

Using of hydrogen cyanide against *Ditylenchus dipsaci* nematode present on garlic

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ABSTRACT

The stem and bulb nematode (*Ditylenchus dipsaci*) is a serious quarantine pest of vegetables spreading worldwide via seed and planting material. Currently, a hot water technique is used as the pre-seed treatment, which is difficult to execute and the risk of seed damage is high. The objectives of this study were to evaluate the (a) penetration of gaseous hydrogen cyanide (HCN) into garlic tissue; (b) HCN phytotoxicity, and (c) nematicide potential of HCN against *D. dipsaci*. Penetration of HCN into the core of the garlic clove was approximately 30% of the concentration inside the fumigation chamber after 30 h of exposure. Decreased emergency was observed only in the exposure treatment lasting 16 and more hours. Garlic cloves naturally infested by *D. dipsaci* were treated with HCN at a concentration of 20 g/m³ for 12, 18 and 24 h in a fumigation chamber, and 99% mortality was achieved in all three exposure times.

Keywords: *Allium sativum*; pesticide; onion vegetable; crop protection

In onion crops, the stem and bulb nematode (*Ditylenchus dipsaci*) is an important pest (Aftalion and Cohn 1990) with quarantine status in many countries. *D. dipsaci* dispersion to new localities is mainly a consequence of its presence in seed material. This consequence occurs especially with vegetables such as onions, where seeds (e.g. of chives or onion) or seed bulbs (mainly the cases of garlic and leek) can be infested. Another crops e.g. hop could be also affected (Lišková and Renčo 2007). Therefore, treatment of the seed plant material is of the key importance and is routinely performed before planting (Hanks and Linfield 1999).

In the previous EPPO protocols (e.g. EPPO 1974), hydrogen cyanide (HCN) was available for fumigation treatment of bulbs, rhizomes and tubers against *D. dipsaci*. That EPPO protocol was withdrawn in 1985, and was substituted with the

protocol nr. PM 3/2(2), which is the fumigation of seed material infested with *D. dipsaci* by methyl bromide (EPPO 1998). As methyl bromide is no longer available as a pesticide, the management of *D. dipsaci* on onion vegetables has relied solely on hot water treatment in recent years (EPPO 2000). This technique is often modified using a hot solution of formaldehyde. As the suspicion of the carcinogenicity of formaldehyde has gained more attention since the 1980s (e.g. Kerns et al. 1983) and some studies have also detected surviving nematodes after the hot water-formaldehyde treatment (Roberts and Greathead 1986), alternative solutions are being investigated for the treatment of seed materials against *D. dipsaci*. Promising results were obtained from sodium hypochlorite (Roberts and Matthews 1995) and peroxyacetic acid (Hanks and Lindfield 1999). However, most

of the investigated methods require dipping of the seed material into liquids. This process has some drawbacks, especially the subsequent drying, which can last up to 24 additional hours after treatment (Roberts and Matthews 1995). Hot water treatment also introduces a risk of nematodes escaping from infested bulbs into the dip tank; these free-swimming nematodes are more difficult to kill by temperature alone (Hanks and Lindfield 1999). The number of newly registered active substances for use as conventional nematicides is currently very low. Therefore, alternative chemical treatments against *D. dipsaci* in seed plant material should be investigated.

One of the promising chemicals applicable as a nematode killing agent is HCN. It occurs naturally in the environment, it features very high penetration properties, and it is highly reactive and therefore easily and quickly degradable. The risks associated with its high toxicity can be decreased by proper cylinder formulation, use in a fumigation chamber, and application by professionally trained staff (Stejskal et al. 2014a). HCN is commercially available and is registered in some European Union countries as a biocide for mills, structural wood and transport fumigation treatment (Ducom 2012, Aulicky et al. 2014). Outside of the European Union, HCN is routinely used as a plant/commodity quarantine fumigant in several countries, e.g. India, New Zealand (Navarro 2006),

