Journal of Helminthology

www.cambridge.org/jhl

Nematicidal activity of *o*-hydroxybenzaldehyde from common buckwheat methanol extract on *Meloidogyne incognita**

N. Aissani[1], R. Aissani[2], F. Zouidi[3] and H. Sebai[1]

[1]Laboratoire de Physiologie Fonctionnelle et Valorisation des Bio-Resssources, Institut Supérieur de Biotechnologie de Béja, Université de Jendouba, Avenue Habib Bourguiba, B.P, 382-9000, Béja, Tunisie; [2]Vitroplant Society, Route el Mahfoura, El Battan 1114, Manouba, Tunisia and [3]Biology Department, College of Sciences and Arts of Muhayil Asir, King Khaled University

Research Paper

Cite this article: Aissani N, Aissani R, Zouidi F and Sebai H (2023). Nematicidal activity of *o*-hydroxybenzaldehyde from common buckwheat methanol extract on *Meloidogyne incognita*. *Journal of Helminthology*, **97**, e60, 1–6
https://doi.org/10.1017/S0022149X23000457

Received: 27 March 2023
Revised: 04 July 2023
Accepted: 05 July 2023

Keywords:
root knot nematodes; buckwheat; salicylaldehyde; DPPH

Corresponding author:
N. Aissani;
Email: aissaninadhem@gmail.com

*The online version of this article has been updated since original publication. A notice detailing the change has also been published.

Abstract

The nematicidal activity of buckwheat (*Fagopyrum esculentum* Moench) on the root-knot nematode *Meloidogyne incognita* was tested. Dried plant methanol extract presented higher nematicidal activity than fresh plant extracts with an EC_{50} = 62.6 ± 26.0 and 40.8 ± 26.1 µg/ml after 48 and 72 hours of immersion, respectively. GC-MS analysis showed the presence of 17 aldehydes, with salicylaldehyde (*o*-hydroxybenzaldehyde) being the most abundant at 16%. Nematicidal activity of the latter and other aldehydes with chemical similarities was then assessed. The most active aldehyde was *o*-hydroxybenzaldehyde followed by *m*-hydroxybenzaldehyde, *p*-hydroxybenzaldehyde and benzeneacetaldehyde with an EC_{50} of about 11.0 ± 1.0, 31.0 ± 22.0, 75.0 ± 23.0 and 168.1 ± 52.3 µg/ml after 1 day of immersion, respectively. Position 2 of the hydroxyl group in the benzene ring seems to be very important for the nematicidal activity, followed by positions 3 and 4. As a complementary experiment, synergistic activity was observed when we added o-hydroxybenzaldehyde to m-hydroxybenzaldehyde and to p-hydroxybenzaldehyde with an EC_{50} after 24 hours of immersion of 8.0 ± 2.5 and 6.1 ± 2.3 µg/ml, respectively. Antioxidant activity assessment showed that this latter is inversely proportional to nematicidal activity. Our results showed that *F. esculentum* and its major compound salicylaldehyde could be integrated into the pest management system.

Introduction

Plants may produce compounds that directly or indirectly affect their biological environment. These compounds are called allelochemicals and have a role in the growth, health, and behaviour of other organisms (Aissani *et al.* 2013). Root-knot nematodes (*Meloidogyne* spp.) are among a vast array of pests continually attacking plants and causing approximately US$70 billion of crop losses in fruit and vegetable production annually (Aissani *et al.* 2015). *Meloidogyne* spp., which is the most common and widespread group of root-knot nematodes in the world, increases the severity of soil borne diseases (Aissani *et al.* 2015; Caboni *et al.* 2012). Many scientific studies have reported data on the biological activity of plant secondary metabolites on root-knot nematodes. Aldehydes from *Ailanthus altissima* as *(E,E)*-2,4-Decadienal and *(E)*-2-Decenal, methylisothiocyanate from caper were active on *Meloidogyne javanica* (Caboni *et al.* 2012a; Caboni *et al.* 2012b), and allyliosthiocyanate from *Armoracia rusticana* was active against *Meloidogyne incognita* (Aissani *et al.* 2013).

