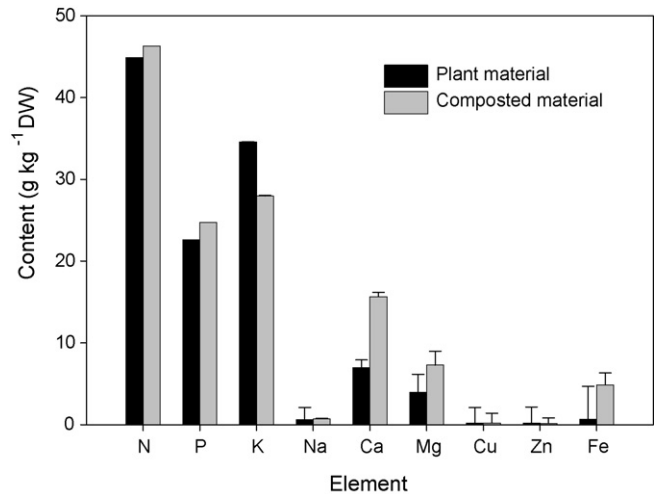

Fig. 1. Mineral element content in Salvinia molesta and material composted for 14 days.

a decrease in the K content and an increase in the Ca, Mg and Fe content with decomposition.

The plant material showed some cytokinin-like activity in the soybean callus bioassay with peaks of activity co-eluting with zeatin, iP and ribotide standards (Fig. 2A). The culture medium (5 l) also showed similar activity (Fig. 2B). The leachate extracts (Fig. 2C–E) were very active with the leachate collected on day 7 showing greater biological activity than the 50 μg g^{-1} kinetin standard. The composted material also had good activity with a large peak of activity co-eluting with the O-glucoside standards to produce cytokinin-like activity equivalent to 10 μg l^{-1} kinetin (Fig. 2F).

The plant material and culture medium had auxin-like rooting activity equivalent to 10^{-4} M IBA (Fig. 3A and B). No activity was measured in the leachate collected on day 0 of the composting experiment (Fig. 3C) while auxin-like activity increased in the leachates collected later during the composting experiment. The leachate collected on day 14 had activity equivalent to 10^{-5} M IBA (Fig. 3E). The composted material showed less auxin-like activity compared to the plant material with activity equivalent to 10^{-5} M IBA (Fig. 3F).