and South Korea (Stejskal et al. 2014b). In the past, HCN was extensively employed as a quarantine treatment of wood against bark beetles in the USA (Quayle 1922) as well as for seed treatment in the Czech Republic (Stejskal 2014b). As previously mentioned, from 1975 to 1984, the EPPO standard for *D. dipsaci* control in onions by HCN was available (EPPO 1974). However, there are insufficient data on the efficacy of HCN fumigation on the mortality of *D. dipsaci* nematodes in garlic cloves. Therefore, the objective of our study was to collect new data on the (a) speed of penetration of HCN gas into garlic tissue; (b) HCN phytotoxicity to seed garlic, and (c) biological efficacy of HCN against *D. dipsaci* in infested garlic cloves.

MATERIAL AND METHODS

Penetration of HCN into garlic tissue. The evaluation of the penetration of HCN into garlic cloves was conducted in a hermetic fumigation chamber. Garlic for testing was prepared by hollowing the single cloves of garlic and attaching a rubber septum onto the hollow area (Figure 1). Treatments were conducted in a stainless hermetic steel fumigation chamber equipped with an air lock, forced ventilation and rubber glove manipulators (Figure 2). An image and technical details of this chamber are described in a previous



Figure 1. Single clove garlic with attached rubber septum

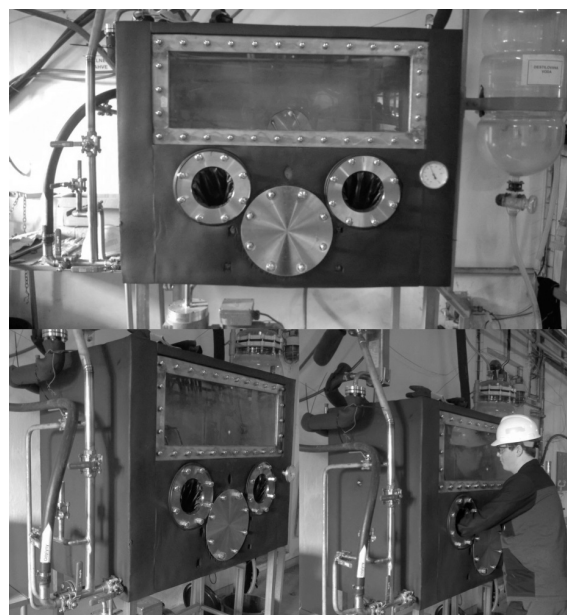


Figure 2. Fumigation chamber

work (Stejskal et al. 2014a). The injected dosage of HCN in the head space of the fumigation chamber was equivalent to 20 g/m^3 . HCN samples were withdrawn from the garlic cloves via the septum using a glass syringe and the HCN concentrations were determined using a gas chromatograph (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm, GC Software Clarity DataApex, Kyoto, Japan) after 6, 12, 18, 24 and 30 h of exposure; the concentration of HCN inside chamber was also measured.

HCN phytotoxicity. A second experiment targeted the evaluation of the phytotoxic effect of HCN on seed garlic cloves. The design of this experiment was similar to the previous one. Nine cultivars of garlic were obtained and treated with HCN (concentration 20 g/m^3) for 10 exposure periods. Seven cloves were treated in each cultivar. After treatment, garlic cloves were planted and numbers of emerging plants were scored 30 days after planting. Untreated control variants of each cultivar were included in the experiment.