Buckwheat (*Fagopyrum esculantum* Moench.) is a well-established special crop that has been grown on Eastern prairies for the last 40 years. Seeds of common buckwheat, or sweet buckwheat, are usually consumed in Asia, Europe, North America, South Africa, and Australia (Li *et al.* 2001). At the present time, the only attributes used to evaluate buckwheat quality are color and flavor related to volatile aldehydes (Przybylski *et al.* 1995). Buckwheat contains high levels of nutritionally beneficial components and can be processed into various functional foods. There have been a large variety of buckwheat-based food products available in the market, such as buckwheat noodles, pasta, bread, tea, spirits, and vinegar (Ikeda *et al.* 2002).

Recent research has found that buckwheat seed extract has strong antioxidant activity (Lin *et al.* 2002). In addition, immunostimulant and antivirulant activities of seeds have been noted (Bai *et al.* 2015; Yuan *et al.* 2015). Besides the work of Sipes and Arakaki dealing with the nematicidal activity of buckwheat against *Meloidogyne javanica* (Sipes *et al.* 1997), there is no available report on the nematicidal activity of *F. esculantum* on *M. incognita*, the most widespread Meloidogyne species.

In the present investigation, we report for the first time (1) the chemical characterization of *F. esculantum* aerial part methanol extract by GC-MS, (2) the nematicidal activity of this latter on *M. incognita* with and without taking into account moisture, (3) the preliminary

structure–activity relationship of the most abundant aldehyde with other selected ones, (4) the correlation between nematicidal activity and antioxidant power, and (5) their synergistic activity.

Material and methods

Chemicals

Aldehydes standards, fosthiazate of purity greater than 98%, Tween-20, and dimethylsulfoxide were obtained from Sigma-Aldrich. Methanol and water were high-performance liquid chromatography (HPLC)-grade.

Plant materials and extraction

Buckwheat seeds were purchased from a local market in Cagliari, Italy, in March 2016. Voucher specimens were deposited at the Laboratory of Functional Physiology and Valorization of Bioresources, Higher Institute of Biotechnology of Beja-University of Jendouba-Tunisia, for species identification. Seeds were germinated in cotton for 2 weeks at a temperature of 24 °C. Fresh aerial plant parts were collected and ground (100 g) and extracted with methanol or water (1:1 w/v) in a sonicator apparatus for 15 min, filtered through a Whatman no. 40 filter, and centrifuged for 15 min at 13,000 rpm. The extracts were then used to assess the nematicidal activity. The same protocol was repeated after drying plant aerial parts at 105 °C for 24 h and moisture determination.

Nematode population

A population of *M. incognita* originally reared from tomato roots (*Solanum lycopersicum* L.) cv. Belladonna, a cultivar that is very susceptible to root-knot nematodes, was collected from a greenhouse in Cagliari, Italy. All plants were maintained in plastic pots (18 cm diameter) in a growth chamber at 25–28 °C with 60% relative humidity and a photoperiod of 16 hours. Plants used for inoculations were 7 weeks old and at the five-leaf stage. After 40 days, the plants were uprooted, and the roots were washed free of soil and cut into 2-cm pieces. Eggs were extracted according to the sodium hypochlorite procedure, and second-stage juveniles (J_2) were allowed to hatch in modified Baermann funnels at 28 °C. All J_2 hatchings in the first 3 days were discarded, and thereafter, J_2 collected after 24 h were used in the experiments (Caboni *et al.* 2014).

