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Chemotaxis of *Ditylenchus destructor* in response to different inorganic ions

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Summary. The chemotactic responses of *Ditylenchus destructor* were studied in salt gradients created in an agarose gel. Nineteen combinations of sodium, calcium, potassium, ferric or ammonium cations and chloride, nitrate, sulphate, hydrogen-phosphate, bicarbonate and carbonate anions were tested at six concentrations from 0.0625×10^{-2} to 2×10^{-2} mol l^{-1}. *Ditylenchus destructor* was attracted to salts that included Cl^-, and NO_3^-, whereas salts with SO_4^{2-} and $H_2PO_4^-$ anions were repellent, but the salts comprising CO_3^{2-} had almost no effect on the nematode movement. The repellent or attraction properties of different salts having the same cations were not consistent. The chemotaxis of nematodes to $Ca(H_2PO_4)_2$, KH_2PO_4, $NH_4H_2PO_4$, K_2CO_3, $FeSO_4$, Na_2SO_4, K_2SO_4, $CaSO_4$, and KNO_3 increased with increasing concentration, whilst the concentration of other salts tested did not influence nematode chemotaxis significantly.

Key words: chemoattraction, chemotaxis, stem nematode, sweet potato.

Ditylenchus destructor is one of the most important pathogens in sweet potato production. The nematodes overwinter in the soil, diseased tubers or organic debris. Once host seedlings have been transplanted, the nematodes orientate to the growing stem and penetrate the stem directly. In a severe infection the younger plants may die, and without any control measures *D. destructor* can cause 30-50% yield losses in China (Zhou & Ma, 2003). The common method for controlling *D. destructor* has been chemical control with aldicarb. However, environmental concerns require alternatives to such toxic compounds, so new control strategies are needed. Understanding the mechanisms and factors involved in host location could provide powerful opportunities for controlling the nematode by disrupting its host finding behaviour.

As early as 1925, Steiner (1925) proposed that plant-parasitic nematodes located their hosts by chemoreception. Subsequently, a series of experiments were conduced on the chemotaxis of nematodes in response to host roots (Bird, 1959; Perry, 1997; Devine & Jones, 2002), pH (Jairajpuri & Azmi, 1978), carbon dioxide (Pline & Dusenbery, 1987; Robinson, 1995), temperature (El-Sherif & Mai, 1969; Robinson, 1994), sex pheromones (Greet *et al.*, 1968) and inorganic ions (Prot, 1979; Castro *et al.*, 1990; Saux & Quénéhervé, 2002). The chemoreception of nematodes in response to different attractants has been reviewed by Perry (1996, 2005). As inorganic salts exist naturally in the soil and are easily altered by crop fertilisers, understanding the effects of inorganic salts on nematode orientation will be important as a potential basis for developing new management approaches.

Inorganic salts of Na^+, Mg^+, Cl^- and OAc^- have been reported to attract *Rotylenchulus reniformis* (Riddle & Bird, 1985), whilst K^+, NH_4^+, Cs^+, NO_3^- and Cl^- are strongly repellent to infective second-stage juveniles (J2) of *Meloidogyne incognita* (Castro *et al.*, 1990). Saux and Quénéhervé (2002) noted that calcium salts had no effect on the orientation of juveniles of *M. incognita*, whilst ammonium salts and ammonium nitrate were strongly repellent. By contrast, the orientation of *R. reniformis* depended on the constitutive anion of the salts, *e.g.*, chloride salts were found to be repellent but sulphate and nitrate salts were attractive. The present study aimed to investigate the chemotaxis of *D. destructor* in response to 19 different inorganic salts.

MATERIAL AND METHODS

Soil nematodes. The diseased tubers were collected from a field in Cangzhou, Hebei, China infested with *D. destructor*. The nematodes were extracted from 1 cm^3 cubes of diseased tubers using an extraction tray (Southey, 1986). Subsequently, the extracted nematodes were cultured on the susceptible sweet potato cv. Dong Fang Hong (Lin, 1989). After one month incubation at 25°C, the nematodes were extracted and sterilised with 0.5% $NaOCl_2$ for 1 min, washed three times with distilled water, and then concentrated by decanting before use in the bioassay.