Sixteen isoprenoid cytokinins including tZ, cZ, DHZ and iP forms were detected in S. molesta to give a total cytokinin content of 81.62 pmol g^{-1} DW. Highest concentrations of iP type cytokinins were recorded while very low concentrations of DHZ type cytokinins were detected. No aromatic cytokinins (BA and topolins) were detected in the plant material (Table 2). Only four cytokinins were detected in the culture medium and these occurred at very low concentrations. Three of these cytokinins were free bases (tZ, cZ and iP; Table 2). Twelve cytokinins were detected in the composted material where the total cytokinin content (67.96 pmol g^{-1} DW) was lower than that of the original plant material. This 17% reduction in cytokinins was mainly due to a decrease in the concentrations of all the tZ type cytokinins, cZROG, cZR5MP, iPR and iPR5MP. Concentrations of the DHZ type cytokinins remained constant while there was an increase in the concentrations of cZ, cZOG and iP in the composted material (Table 2).
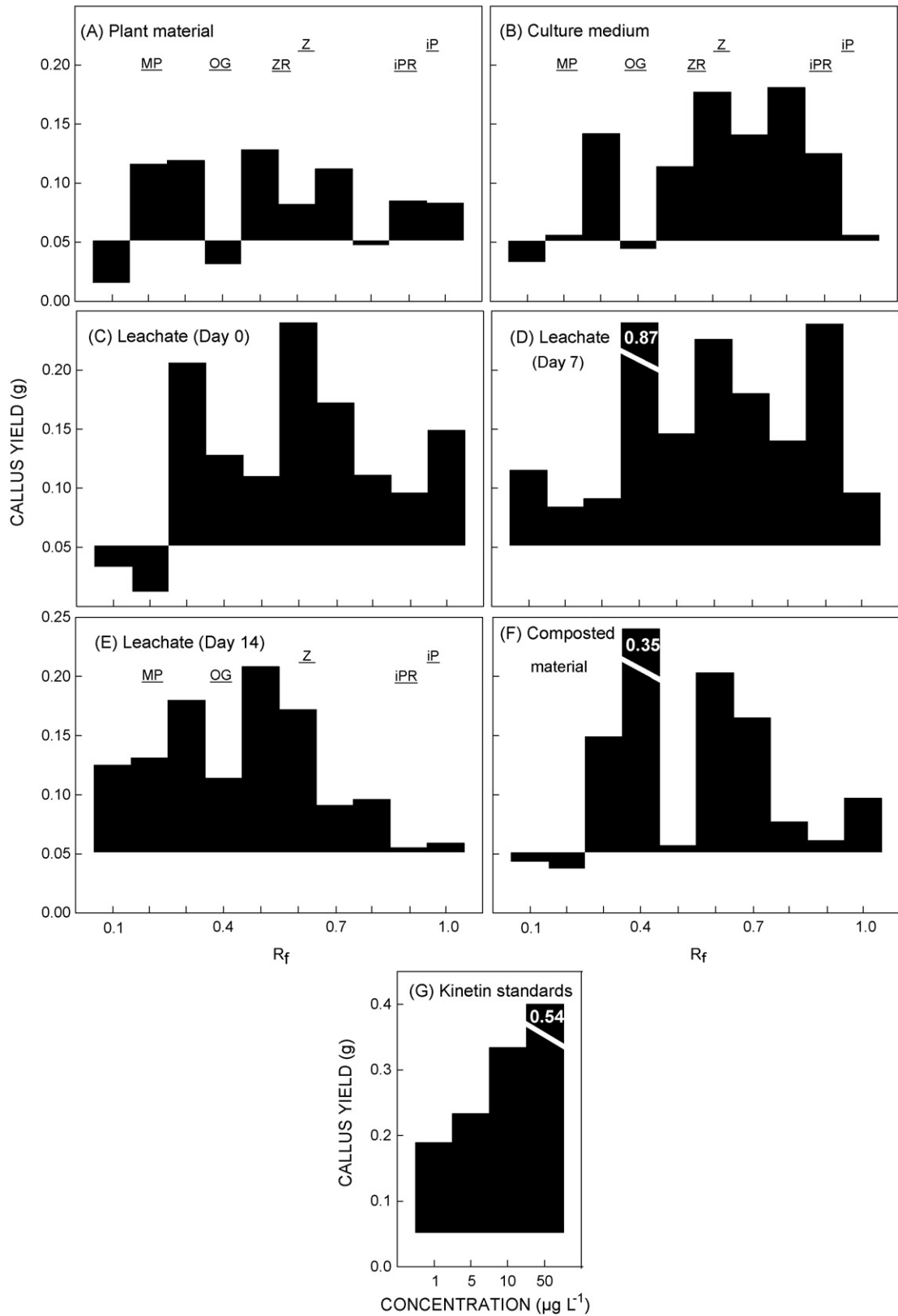
Fig. 2. Cytokinin-like activity detected using the soybean callus bioassay in various *Salvinia molesta* extracts. R_f values of some authentic cytokinin standards are indicated (MP, monophosphates; OG, *O*-glucosides; ZR, zeatin riboside; Z, zeatin; iPR, isopentenyladenosine; iP, isopentenyladenine). Note the different scale of the *Y*-axis for the kinetin standards (G).
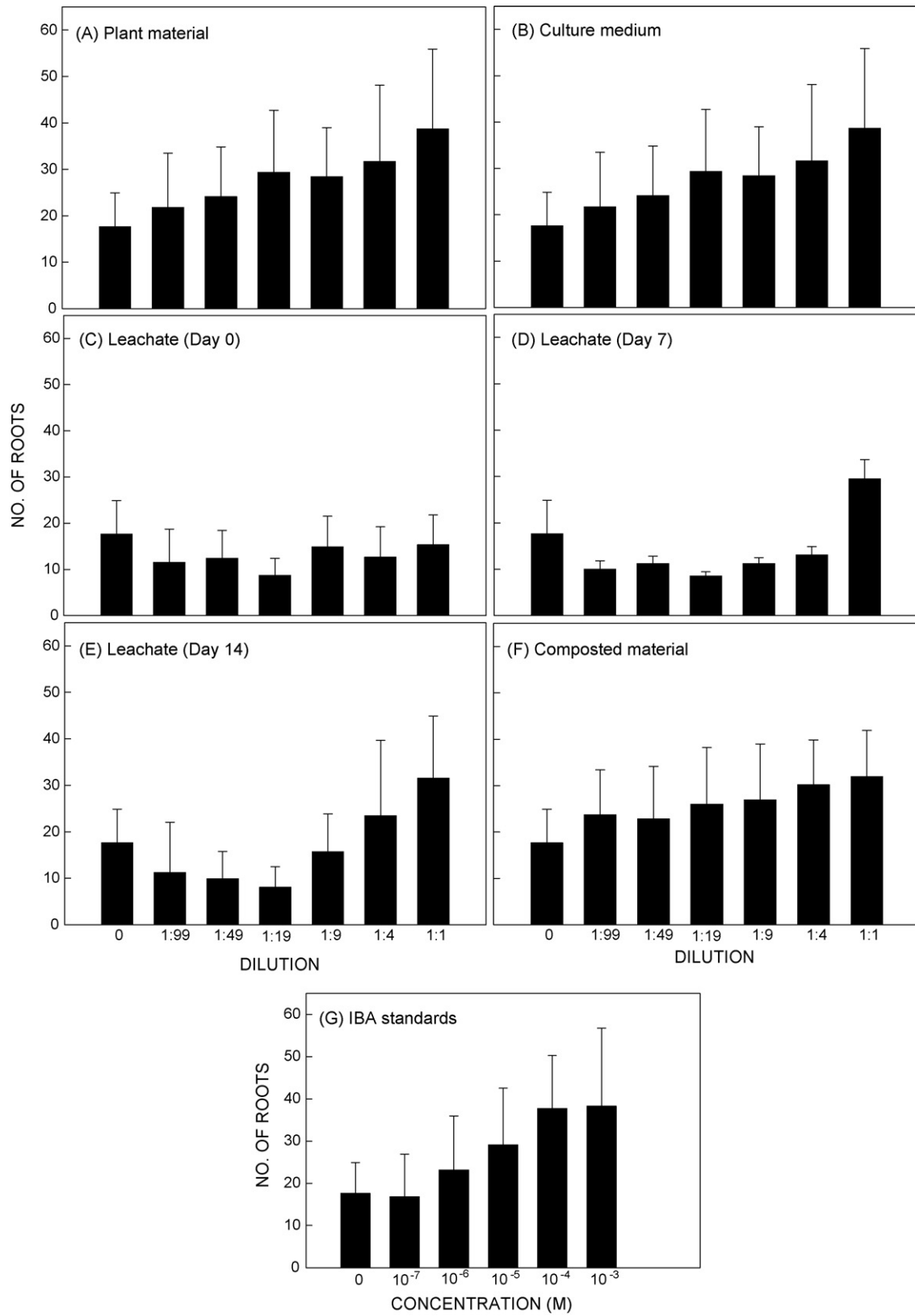
Fig. 3. Auxin-like activity detected using the mungbean rooting bioassay in various *Salvinia molesta* extracts.