Nematicidal assay

The nematicidal activity of methanol and aqueous extracts and compounds on nematode juvenile infestive stage (J_2) paralysis was tested, and the EC_{50} values were calculated. Stock solutions were prepared by dilution with dimethyl sulfoxide, whereas working solutions were obtained by dilution with distilled water containing the polysorbate surfactant 20 (Tween-20). Final concentration of dimethyl sulfoxide in each well never exceeded 2% v/v because preliminary trials showed that paralysis of nematodes exposed at those concentration levels was similar to paralysis of nematodes maintained in distilled water (Aissani *et al.* 2013). Distilled water, as well as a mixture of water with dimethyl sulfoxide and Tween-20 at concentrations equivalent to those in the treatment wells, served as a negative control, whereas fosthiazate at the same concentrations was used as a positive control. Twenty-five juveniles were used per treatment well in Cellstar 96-well plates (Greiner bio-one, Milan-Italy). The plates were covered to prevent evaporation and were maintained in the dark at 28 °C. Border wells containing plain water with nematodes were placed around the wells of each treatment to check the vapor drift among wells that could possibly interfere with the efficacy results. Juveniles were observed with the aid of an inverted microscope (Zeiss, West Germany- Oberkochen) at 40x after 24 h and were ranked into two distinct categories: motile or paralyzed. After the assessment, the nematodes were transferred into plain water, after washing in tap water through a 20 µm pore screen to remove the excess of tested compounds, and they were assessed again after 24, 48, and 72 h for the re-obtainment of motility. Dead nematodes were characterized by the presence of internal vacuoles. In another experiment, nematodes were treated with single aldehydes found by GC-MS analysis or for synergistic activity.

GC-MS analysis

A gas chromatograph HP 5890 Series with a mass spectrometric detector (MSD) 5972 from Hewlett-Packard (Palo Alto, CA, USA) was used. Column: VOCOL, 60 m 0.25 mm (i.d.), film thickness 1.5 µm (Supelco, Bellefonte, PA, USA). Temperature program: starting temperature 50 °C (2 min), heating rate 5 °C /min, final temperature 210 °C (40 min). T_{inj}: 250 °C; T_{det}: 280 °C; injection volume: 1 µl. Carrier gas: He, flow 1 ml/min. MS conditions: electron impact mode, total ion current (TIC) recorded. Mass spectra of the compounds were compared to the spectra from the NIST02 spectral library and with mass spectra of reference compounds. Identities of compounds were confirmed by comparison of their retention times with the retention times of reference compounds.

Antioxidant assay

The antioxidant capacity of the tested compounds was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, as described previously by Grzegorczyk *et al.* (2007). Briefly, various concentrations of aldehydes (0.3–200 mg/ml) were added to 1 ml of 0.1-mM methanol solution of DPPH and incubated at 27 °C for 30 min. The optical density of the sample was quantified at 517 nm. DPPH radical-scavenging activity (RSA), expressed as percentage, was estimated utilizing the following formula:

$$\mathrm{RSA}(\%) = \left[A_{\mathrm{DPPH}} - \left(A_{\mathrm{sample}} - A_{\mathrm{control}}\right)/A_{\mathrm{DPPH}}\right)\right] \mathrm{X}\, 100$$

Ascorbic acid (Sigma-Aldrich) was used as a reference molecule in the same concentrations as the tested extract. All the analyses were carried out in triplicates. The EC_{50} value was determined as the concentration of the compound required to scavenge 50% of the DPPH radicals.

Statistical analysis

Treatments of paralysis experiments were replicated six times, and each experiment was performed twice. The percentages of paralyzed J_2 in the microwell assays were corrected by elimination of the natural death/paralysis in the water control according to the Schneider Orelli formula, corrected (Puntener *et al.* 1981):

$$\% = [(\text{paralysis}\% \text{in treatment-paralysis}\% \text{in control})$$

https://doi.org/10.1017/S0022149X23000457 Published online by Cambridge University Press

/(100-paralysis%in control)}]x100.

They were analysed (ANOVA) after being combined over time. Because ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Corrected percentages of paralyzed J_2 treated with test compounds were subjected to nonlinear regression analysis using the log-logistic equation proposed by Seefeldt *et al.* (1995):

$$Y = C + (D\text{-}C)/\{1 + \exp[b(\log(x)\text{-}\log(EC50))]\},$$

where C = the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = the test compounds concentration required for 50% paralyzed nematodes after elimination of the control (natural death/paralysis). In the regression equation, the test compounds concentration (% w/v) was the independent variable (x), and the paralyzed J_2 (percentage increase over water control) was the dependent variable (y). The mean value of the six replicates per compound concentration and immersion period was used to calculate the EC_{50} value.

Results

Moisture determination

The two weeks aged aerial part plants moisture was calculated at 60.5% dealing with the richness of this plant on water and allowing the assessment of the nematicidal activity of the extract before and after drying the plant.