Biological efficacy on *D. dipsaci*. Garlic cloves infested with *D. dipsaci* were obtained from a farm in Central Bohemia. The presence and quantification of the species was confirmed using a Baermann funnel extraction technique. To determine the location of the nematodes within the cloves, 56 cloves with peels removed were examined and the removed peels were examined separately. On average, one clove contained 7 422 *D. dipsaci* specimens, 7 406 in peels and 16 in the bare clove. Ten infested cloves were inserted into fabric sacks (mesh size $45 \mu\text{m}$) for fumigation. Five replicates were treated for each cultivar. The injected dosage of HCN in the head space of the fumigation chamber was equivalent to 20 g/m^3 . The temperature inside the chamber was maintained at 24°C during the trials. The three exposure times tested were 12, 18 and 24 h, and HCN samples were also withdrawn from the chamber at those intervals. After 24 h, the HCN was ventilated from the samples, and surviving nematodes were extracted from treated cloves using the Baermann funnel technique and counted under a stereomicroscope. An untreated control was placed beside the fumigation chamber during the trials and was evaluated in the same manner. Obtained data underwent statistical analysis (two way and one-way ANOVA, regression analysis; Statistica 12, StatSoft, Inc., Tulsa, USA, 2013).

RESULTS AND DISCUSSION

The concentration of HCN in the headspace of the fumigation chamber during the treatments and inside the garlic tissue is summarised in Figure 3. The HCN concentration in the core of the garlic cloves was approximately 30% of the initial concentration inside the chamber headspace after 30 h of treatment. Strong and simple relationship of exposure time and concentration of HCN inside garlic tissue (correlation coefficient 0.98) as well as in fumigation chamber (correlation coefficient 0.90) enabled estimation of equalizing of concentrations in both environments at 50 h of hypothetical treatment. Our tests on phytotoxicity (Table 1) further showed that HCN did not affect the viability of garlic in the short treatments (up to 14 h); however, there was a decrease in germination after longer HCN exposures.

Overall, there was good efficacy of the HCN treatment on *D. dipsaci* mortality (Table 2). Even the shortest treatment period significantly decreased the number of nematodes in the garlic tissue. No significant differences were observed among the three exposure times. After all three tested exposure times, some living nematodes were extracted; however, the number of survivors was only approximately 1% of the numbers recorded in the untreated control.

The HCN concentration in the core of the garlic cloves was lower than in the fumigation chamber;

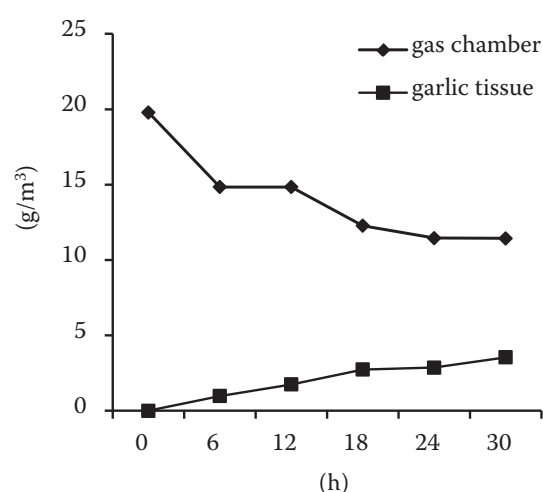


Figure 3. Concentration of HCN in the headspace of the fumigation chamber and inside garlic tissue (measured in the centre of the garlic clove via septum as depicted in Figure 1 during treatment (30 h))

Table 1. Number of emerging garlic plants 30 days after treatment

| Cultivar | Exposure time (h) | | | | | | | | | | control |
|----------|-------------------|---|----|----|----|----|----|----|----|----|---------|
| | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | |
| Vekan | 7 | 7 | 6 | 7 | 6 | 4 | 2 | 2 | 0 | 1 | 7 |
| Bjetin | 7 | 5 | 6 | 7 | 4 | 6 | 4 | 6 | 7 | 6 | 6 |
| Blanin | 6 | 7 | 6 | 3 | 1 | 2 | 0 | 0 | 0 | 0 | 7 |
| Benátčan | 6 | 5 | 4 | 1 | 2 | 2 | 2 | 0 | 1 | 0 | 4 |
| Stanik | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Slavin | 7 | 7 | 6 | 6 | 6 | 4 | 2 | 2 | 0 | 0 | 7 |
| Jovan | 7 | 7 | 7 | 5 | 5 | 1 | 4 | 7 | 4 | 1 | 5 |
| Japo II | 5 | 4 | 6 | 4 | 6 | 6 | 3 | 4 | 3 | 4 | 4 |
| Matin | 6 | 4 | 3 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |

7 replicates were tested from each cultivar. The HCN concentration was 20 g/m³. Statistically significant (two-way ANOVA; $\alpha = 0.05$) decrease of the number of emerging plants at exposure times from 16 to 24 h was detected with exceptions of Bjetin and Stanik cultivars

however, this factor did not seriously diminish the effect of the treatment, as most of the nematodes occurred in or near the garlic peel. This finding was also apparent from the results of testing the biological activity of HCN. Our results show good efficacy (i.e. 99%) of the HCN fumigation treatment on *D. dipsaci*. It may be possible to use seed garlic with such low *D. dipsaci* numbers for the establishment of commercial field cultures without many problems, because the numbers of nematodes will not increase beyond the threshold of severe economic impact until the end of the vegetation period. According to the early HCN protocol, the HCN initial dosage was 4 g/m³ and the exposure time was 1 h (EPPO 1974), approximately four times lower than the concentration used in our study. According to our results, where living nematodes were found even after the treatment of 20 g/m³ of HCN inside the host plant tissue and after 24 h of exposure, the dosage and exposure time recommended in this standard seem rather insufficient.

The high efficiency of HCN on *D. dipsaci* mortality is consistent with our previous findings with two other nematode species *Caenorhabditis elegans* (Maňasová et al. 2013) and *Bursaphelenchus*

xylophilus (Stejskal et al. 2014a). Treatment using a gas fumigant seems to overcome the drawbacks of the currently used hot water dip treatment. Although the efficacy of HCN on nematodes is sufficient, there are still remaining issues to address concerning its potential phytotoxicity. It was previously found that HCN has a very low phytotoxicity on grain germination, which it did not diminish but rather slightly enhanced (Ren et al. 1996). A stimulating effect of the short HCN treatment on garlic germination was also detected in our study, especially in the case of cvs. Matin and Benátčan (Table 1). According to our findings, exposures up to 14 h with a HCN concentration of 20 g/m³ seem to be relatively safe in terms of phytotoxicity. Only one cultivar (Stanik) was negatively affected even under the short exposure conditions. It is hypothesised that this outcome could be the result of different dormancy period of this cultivar. On the other hand the cv. Bjetin was not affected even by the 24 h treatment. Anyway it is important to note that garlic must be in the dormant stage during treatment, as it is likely that damage would occur during the vegetative stage. According to our statistical analysis from

Table 2. Number of nematodes in 10 garlic cloves after HCN treatment

| Exposure time (h) | 12 | 18 | 24 | Untreated control |
|-------------------------------|------------------------|------------------------|------------------------|----------------------------|
| Number of surviving nematodes | 615 ± 577 ^a | 379 ± 265 ^a | 164 ± 150 ^a | 40220 ± 24723 ^b |

Average ± standard deviation; the average weight of 10 garlic cloves was 54.03 ± 1.84; all three exposure times grouped together and were significantly different (indicated by *a* and *b* letters) from untreated control (one-way ANOVA, $\alpha = 0.05$). Ct product for seed garlic after 24 h exposure to HCN concentration 20 g/m³ was < 378.16 g × h/m³

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both factors involved (exposure time, cultivar) the first one plays definitely a major role in plant emergence.

It seems clear that dormancy of the treated garlic is of critical importance. This issue could be solved by timing the treatment during the 4–6 week period after harvest. Though the physiology of dormancy is not completely understood in garlic, it is known generally that it has an innate dormancy period (Volk et al. 2004). However, the main task would be the implementation of appropriate legislation regarding HCN utilisation including corresponding technical guidelines. This task can be conducted only after further research focused on the detailed evaluation of the effects of HCN on garlic physiology and viability is conducted.

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