Salts. The salts tested were NaCl, NH_4Cl, KCl, $CaCl_2$, KNO_3, NH_4NO_3, $NaNO_3$, $FeSO_4$, Na_2SO_4, K_2SO_4, $(NH_4)_2SO_4$, $CaSO_4$, $Ca(H_2PO_4)_2$, KH_2PO_4, $NH_4H_2PO_4$, K_2CO_3, $(NH_4)_2CO_3$, $NaHCO_3$, and $CO(NH_2)_2$. For each salt, six concentrations were tested: 2×10^{-2}, 1×10^{-2}, 0.5×10^{-2}, 0.25×10^{-2}, 0.125×10^{-2} and 0.0625×10^{-2} mol l^{-1}.

Bioassay. The experimental set up used in this study was modified from Wuyts *et al.* (2006). In brief, 5-cm-diam. Petri dishes were divided into 16 sections in two circles, *viz.* an inner and outer circle (Fig. 1). The Petri dishes were filled with 0.8% agarose. In the outer circle of each dish, 50 μl of the salt being tested and distilled water were inoculated on opposite sides (S and W, respectively, in Fig. 1) and incubated for 1 h at 25°C. Ten mixed stages of *D. destructor* in 5 μl were eventually transferred to the centre of the test arena and incubated at 25°C for 5 h. After this period, movement of nematodes was stopped by spraying the plates with ethanol. The number of nematodes from section 1-8 were counted. Five plates were tested for each concentration and a control in which the salt was replaced by distilled water. All the experiments were repeated four times. The pH gradient established across the agar plates was tested with pH indicator (Unisol 410, Macherey Nagel). The pH gradient were only detectable when $0.5\text{-}2\times10^{-2}$mol .$l^{-1}$ $(NH_4)_2CO_3$ were added to the plated with pH 8 in the location of $(NH_4)_2CO_3$ dropped.

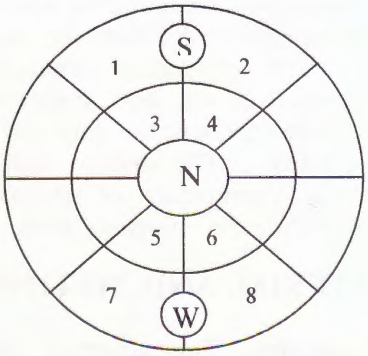

Fig. 1. Test arena for investigating the chemotaxis of *Ditylenchus destructor.* S: salt; W: distilled water; N: nematodes.

Data analysis. The chemotactic index was defined as a positive number (attractant) or negative (repellent) number ranging from +2 to -2 (which means the average distance of the nematodes travelled from the centre of Petri dish). The chemotactic index was calculated based the equation as followed:

Chemotactic index (A+R) = $\sum$(p.Id) (Saux & Quénéhervé, 2002) where: p = nematode proportion in the selected sections 1-8 (the number of nematodes in these sections expressed as a percentage of the total number of nematodes inoculated onto the Petri dish);

Id = distance index (2 for section 1-2, 1 for section 3-4, -1 for section 5-6, -2 for section 7-8).

The Wilcoxon Test was used to analysis the significance between treatment and control ($P < 0.05$).

RESULTS

The chemotactic indices of *D. destructor* in response to 19 different salts at six concentrations are shown in Figs 2-6. In general, the salts containing Cl^- and NO_3^- anions were attractive to *D. destructor*, whilst salts with SO_4^{2-} and $H_2PO_4^-$ anions were repellent. The salts comprising CO_3^{2-} had almost no effect on the nematode movement (Table 1). The effects of different salts with the same cation were not consistent. The order of repellence for the anion was $SO_4^{2-} > H_2PO_4^- > CO_3^{2-}$. Nematodes were more strongly attracted to salts containing Cl^- than to those with NO_3^-.