J_2 paralysis bioassays

Without taking into account plant compound bioavailability or the synergetic effect when the extract was tested against *M. incognita*, a linear dose–response relationship was established, and significant paralysis/death of nematodes was evident after 48 and 72 hours of exposure to methanol extract with an EC_{50} calculated value of about 62.6 ± 26.0 (R^2 = 0.99) and 40.8 ± 26.1 µg/ml (R^2= 0.96), respectively (Figure 1). Nematicidal activity of the methanol extract of fresh plant was EC_{50} = 127.7 ± 67.2 and 98.3 ± 54.0 µg/ml after the same period of immersion, respectively, whereas aqueous extracts of the fresh and dried plant were not active, with EC_{50} > 1000 µg/ml during the same period (Table 1).

GC-MS analysis of methanol extract

Using GC-MS of these experimental conditions, we were able to identify and quantify 17 aliphatic and aromatic aldehydes. The most abundant aldehyde was salicylaldehyde at 16 % (Table 2).

J_2 paralysis of the tested compounds

Compared with the selected aldehydes previously tested against J_2, *o*-hydroxybenzaldehyde was the most active one, followed by *m*-hydroxybenzaldehyde **(3)**, *p*-hydroxybenzaldehyde **(4),** benzeneacetaldehyde **(5)**, and then benzaldehyde **(1)** with an EC_{50} after 24 hours of immersion of about 11.0 ± 1.0, 31.0 ± 22.0, 75.0 ± 23.0, 168.1 ± 52.3, and 300.2 ± 110.0 µg/ml, respectively (Figure 2, Table 2).

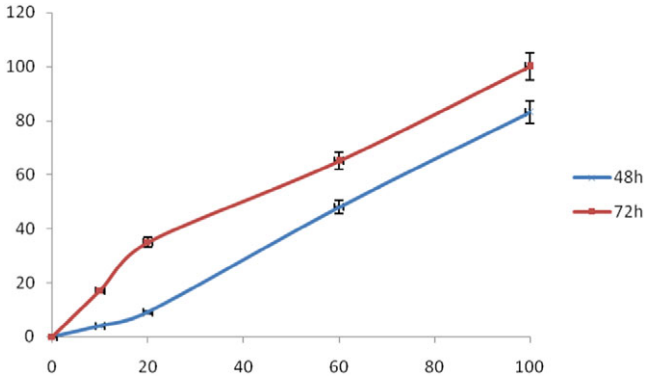

Figure 1. Curves of nematode death after 48 and 72 hours of immersion in 10, 20, 60, and 100 µg/ml of buckwheat methanol extract. No death was noted in control (Tween 20).

Table 1. EC_{50} of tested extracts on *M. incognita* J_2

Extract	$EC_{50/48h}$ (µg/ml)	$EC_{50/72h}$ (µg/ml)
Methanol extract/dried plant	62.6 ± 26.0	40.8 ± 26.1
Methanol extract/fresh plant	127.7 ± 67.2	98.3 ± 54.0
Aqueous extract/dried plant	> 1000	> 1000
Aqueous extract/fresh plant	> 1000	> 1000

Synergistic activity assessment

A synergistic activity of *o*-hydroxybenzaldehyde and *m*-hydroxybenzaldehyde; *o*-hydroxybenzaldehyde and *p*-hydroxybenzaldehyde was observed with EC_{50} of 8.0 ± 2.5 and 6.1 ± 2.3 µg/ml, respectively. The addition of o-hydroxybenzaldehyde to benzeneacetaldehyde significantly enhances the activity of this latter to become 23.3 ± 4.0 µg/ml after the same immersion period.

Antioxidant assay

The DPPH radical-scavenging activity method showed that the most antioxidant compound was benzaldehyde followed by *p*-hydroxybenzaldehyde and *(E)*-2-octenal with $EC_{50/24h}$ values of 1.4 ± 0.3, 3.5 ± 0.7, and 5.2 ± 0.9 µg/ml, respectively. However, the least antioxidant compound was furfural followed by *o*-hydroxybenzaldehyde and *m*-hydroxybenzaldehyde with $EC_{50/24h}$ values of 12.7 ± 2.6, 9.1 ± 1.2, and 5.1 ± 0.9 µg/ml, respectively.