Table 2
Cytokinins and auxins quantified in the fresh and composted material of *Salvinia molesta* and the media in which it was grown

Hormone	Plant extract (pmol g^{-1} DW)	Culture medium (pmol 500 ml^{-1})	Composted material (pmol g^{-1} DW)
*t*Z	5.98 ± 0.11	0.55 ± 0.04	2.85 ± 0.11
*t*ZR	4.00 ± 0.59	<LOD	0.34 ± 0.05
*t*ZOG	5.44 ± 0.48	<LOD	1.04 ± 0.14
*t*ZROG	3.47 ± 0.04	<LOD	<LOD
*t*ZR5MP	3.70 ± 0.65	<LOD	<LOD
Total *t*Z derivatives	22.59	0.55	4.23
*c*Z	1.44 ± 0.04	0.57 ± 0.08	20.02 ± 1.74
*c*ZR	3.04 ± 0.35	<LOD	2.58 ± 0.16
*c*ZOG	1.12 ± 0.02	<LOD	9.52 ± 0.07
*c*ZROG	5.89 ± 0.17	<LOD	1.45 ± 0.11
*c*ZR5MP	11.73 ± 0.28	<LOD	<LOD
Total *c*Z derivatives	23.22	0.57	33.57
DHZ	0.84 ± 0.05	<LOD	0.63 ± 0.06
DHZR	1.22 ± 0.03	<LOD	<LOD
DHZOG	1.73 ± 0.31	<LOD	1.53 ± 0.32
Total DHZ derivatives	3.79	–	2.16
iP	9.42 ± 0.98	2.85 ± 0.33	22.15 ± 0.80
iPR	9.91 ± 0.69	0.34 ± 0.05	4.34 ± 0.05
iPR5MP	12.69 ± 0.22	<LOD	1.51 ± 0.18
Total iP derivatives	32.02	3.19	28.00
Total cytokinins	81.62	4.31	67.96
Total IAA	393.6 ± 37.8	30.8 ± 4.3	148.1 ± 12.1

No aromatic (BA and topolin derivatives) and -9G cytokinins were detected. The results are presented as mean ± S.D. of duplicate measurements. <LOD = below the limit of detection.

A high concentration of IAA was detected in the plant material compared to very low concentrations detected in the culture medium. IAA concentrations were also lower in the composted material with a 62% loss being recorded when compared to the plant material (Table 2).