For each salt the chemotactic indices varied with the concentration tested. The highest chemotactic index was 0.97 (the maximum value would be 2.0) and the lowest chemotactic index was -0.74 (minimum -2.0) from all the salts tested. Nematodes were significantly attracted to salts containing Cl^- and NO_3^- anions at all test concentrations except at 2×10^{-2} mol l^{-1} for $NaNO_3$ (Table 1). For the salts with SO_4^{2-}, the chemotactic indices of both $FeSO_4$ and $NaSO_4$ at all test concentrations were significantly lower than those from the control, whilst the chemotactic index was only significantly different from the control for the medium concentration K_2SO_4; with $CaSO_4$ and $(NH_4)_2SO_4$ the chemotactic indices decreased with increasing concentration (Fig. 4). The salts containing $H_2PO_4^-$ were also repellent to the nematodes and, in general, the greater the concentration of $H_2PO_4^-$ concentration, the stronger the repellent effect (Fig. 5). For the salts containing CO_3^{2-}, only the higher concentration of 1×10^{-2} mol l^{-1} of K_2CO_3 gave a chemotactic index

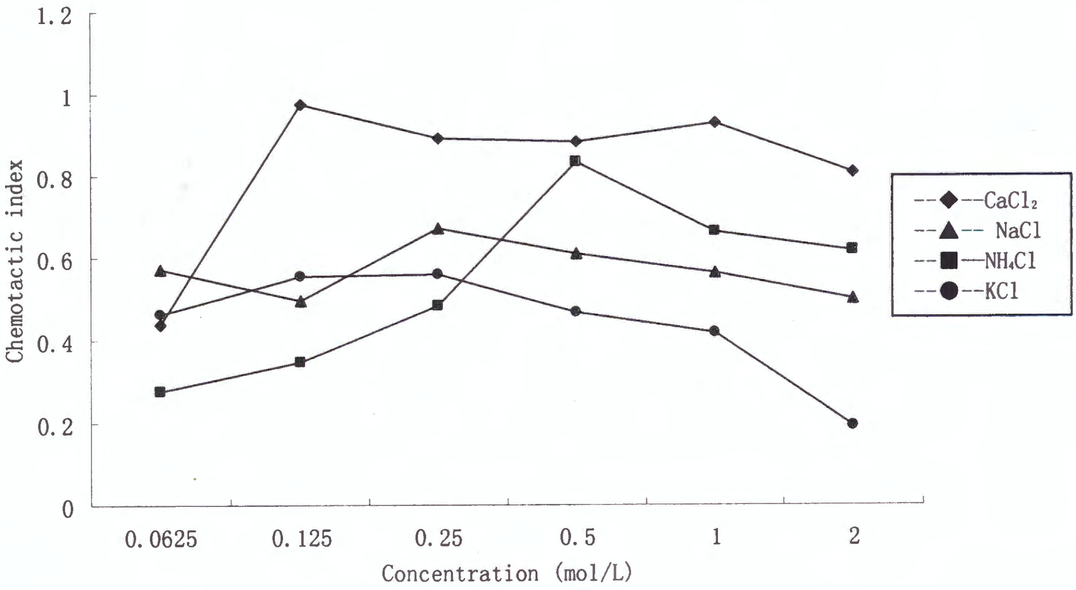

Fig. 2. Effect of salts containing chlorine anion on the chemotaxis of mixed stage *Ditylenchus destructor*.

Table 1. Significant tests for the chemotactic index of different inorganic ions at different concentrations to *Ditylenchus destructor* compared with the control.

Salts	Concentration (x 10^{-2} mol l^{-1})					
	0.0625	0.125	0.25	0.5	1	2
$Ca(H_2PO_4)_2$	ns	*	ns	*	*	*
KH_2PO_4	*	ns	ns	*	*	*
$NH_4H_2PO_4$	ns	ns	ns	ns	ns	*
$NaHCO_3$	ns	ns	ns	ns	ns	ns
$(NH_4)_2CO_3$	ns	ns	ns	ns	ns	ns
K_2CO_3	ns	ns	ns	ns	*	ns
$CaSO_4$	ns	ns	*	ns	*	*
$(NH_4)_2SO_4$	ns	ns	*	ns	*	*
K_2SO_4	ns	*	*	*	ns	ns
Na_2SO_4	*	*	*	*	*	*
$FeSO_4$	*	*	*	*	*	*
KCl	*	*	*	*	*	*
NaCl	*	*	*	*	*	*
NH_4Cl	*	*	*	*	*	*
$CaCl_2$	*	*	*	*	*	*
KNO_3	*	*	*	*	*	*
$NaNO_3$	*	*	*	*	*	ns
NH_4NO_3	*	*	*	*	*	*
$CO(NH_2)_2$	ns	ns	ns	*	ns	ns

ns = the chemotactic index is not significantly different from that of control; * = the chemotactic index is significantly different from that of control at $P < 0.05$.