Discussion

In this study, aerial fresh and dried parts of common buckwheat *F. esculentum* were extracted using methanol and water, and the nematicidal activity of the extracts was assessed against juveniles of *M. incognita*. Results showed that the methanol extract had more activity when the plant was dried, with EC_{50} = 40.8 ± 26.1 µg/ml after 72 hours. This nematicidal activity is high, taking into account the effect of *Capparis spinosa* stems methanol extracts with an EC_{50} value of 215.0 ± 36.0 µg/ml (Caboni *et al.* 2012b). The aqueous extract was not active, with EC_{50}>1000 µg/ml.

https://doi.org/10.1017/S0022149X23000457 Published online by Cambridge University Press

Table 2. Nematicidal activity of the tested compounds (n = 3)

Compound	R_t (min)	$EC_{50/24h}$ (µg/ml) ± SD	EC_{50} DPPH (µg/ml)
pentanal	26.3	nt	nt
hexanal	27.5	na	nt
heptanal	38	na	nt
furfural	38.3	8.0 ± 1.0[a]	12.7 ± 2.6
octanal	47.2	nt	nt
5-methylfurfural	48.3	nt	nt
benzaldehyde	48.9	300.2 ± 110.0	1.4 ± 0.3
(E)-2-octenal	52.3	30.4 ± 17.2	5.2 ± 0.9
o-hydroxybenzaldehyde	54.5	11.0 ± 1.0[a]	9.1 ± 1.2
benzeneacetaldehyde	54.7	168.1 ± 52.3	nt
nonanal	55.2	na	nt
2-nonenal	60.4	nt	nt
decanal	63.7	nt	nt
(E)-2-decenal	68.3	22.0 ± 10.0	nt
(E,E)-2,4-decadienal	73.8	12.0 ± 2.0	nt
2-undecenal	76.3	nt	nt
p-hydroxybenzaldehyde	77.8	75.0 ± 23.0[a]	2.5 ± 0.7
m-hydroxybenzaldehyde	–	31.0 ± 22.0[a]	5.1 ± 0.9
salicyladehyde + benzeneacetaldehyde	–	23.3± 4.0	–
salicyladehyde + 3-hydroxybenzaldehyde	–	8.0 ± 2.5	–
salicyladehyde + 4-hydroxybenzaldehyde	–	6.1 ± 2.3	–
fosthiazate	–	0.4 ± 0.3[a]	nt
ascorbic acid	–		1.15 ± 0.9[b]

[a]Result reported by Caboni *et al.* (2013)
[b]Data reported by Aissani *et al.* (2017)

Using GC-MS analysis, we were able to identify 17 aliphatic and aromatic aldehydes,with salicylaldehyde (*o*-hydroxybenzaldehyde) being the most abundant at 16%. In the same fashion, the analysis of buckwheat volatilome by headspace technique showed that this plant is rich with aldehydes and ketones (Prosen *et al.* 2010).

According to Janes and Kreft (2008) and Janes *et al.* (2012), salicylaldehyde is a characteristic aroma component of common buckwheat and presents the highest concentration with 1.6 µg/ml. Aldehydes from *Ailanthus altissima* presented a strong nematicidal activity against root knot nematodes (Caboni *et al.* 2012a).

In our previous study, salicylaldehydes presented a nematicidal activity against the J_2 stage of *M. incognita* of 11.0 ± 1.0 µg/ml after 24 h of immersion (Caboni *et al.* 2013). Table 2 summarizes the nematicidal effect of tested compounds found by GC-MS analysis.