4. Discussion

S. molesta contained a wide spectrum of mineral elements as found in most plants as well as a number of endogenous cytokinins and IAA. These extracts also had biological activity that enhanced both cell division in the soybean callus bioassay (cytokinin-like activity) and rooting in the mungbean bioassay (auxin-like activity). Cytokinins have previously been reported to occur in ferns. For example, iPR was identified in the fertile fronds of *Dryopteris crassirhizoma* (Yamane et al., 1983) and Z, ZR, iP and iPR were detected in the fertile fronds of the aquatic *Marsilea drummondii* (Pilate et al., 1989). The cytokinin complement of *S. molesta* was similar to that found in other lower order plants such as algae and mosses where a literature survey by Auer (1997) found that these groups of plants generally contained mainly Z and iP free bases and their respective riboside and ribotide conjugates. Recent advances in the ana-

lytical techniques used in plant growth regulator research now enable lower concentrations of compounds to be detected. The present study was able to detect *O*-glucoside derivatives and very low concentrations of DHZ derivatives in *S. molesta*. Aromatic cytokinins (BA and topolin) were absent or occurred at concentrations below the limit of detection.

Air-dried *S. molesta* underwent rapid decomposition with the plant matter being greatly reduced in biomass within 7 days. There was a rapid release of cytokinin-like and auxin-like compounds with biological activity being detected in the leachate collected after 7 days composting (Figs. 2 and 3). By day 14, there had been a 17% decrease in cytokinin and 62% decrease in auxin concentrations found in the plant matter indicating that these metabolites are rapidly released, making them available for uptake from the soil. The increase in the *c*Z type cytokinins in the composted material (Table 2) may be due to an increase in the bacteria population. Bacteria contain *c*Z type cytokinins that are derived from tRNA (Zažímalová et al., 1999). Using the same composting methods as the present study, visual signs of decomposition were only observed 2 months after composting in manually shredded *Gingko biloba* leaves. Cytokinin-like compounds were only detected in the leachate collected after 4 months (Arthur et al., 2001). These results highlight the rapid decomposition and release of cytokinins and auxins from *S. molesta*.

The mineral content of *S. molesta* changed after 14 days decomposition with there being an increase in the Ca, Mg and Fe content, a decrease in the K content and little change in the other mineral elements (N, P, Na, Cu and Zn). This suggests that very few nutrients had yet been released into the leachate by day 14. This increase in certain mineral elements may be due to an increase in the bacterial population on the decomposing leaves. Like *S. molesta*, dried *Azolla* decomposed within 8–10 days when incorporated into rice paddy soil although it took 5–8 weeks for two thirds of the *Azolla*'s nitrogen content to be released (Lumpkin and Plucknett, 1980). Pretreatment of *Azolla filiculoides* by heating or air-drying enhanced its effectiveness in improving wheat growth and yield compared to fresh *A. filiculoides* plants applied directly to the soil. This was attributed to the drying treatments enhancing decomposition rates (Ripley et al., 2003).

The main benefit to using *Azolla* as a biofertilizer is its nitrogen-fixing ability. However, a comparative study using *Azolla microphylla* and *Sesbania rostrata* showed that on a dry weight basis, *S. rostrata* released more nitrogen (NH_4^+) than *A. microphylla*, probably owing to the higher lignin content of *A. microphylla* which is known to retard mineralization (Nagarajah et al., 1989). Thus other non-nitrogen-fixing plants with a high productivity and biomass such as *S. molesta* should also be considered as potential biofertilizers.

One major concern with using *Azolla* as a biofertilizer is the possibility that this practice can promote spore dispersal. Viable spores were found in soils that had been fertilized with *A. filiculoides* (Ripley et al., 2003). By promoting the use of dried *S. molesta*, a sterile hybrid, this major concern is nullified. Also, sustained harvesting could help lessen the negative impact of this weed on the environment.

There is increasing evidence that phytohormones may be recycled through the environment in a similar manner to that of nutrients. In a previous study using *S. molesta* and *A. filiculoides*, cytokinin-like activity was detected in the culture medium (Stirk and van Staden, 2003). Similar cytokinin-like activity was obtained in the current study with *S. molesta* although a different purification method was used. Auxin-like activity was also detected in the current study. Compound identification and quantification by analytical techniques confirmed that low concentrations of cytokinins and auxins were present in the culture medium. These results show that *S. molesta* does exude compounds which alter the culture medium in which it is growing and can thus potentially influence the growth of other aquatic plants growing in the same water system.

The results confirm the hypothesis that *S. molesta* can potentially be used as an organic fertilizer. *S. molesta* undergoes rapid vegetative growth as seen by the rapid doubling time of the plant biomass in the present experiment and thus has a large biomass making it a prolific and productive species. It contains many mineral elements as well as plant hormones (cytokinin and auxins). Once dried, it quickly decomposes so that metabolites such as cytokinins and auxins are available for uptake by crop plants although the release of mineral elements appears to be a slower process. Also, as it is a sterile hybrid, there is no danger of agricultural practices aiding its dispersal. Thus *S. molesta* has great potential as a sustainable environmentally friendly, cheap alternative to agro-chemicals and its use could be promoted amongst small-scale farmers as a way of increasing crop yield while minimizing costs of chemical fertilizers.

Acknowledgements

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