PHOSPHONATE FERTILIZERS SUPPRESSED ROOT KNOT NEMATODES MELOIDOGYNE JAVANICA AND M. INCOGNITA

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Abstract

The efficacy of the phosphonate fertilizers, Calphos (a.i. calcium phosphonate), Magphos (a.i. magnesium phos- phonate and potassium phosphonate) and Phosphoros (a.i. potassium phosphonate) against two species of root knot nematodes (RKN), Meloidogyne javanica and M. incognita is evaluated. Laboratory experiments showed that Calphos, Magphos and their main components inhibited egg hatching and caused 100% mortality of the second stage juveniles (J2s) of the two RKN species; the hatching inhibition effects persisted after transferring the egg masses of both species to water. However, Phosphoros (0.5%) did not suppress egg hatching or the survival of J2s of both RKN species. No hatching occurred when egg masses were treated for one week with the nematicide Vydate L (2 ml/l), however, J2s hatched when the Vydate L treated egg masses were moved to water. The glasshouse study indicated that Magphos , Calphos and Phosphoros reduced root galling caused by M. javanica by 98, 66 and 47%, respectively, in comparison to the untreated controls. Magphos resulted in the lowest number of root galls formed by M. incognita, the reduction was 84%. In contrast, Calphos and Phosphoros reduced galling by 47 and 39%, respectively. The Magphos treatment resulted in the lowest numbers of egg masses and the lowest reproductive factor (RF) of both nematode species. However, plants treated with Phosphoros resulted in higher foliage weights compared with the application of the other two fertilizers and the untreated plants.

Key words: Calphos, Magphos, Phosphoros, RKN, hatching, mortality.

INTRODUCTION

Root knot nematodes (RKN) attack several economic crops in Jordan (Mamluk et al., 1984). Suppression of these nematodes has been achieved using mostly fumigant and non-fumigant nematicides (Abu-Gharbieh, 1994). Some soil solarization is being used only during the hottest summer periods in the Jordan Valley (Abu- Gharbieh, 1994). Because of the environmental impact of synthetic nematicides and the limitations of using soil solarization, other alternatives should be employed. Recently, many studies showed that organic or synthetic fertilizers had a suppressant effect on RKN and other nematodes (Kaplan and Neo, 1993; Sarathchandra et al., 2001; Oka and Pivonia, 2002). It was reported that the application of fertilizers affected nematode populations indirectly by increasing the nematode feeding or by providing nutrition to compensate the plant from the nematode feeding (McIntoch et al., 1999). On the other hand, nutrient deficiency may make the plant weak and more susceptible to nematode attack (Melakeberhan et al., 1997). The direct effect of fertilizers on nematodes may alter nematode behavior and reproduction which may result in either a decrease or increase of the population. The effectiveness of fertilizers in changing nematode population depends on the fertilizer components and their active ingredients. Studies on the mechanism by which the fertilizers affect nematode population concluded that the fertilizer components might be lethal directly to nematodes or they might alter both pH and salinity of the soil harboring nematodes (Oka and Pivonia, 2002; Tenuta and Ferris, 2004).

MATERIALS AND METHODS

Nematode culture: Populations of two species of root knot nematodes (RKN), M. javanica (Trueb) Chitwood and M. incognita (Kofoid & White) Chitwood, were isolated frominfected cucumber and eggplant plants grown in Albaq'a and Deir Alla/ Jordan valley, respectively. Based on perennial patterns, the species were identified using original descriptions and diagnostic keys (Nickle, 1991). Cultures of the two populations of RKN were established by placing handpicked eggmasses near roots of healthy tomato plants (cv GS12) grown in pots in the glasshouse. The nematode cultures were regularly

subcultured and maintained on susceptible tomato plants (cv GS12) in the glasshouse at 25 ± 5 8C at the Faculty of griculture, University of Jordan at Jubeiha. Inocula of the cultures were used for further studies.

Table 1. The pH and the EC of the tested fertilizers and their main components.