The structure-activity study showed that salicyaldehyde (*o*-hydroxybenzaldehyde) is three times more active than *m*-hydroxybenzaldehyde and seven times more active than *p*-hydroxybenzaldehyde. Thus, position 2 of the hydroxyl group in the benzene ring seems to be very important for nematicidal power, followed by positions 3 and 4. Using aldehydes with linear chains, our results clearly indicate that *α,β,γ,δ*-unsaturated aldehydes are generally more nematicides than aromatic counterparts against *M. incognita* confirming results found by Caboni *et al.* (2012a) using *M. javanica*. In a recent study, *(E)-2*-decenal degenerated the nematode pseudocoel cells and caused malformations of somatic muscles (Ntalli *et al.* 2016). Caboni *et al.* (2013) showed that nematodes treated with salicylaldehyde and aromatic aldehydes presented serious cuticle damages with marked macroscopic fractures and paralysis in a straight shape and evident internal vacuolization compared to the controls (Caboni *et al.* 2013). This observation was noted also after immersion of nematodes in buckwheat methanolic extract (Figure 3). Previous studies reported similar results when nematodes were treated with isothiocyanates and some vacuolar-type proton-translocating adenosine triphosphatase (V-ATPase) inhibitors such as pyocyanin (Aissani *et al.* 2013; Aissani *et al.* 2015). Conversely, nematodes treated with the organophosphorous fosthiazate were paralyzed in a coiled shape (Aissani *et al.* 2015). Salicylaldehyde produced an increased pH in lysosomal-like organelles on HeLa human cell line, and this alteration was most likely related to a V-ATPase impairment (Caboni *et al.* 2014). In another experiment, synergistic activity of *o*-hydroxybenzaldehyde and *m*-hydroxybenzaldehyde; *o*-hydroxybenzaldehyde and *p*-hydroxybenzaldehyde was observed with EC_{50} of 8.0 ± 2.5 and 6.1 ± 2.3 µg/ml, respectively, after 24 h. Interestingly, the addition of o-hydroxybenzaldehyde to benzeneacetaldehyde significantly enhances the activity of this latter from 168.1 ± 52.3 to 23.3 ± 4.0 µg/ml after the same immersion period.

Remarkably, salicylaldehyde showed the highest nematicidal activity with the corresponding lowest antioxidant activity with EC_{50}= 9.1 ± 1.2 µg/ml. Conversely, benzaldehyde presented the lowest nematicidal activity with the corresponding highest antioxidant effect (Table 1, Figure 4). These results confirm those found in our previous studies when we correlated nematicidal and antioxidant activities of selected isothiocyanates and phenolic compounds (Aissani & Sebai 2022; Aissani *et al.* 2017).

This study is the first to investigate the nematicidal activity of common buckwheat methanol extract against *M. incognita.* This plant is rich in aldehydes, such as salicylaldehyde, which has nematicidal activity against this root-knot nematode and allows us to use this plant for crop protection against pests. Comparison with other aldehydes showed that position 2 in the phenolic ring of the aldehyde is important for nematicidal activity. This gives insight into the development of new potent nematicides.

Acknowledgements. The author extends his appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the General Research Project under Grant number (RGP.2/83/44).

Competing interest. The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical standard. The authors assert that all procedures contributing to this study comply with the ethical standards of the relevant national guidelines on the care and use of laboratory animals and have been approved by the institutional committee of the Institut Pasteur, Tunis.

https://doi.org/10.1017/S0022149X23000457 Published online by Cambridge University Press