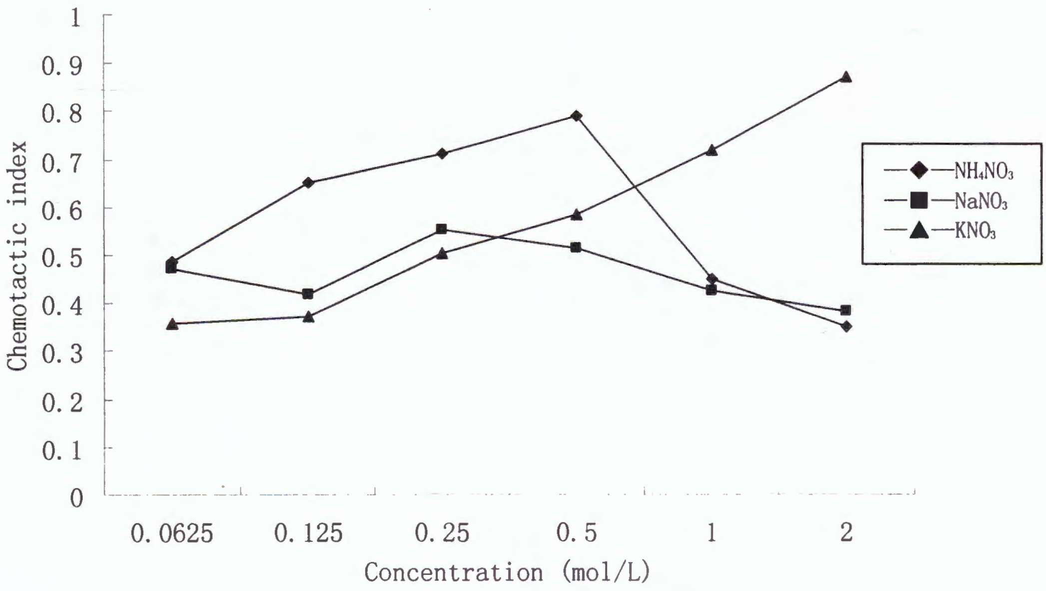

Fig. 3. Effect of salts containing nitrate anion on the chemotaxis of mixed stage *Ditylenchus destructor*.