Fertilizers	Fertilizer pH	EC (mS/cm)
Calphos (0.5%)	2.2	5.63
Magphos (0.5%)	3.46	3.99
Phosphoros (0.5%)	6.22	4.32
Calcium phosphonate (0.5%) - component of Calphos	2.09	6.28
Magnesium phosphonate (0.5%) - component of Magphos	2.25	5.54
Potassium phosphonate (0.5%) - component of Phosphoros	6.35	3.36

Fertilizers: Three locally manufactured phosphonate fertilizers were used in this study and were supplied by Al-Qawafel IND.AGR.EST. Calphos fertilizer consists of 36% phosphorus as phosphonate and 6% calcium as cal- cium phosphonate; Magphos fertilizer consists of 19% phosphorus as phosphonate, 6% potassium as potassium phosphonate and 6% magnesium as magnesium phos- phonate; and the third fertilizer, Phosphoros , consists of 3% nitrogen, 27% phosphorus as P2O5, 18% potassium as K2O, and mono and dipotassium phosphonate. Pure chemical components of these fertilizers, calcium phos- phonate, magnesium phosphonate and potassium phos- phonate also obtained from Al-Qawafel IND.AGR.EST to be tested for possible nematicidal properties. The pH and the EC of each fertilizer and their main chemical components were measured (Table 1). The effective con- centration (0.5%) of the fertilizers and their main phos- phonate components was selected after conducting preliminary tests on egg hatching and RKN J2 mortality. Effect of phosphonate fertilizers on egg

hatching of M. jav- anica and M. incognita: Two assays were performed using M. javanica and two separate assays using M. incognita. Three RKN egg masses were handpicked from galled tomato roots and placed in separate plastic Petri dishes that contained 5 ml of each treatment. These egg masses were exposed to the fertilizers or their main chemical components at a concentration of 0.5%. Egg masses placed in water only or treated with the nematicide Vy- date L (oxamyl a.i. 24%) at a concentration of 2 ml/L were served as controls. The treated egg masses were incubated for one week at 25 ±2 8C. The hatched J2s were counted after two, four and seven days of exposure using a dissecting microscope. After one week of expo- sure to the treatments, the egg masses were removed and incubated in fresh water to check recovery, i.e. resume hatching, to ascertain if the fertilizers have nematostatic or nematicidal properties. Each treatment was replicated
three times and the results were tabulated.

Effect of phosphonate fertilizers on J2 mortality of M. jav- anica and M. incognita: Two assays were performed using M. javanica and two separate assays using M. incognita. Egg masses were handpicked from tomato roots under dissecting microscope and then were placed in a plastic Petri dish containing fresh water and incubated until J2s hatched. The hatched J2s (about 100 J2s /replicate) were exposed to the fertilizers or their main chemical components at a concentration of 0.5 % for 3 days. Dead J2s were counted daily and up to three days using a dis- secting microscope, and then the mortality (%) was cal- culated. J2s were placed in water only or treated with the nematicide Vydate L at a concentration of 2 ml/L served as controls. The treatments were incubated at 25 ±2 8C. Each treatment was replicated three times.

Morphological changes of M. incognita J2 exposed to phos-phonate fertilizers: Temporary mounts of 10 J2s exposed to each fertilizer treatment were prepared and were ex- amined using a compound microscope after 6, 24 and 48 hours to assess any morphological changes. The length and

width of the nematode body, and their esophagus and intestinal changes were monitored.

Effect of phosphonate fertilizers on tomato root galling and egg mass production of M. javanica and M. incognita: A susceptible tomato cultivar (GS12) was used for this as- say. One assay was performed on M. javanica and a sep- arate assay on M. incognita. Nematode inoculums (10 egg masses/pot) was added to pots containing 500 cc soil mix (equal volume of sand and peat moss). Concur- rently, the fertilizers (250 ml at a concentration of 0.5%) were added to the nematode inoculated pots. The pH and EC of the soil mix used in the pot experiment was measured before and after the application of phospho- nate fertilizers. Results showed that the soil pH de- creased spontaneously after the application of calphos, magphos and phosphoros and reached 6.75, 6.43, 7.7, respectively. The pH values of the treated soil did not change even after 3 days of application; the pH of the untreated soil was 7.89. The application of the three fertilizers spontaneously raised EC almost 3 times of the untreated sand mix. The EC in the phosphoros treat- ment did not change over the 3 day period. However, the EC of both calphos and magphos treatments decreased slightly over the 3 day period. Pots treated with water only or with Vydate L at a concentration of 0.5ml/L served as controls. Pots were placed in the glasshouse at 25 ±5 8C for one week and then a tomato seedling was transplanted into each pot. Plants were maintained under optimum growing conditions for approximately two months in the glasshouse. Thenthe tomato plants were harvested and roots were examined for galling and egg mass production. Tomato fresh shoot and root weights were also determined for each replicate. Each treatment was replicated four times in a completely randomized design. The data was tabulated and analyzed using ANOVA / SAS program version 7 and the means were separated using Duncan multiple range test (Little and Hills, 1974).