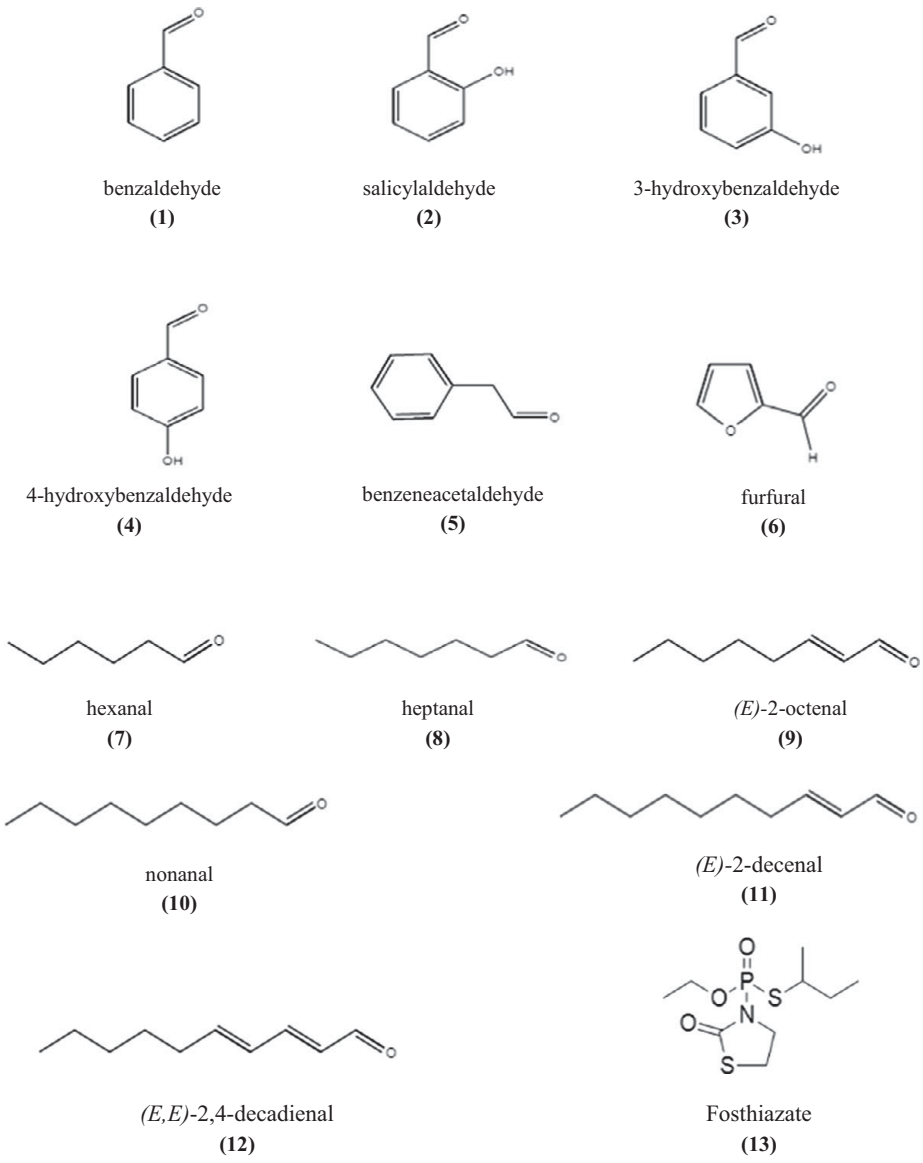

Figure 2. Chemical structure of tested compounds.

Figure 3. Nematodes before (A) and after (B) treatment with Buckwheat methanol extract (internal vacuole is evident).

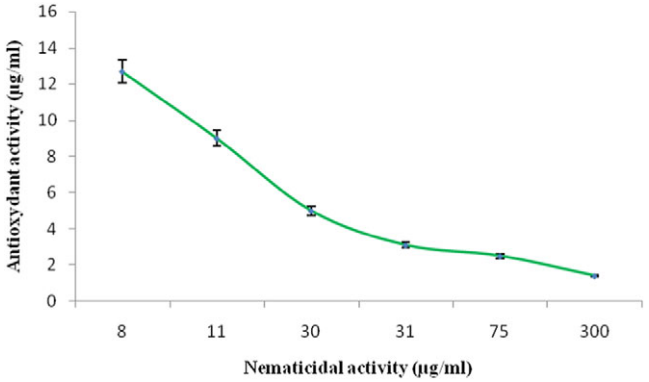

Figure 4. Correlation between nematicidal activity and antioxidant effect of the tested aldehydes.

https://doi.org/10.1017/S0022149X23000457 Published online by Cambridge University Press

References

Aissani N, Balti R, and Sebai H (2017) Potent nematicidal activity of phenolic derivatives on *Meloidogyne incognita*. *Journal of Helminthology*. DOI: 10.1017/S0022149×17000918.

Aissani N and Sebai H (2022) Nematicidal effect of Raphasatin from *Raphanus sativus* against *Meloidogyne incognita*. *Journal of Nematology* **54**. DOI: 10.2478/jofnem-2022-0050.

Aissani N, Tedeschi P, Maietti A, Brandolini V, Garau VL, and Caboni P (2013) Nematicidal activity of allylisothiocyanate from horseradish (*Armoracia rusticana*) roots against *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* **61**, 4723–4727.