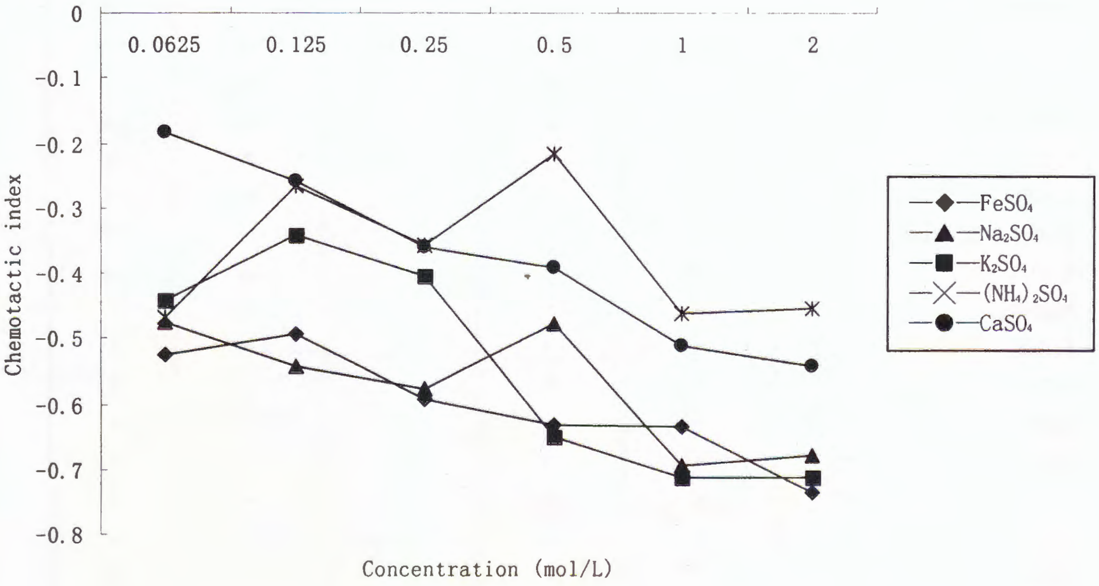

Fig. 4. Effect of salts containing sulphate anion on the chemotaxis of mixed stage *Ditylenchus destructor.*

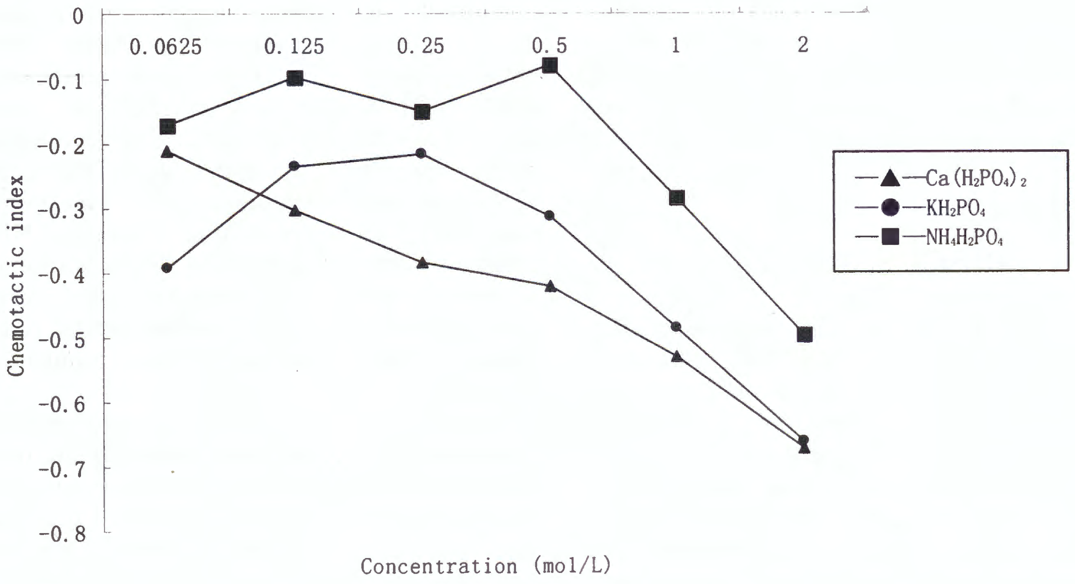

Fig. 5. Effect of salts containing hydrogen-phosphate anion on the chemotaxis of mixed stage *Ditylenchus destructor*.