RESULTS

Effect of phosphonate fertilizers on egg hatching of M. javanica and M. incognita: The treatment of egg masses of M. javanica and M. incognita with 0.5% Calphos and 0.5% Magphos inhibited J2 hatching even after seven days of exposure. Inhibition of J2 hatching by Calphos and Magphos continued for five days after trans- ferring of the treated egg masses to fresh water for both RKN species. No hatching was observed in Vydate L treated egg masses, however, J2s hatched when these egg masses were transferred to water for an incubation period of one week. Phosphoros did not affect egg hatching; a total of 153, 339 and 490 J2s of M. javanica and 231, 389 and 492 J2s of M. incognita hatched from egg masses exposed to 0.5% Phosphoros for two, four and seven days, respectively (Table 2). Furthermore, J2s hatched from egg masses of M. javanica and M. in- cognita treated with water only and reached 457 and 541, respectively. Treated egg masses of M. javanica and M. incognita with 0.5% calcium phosphonate and 0.5% magnesium phosphonate, themain components of the tested fertilizers, inhibited egg hatching even after seven days of incubation. No egg hatching occurred when egg masses of both RKN species were transferred to fresh water even after five days of incubation. How- ever, 292 and 427 J2s hatched when M. javanica and M. incognita egg masses were treated with 0.5% potassium phosphonate for one week, respectively (Table 2). J2s hatched from M. javanica and M. incognita egg masses treated with water only and reached 254 and 1325 J2s, respectively.

Table 2. Effect of fertilizers and their chemical components on J2s emerging from eggs *of Meloidogyne javanica* and *M. Incognita* at different exposure times

Treatments	Numbers of hatched J2s					
	M. javanica			*M. incognita*		
	2 days	4 days	7 days	2 days	4 days	7 days
Calphos (0.5%)	0	0	0	0	0	0
Magphos (0.5%)	0	0	0	0	0	0
Phosphoros (0.5%)	153	339	490	231	389	492
Vydate L	0	0	0	0	0	0
Water only	206	398	457	253	409	541
Calcium phosphonate	0	0	0	0	0	0
Magnesium phosphonate	0	0	0	0	0	0
Potassium phosphonate	263	275	292	141	235	427
Water only	229	237	254	399	949	1325

Effect of phosphonate fertilizers on J2s mortality of M. javanica and M. incognita: Calphos and Magphos treatments caused 100% mortality of M. javanica and M. incognita J2s after one day of exposure. Mortality was lower when J2s were exposed to 0.5% Phosphoros . Similarly, Vydate L has the same nematicidal effect on the J2s as Calphos and Magphos. J2s of both RKN species, exposed to 0.5% Calphos , 0.5% Magphos or Vydate L did not recover i.e. did not resume mobility after being transferred to water in comparison to controls. Mortality in 0.5% Phosphoros treated J2s was low even after three days of exposure (Table 3). Similarly, 100 % mortality resulted from the first day of exposure when J2s of both RKN species treated with 0.5% calcium phosphonate or with 0.5% magnesium phosphonate. None of the J2s of both RKN died after one day when they were placed in 0.5% potassium phosphonate or water, whereas, few J2s died after three days of exposure (Table 3). J2s of both nematode species, exposed to 0.5% calcium phospho- nate or 0.5% magnesium phosphonate did not resume mobility after being transferred to fresh water.

Table 3. Effect of fertilizers and their chemical components on J2s mortality of Meloidogyne javanica and M. incognita at different ex- posure times.

Treatments	Mortality (%) of J2s					
	M. javanica			M. javanica		
	1 day	2 days	3 days	1 day	2 days	3 days
Calphos (0.5%)	100	100	100	100	100	100
Magphos (0.5%)	100	100	100	100	100	100
Phosphoros (0.5%)	0.7	,0.9	4.5	3.9	4.2	4.7
Vydate L	100	100	100	100	100	100
Water only	0	2.1	10.7	1.1	3.4	5.2
Calcium phosphonate	100	100	100	100	100	100
Magnesium phosphonate	100	100	100	96.8	100	100
Potassium phosphonate	0	1.4	11.6	0	0.6	1.2
Water only	0	2.3	6.6	0	0	0