Aissani N, Urgeghe PP, Oplos C, Saba M, Tocco G, Luigi Petretto GG, Eloh K, Menkissoglu-Spiroudi U, Ntalli N, and Caboni P (2015) Nematicidal activity of the volatilome of *Eruca sativa* on *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* **63**, 6120–6125.

Bai CZ, Ji HJ, Feng ML, Hao XL, Zhong QM, Cui XD, and Wang ZH (2015) Stimulation of dendritic cell maturation and induction of apoptosis in lymphoma cells by a stable lectin from buckwheat seeds. *Genetics and Molecular Research* **14**, 2162–2175.

Caboni P, Aissani N, Cabras T, Falqui A, Marotta R, Liori B, Ntalli N, Sarais G, Sasanelli N, and Tocco G (2013) Potent nematicidal activity of phthaldehyde, salicylaldehyde, and cinnamic aldehyde against *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* **61**, 1794–1803.

Caboni P, Ntalli NG, Aissani N, Cavoski I, and Angioni A (2012a) Nematicidal Activity of (E,E)-*2,4*-Decadienal and (E)-*2*-Decenal from *Ailanthus altissima* against *Meloidogyne javanica*. *Journal of Agricultural and Food Chemistry* **60**, 1146–1151.

Caboni P, Sarais G, Aissani N, Tocco G, Sasanelli N, Liori B, Carta A, and Angioni A (2012b) Nematicidal activity of 2-thiophenecarboxaldehyde and methylisothiocyanate from caper (*Capparis spinosa*) against *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* **60**, 7345–7351.

Caboni P, Tronci L, Liori B, Tocco G, Sasanelli N, and Diana A (2014) Tulipaline A: structure-activity aspects as a nematicide and V-ATPase inhibitor. *Pesticide Biochemistry and Physiology* **112**, 33–39.

Ikeda K (2002) Buckwheat: composition, chemistry, and processing. *Advances in Food and Nutrition Research* **44**, 395–434.

Janes D and Kreft S (2008) Salicylaldehyde is a characteristic aroma component of buckwheat groats. *Food Chemistry* **109**, 293–298.

Janes D, Prosen H, and Kreft S (2012) Identification and quantification of aroma compounds of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) and some of its milling fractions. *Journal of Food Science* **77**(**7**), 746–751.

Lin RF, Cai Y, and Liao Q (2002) *Minor grain crops in China*. Beijing, China Agricultural Scientech Press. Pp. 27–64.

Li SQ and Zhang QH (2001) Advances in the development of functional food from buckwheat. *Critical Reviews in Food Science and Nutrition* **41**, 451–464.

Ntalli N, Ratajczak M, Oplos C, Menkissoglu-Spiroudi U, and Adamski Z (2016) Acetic acid, *2*-undecanone, and *(E)-2*-Decenal ultrastructural malformations on *Meloidogyne incognita*. *Journal of Nematology* **48**, 248–260.

Prosen H, Kokalj M, Janeš D, and Kreft S (2010) Comparison of isolation methods for the determination of buckwheat volatile compounds. *Food Chemistry* **121**, 298–306.

Przybylski R, Woodward L, Eskin NAM, Malcolmson LJ, and Mazza G (1995) Effect of buckwheat storage and milling on flavor compounds. *Current Advances in Buckwheat Research*, 783–787.

Puntener W (1981) *Manual for field trials in plant protection.* 2nd ed. Basel, Switzerland, Ciba Geigy. p. 205.

Seefeldt SS, Jensen JE, and Fuerst EP (1995) Log-logistic analysis of herbicide rate response relationships. *Weed Technology* **9**, 218−227.

Sipes BS and Arakaki AS (1997) Root-knot nematode management in dryland taro with tropical cover crops. *Supplement to the Journal of Nematology* **29**, 721–724.

Yuan S, Yan J, Ye X, Wu Z, and Ng T (2015) Isolation of a ribonuclease with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from Japanese large brown buckwheat seeds. *Applied Biochemistry and Biotechnology* **175**, 2456–2467.

https://doi.org/10.1017/S0022149X23000457 Published online by Cambridge University Press