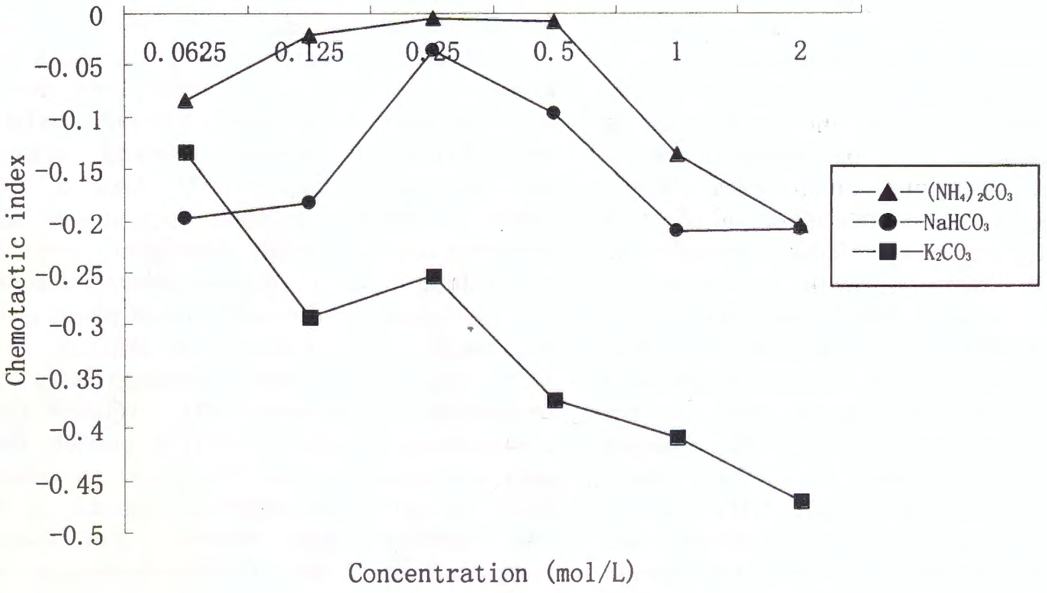

Fig. 6. Effect of salts containing bicarbonate or carbonate on the chemotaxis of mixed stage *Ditylenchus destructor*.

that was significantly lower then that of the control (Table 1).

If comparing the repellent or attraction responses of *D. destructor* to the same salts at different concentration, the chemotactic index of $Ca(H_2PO_4)_2$, KH_2PO_4, $NH_4H_2PO_4$, K_2CO_3, $FeSO_4$, Na_2SO_4, K_2SO_4, $CaSO_4$ and KNO_3 increased with increasing concentration, whilst for the other salts tested the concentration did not influence nematode chemotaxis significantly (Figs 2-6).

DISCUSSION

Sweet potato is one of the most frequently planted crops in the dry and hill regions of Northern China. In most of these areas this crop has been continuously cultivated for several decades. In recent years, farmers are unlikely to rotate sweet potato with other crops because of its high value and drought resistance. As a consequence, the stem nematode *D. destructor*, the most serious pest for the sweet potato production, increases in importance. Controlling this nematode has been a challenge. In practice, chemical control is the routine way to manage the pest; over the years the dosage of aldicarb has been increased from 1.5 kg a.s. ha^{-1} in the 1990s to 4.5 kg a.s. ha^{-1} at present. The local government has suggested that aldicarb should be removed from the market for the control of pests of sweet potato because of its high toxicity and long persistence in the soil.

Application of fertiliser is a standard practice in sweet potato production and, as inorganic salts in the soil are easily altered by crop fertilisers, understanding the effects of inorganic salts on nematode orientation will be important as a potential basis for disrupting nematode orientation.

Previous studies showed the repellent effect of some cations and anions to infective second-stage juveniles of *M. incognita*, with the order of repellence as $K^+ > Cs^+$, NH_4^+ and $NO_3^- > Cl^-$ (Castro *et al.*, 1990). Saux and Quénéhervé (2002) noted that the response of *M. incognita* is governed more by the constitutive cation than by the constitutive anion after testing all possible combinations of three anions, Cl^-, NO_3^-, SO_4^{2-}, and four cations, Ca^{2+}, K^+, Na^+, NH_4^+. In the same study, the response of *R. reniformis* was governed by the constitutive cations. The current study provides information on the response of *D. destructor* to a range of inorganic salts. It demonstrates the repellent effect of salts containing SO_4^{2-} and $H_2PO_4^-$ and the attractant effect of salts containing Cl^- and NO_3^- to mixed stages of *D. destructor*. The effects of cations on movement of *D. destructor* are not consistent, the same cations in different salts eliciting different chemotatic responses. We assume that the chemotaxis of different species of nematodes may show different responses to either anions or cations. Both $CO(NH_2)_2$ and salts comprising CO_3^{2-}, which are components of the most frequently used fertilisers, have a very weak effect on the movement of *D. destructor*. This is the first report of their chemotactic effect on nematodes. Obviously, salts containing Cl^- and NO_3^- are attractants for *D. destructor*. When combined with a chemical nematicide these salts may increase the effect of the nematicide and therefore reduce the dosage required for an efficient control of the nematode.