Morphological changes of J2s of M. incognita exposed to phosphonate fertilizers: Examining M. incognita within 2 days of exposure to the fertilizers revealed that no changes appeared in the length or width measurements of the treated J2s whereas, changes were observed along the esophagus and intestine of J2s treated with 0.5% Calphos and 0.5% Magphos . J2s exposed for six hours to 0.5% Calphosor 0.5% Magphos were dead, however, J2s treated with 0.5% Phosphoros or water were alive even after three days of treatment. After 6 hours of exposure, no observable changes were de- tected in the esophageal region of J2s in all treatments. However, there were vacuoles present on small regions of the intestine of J2s exposed to 0.5% Calphos . Magphos (0.5%) treated J2s had small regions of in- testine that had deteriorated. Intestine of J2s treated with 0.5% Phosphoros appeared normal as the un- treated ones with dense cells and without vacuoles. Af- ter 24 hours of exposure, only small degeneration appeared in the anterior part including the stylet and the esophagus of 0.5% Calphos treated J2s. No changes were observed in the esophageal region of the

nematodes in the other treatments. Large vacuoles were observed along the intestine of J2s exposed to ei- ther 0.5% Calphos or 0.5% Magphos . Moreover, a constriction was noticed in the intestine of 0.5% Calphos treated nematodes. Normal intestines were found in J2s exposed to either 0.5% Phosphoros or water. After 2 days of exposure, the esophagus of every nematode exposed to either 0.5% Calphos (Fig. 1A) or 0.5% Magphos (Fig. 1B) exhibited a complete deterioration.

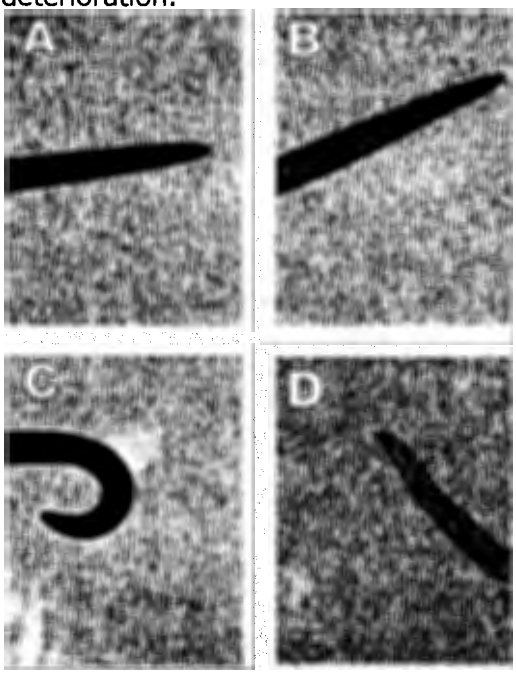

FIG. 1. Anterior part of the J2s of M. incognita exposed for 48 hours to A: Calphos ; B: Magphos ; C: Phosphoros , and D: Water (the scale bar of A, B, C, D was 20 mm).

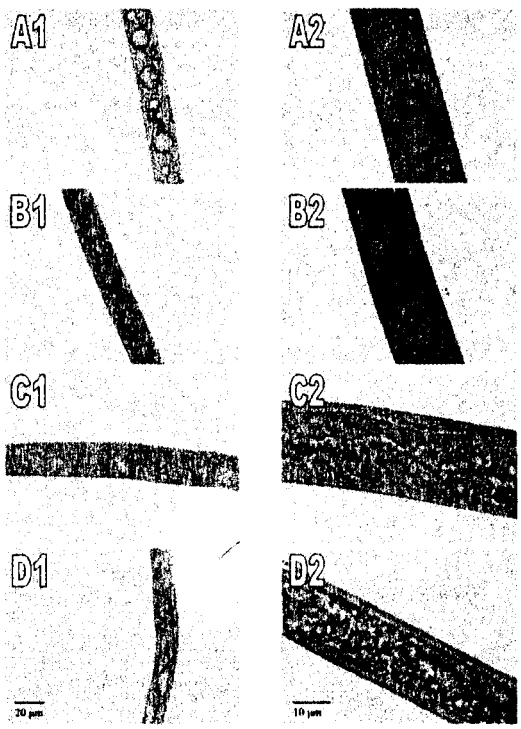

FIG. 2. Intestinal region of J2s of M. incognita exposed for 48 hours to A1 and A2 Calphos ; B1 and B2 Magphos ; C 1 and C2 Phosphoros , and D1 and D2 Water only (the scale bar of A1, B1, C1, D1 was 20 mm whereas for A2, B2, C2, D2 it was 10 mm).