Based on farm practice, damage caused by *D. destructor* is decreased after applying the powder of fired brick made of clay and cereal fibre (Jun Li, personal communication). The fired brick contains mainly K_2CO_3 and KCl; however, the mode of action in decreasing stem nematode damage is not clear. As the K^+ and Cl^- concentration in the soil after application of the fired brick is not known we cannot relate the observation to our data. It is possible that if the nematodes were exposed to a solution of a chemical that was attractive to them; this may compete with the plant as an attractant and, as a consequence, fewer nematodes may succeed in locating the host.

Inorganic ions not only affect nematode movement but also can affect nematode survival. The nematicidal activity of ammonia has been known for a long time. Among ten ammonia-releasing compounds tested, NH_4OH, $NH_4H_2PO_4$ and NH_4HCO_3 showed marked nematicidal activities in pot experiments (Oka & Pivonia, 2002). Ammonium sulphate applied with alkaline stabilised biosolid (ASB) significantly reduced the root-galling index of tomato plants infested with *M. javanica* compared with that of plants grown in soil treated with ammonium sulphate or ASB alone (Oka *et al.*, 2006). Although NH_4^+ is not nematicidal, it can form NH_3, which is toxic to nematodes in alkaline soil. It is possible that the salts containing cation NH_4^+ and an anion that elicits an attraction response, *e.g.* Cl^- or NO_3^-, may have a better control effect than salts comprising other ions. Further studies are needed on this aspect.

The soil is a complicated environment. The complex interaction between plants, nematodes, abiotic factors and biotic factors, for example, may mask the interaction between the two individual factors. It has been shown that mineral nutrition is one of the factors influencing the production of antibiotics by PGPR strains (Gutterson, 1990; Duffy & Défago, 1999) and enhancing its control effect. Zinc stimulated the production of pyochelin and pyoverdin in *Pseudomonas aeruginosa* (Höfte *et al.*, 1994), antibiotic production by *P. fluorescens* (Keel *et al.*, 1996) and nematicide activities in pot experiments (Siddiqui & Shaukat, 2002; Siddiqui *et al.*, 2002). Interaction between the repellent or attractive ions tested in the present study and biocontrol agents need to be tested. Optimising the combination of inorganic ions with other biotic factors or abiotic factors may enhance the overall nematicidal activity.

Repellence of nematodes in response to certain salts can be influenced by direct chemical effects (Prot, 1979), pH (Bird, 1959) or redox potential (Bird, 1959, 1962). In the present study, the chemotaxis of *D. destructor* in response to different concentration of salts is variable. For most salts tested, such as $Ca(H_2PO_4)_2$, K_2SO_4 and $CaSO_4$, their chemotactic indices increased with increasing concentration, whilst for some salts tested their concentration does not influence nematode chemotaxis significantly.

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Qi Yonghong, Li Xiuhua, Ma Juan, Li Minquan, Chen Shulong. Хемотаксис *Ditylenchus destructor* в ответ на различные неорганические ионы.

Резюме. Хемотаксис *Ditylenchus destructor* был изучен в градиенте различных солей в агарозном геле. Девятнадцать комбинаций катионов натрия, кальция, калия, железа и аммония, а также анионов хлорида, нитрата, сульфата, гидрофосфата, бикарбоната и карбоната были испытаны при 6 концентрациях от 0.0625×10^{-2} до 2×10^{-2} mol l^{-1}. Соли, содержащие анионы Cl^- и NO_3^-, привлекали *Ditylenchus destructor*, тогда как соли, содержащие анионы SO_4^{2-} и $H_2PO_4^-$, – отпугивали. Соли, содержащие анион CO_3^{2-}, не воздействовали на передвижение нематод. Привлечение и отпугивание дитиленхов солями со сходными катионами носило непредсказуемый характер. Хемотаксис в градиенте $Ca(H_2PO_4)_2$, KH_2PO_4, $NH_4H_2PO_4$, K_2CO_3, $FeSO_4$, Na_2SO_4, K_2SO_4, $CaSO_4$, и KNO_3 возрастал с повышением концентрации, тогда как концентрация других солей не показывала воздействия на уровень хемотаксиса.