Normal esophagi appeared in J2s treated with 0.5% Phosphoros and water only (Fig. 1C, D). The intestine was completely degraded in nematodes exposed to 0.5% Calphos and 0.5% Magphos and vacuoles were abundant along the intestinal region (Figs.2A1, A2, B1, B2). Normal intestine was noticed in 0.5% Phosphoros and water only treated J2s (Fig. 2C1, C2, D1, D2).

Effect of phosphonate fertilizers on root galling of tomato and egg mass production of M. javanica and M. incognita: Results showed that the reduction of root galling caused by M. javanica treated by Magphos, Phosphoros and Calphos was 98, 66, and 47%, respectively compared with the untreated control ($P = < 0.0001$). No galls were found on roots of tomato plants treated with the nem- aticide Vydate L (Table 4). The average numbers of egg masses produced on tomato roots inoculated with M. javanica were significantly lower ($P = 0.0001$) in pots treated with the phosphonate fertilizers than the un- treated pots. The lowest numbers of egg masses was produced in plants treated with the phosphonate fertilizers. Magphos treatment resulted in the lowest numbers of egg masses and consequently the lowest value of the reproductive factor in comparison to Calphos and Phosphoros ($P = 0.0001$) (Table 4). On the other hand, the Phosphoros fertilizer treatment produced the highest tomato foliage weights which were significantly higher than those of the other two fertilizers and the untreated plants. Application of Vydate L and Magphos significantly ($P = <0.0001$) reduced the foliage weights. Root weights of Magphos and Calphos treatments were lower than the untreated roots (Table 4). The number of galls formed by M. incognita on tomato roots in all treat- ments was higher thanthose infected with M. javanica, alone. However, the effect of Magphos treatment was similar to the results obtained on M. javanica since pots treated with Magphos resulted in the lowest number of roots galls ($P = 0.0029$); this attributed to a total gall re- duction of 84%. In contrast, gall numbers in tomato roots treated with Calphos and Phosphoros were lower than the control, 47% and 39% respectively; however, these differences were not significant. No galls were formed in tomato roots treated with Vydate L (Table 4). The re- production of M. incognita females expressed as egg masses was the lowest ($P = 0.0082$)

when soil was amended with Magphos followed by Phosphoros. Calphos soil amended resulted in lower number of egg masses when compared with untreated soil with no significant differ- ences. The number of egg masses produced reflected the reproductive factor values (Table 4). On the other hand, the plants treated with Phosphoros resulted in higher foliage weights compared with the other two fertilizers applications and untreated plants. Calphos increased the foliage weight but was not significantly different from untreated plants. Magphos decreased the tomato fo- liage weights but with no significant difference from the untreated plants. Tomato root weights were higher for plants treated with Phosphoros and Vydate L in comparison with all the other fertilizers (Table 4).

Table 4. The effect of Calphos , Magphos and Phosphoros on foliage and root weight of tomato plants (cv GS12) infected with M. javanica and M. incognita, and root galling of tomato roots egg mass production and reproductive factor of M javanica and M. incognita.

Treatment	Galls#	Egg masses #	Reproductive factors (RF)	Foliage weight (gm)	Root weight (gm)
M. javanica					
Calphos	87.0 bc	25.3 b	2.53 b	10.3 bc	3.5 a
Magphos	4.3 a	2.5 a	0.25 ab	8.5 c	3.5 a
Phosphoros	55.5 b	20.5 b	2.05 ab	25.9 a	4.1 a
Vydate L	0.0 a	0.0 a	0.00 a	7.4 c	4.3 a
Control	163.3 c	102.3 c	10.25 c	13.3 b	4.1 a
M. incognita					
Calphos	192.7 bc	87.8 bc	8.78 bc	9.9 b	5.6 b
Magphos	59.5 ab	9.3 ab	0.93 ab	6.7 b	4.3 b
Phosphoros	226.3 bc	26.5 ab	2.65 bc	19.9 a	9.4 a
Vydate L	0.0 a	0.0 a	0.00 a	11 b	9.6 a
Control	366.3 c	167.5 c	16.70 c	8.3 b	6.2 b

DISCUSSION

The nematicidal effect of Calphos and Magphos might be due to their low pH values, their salinity or to the combined effect of both; the pH of the materials containing calcium phosphonate and magnesium phosphonate is 2.2 and 3.46, respectively. Loewenberg et al. (1960) reported that pH 6.5 was optimum for both hatching and survival of M. incognita J2s while de- creasing the pH adversely affected egg hatching and J2 survival. In addition, several reports indicated that the salinity has adverse effects on plant parasitic nematodes (Edongali and Ferris, 1981; Karajeh and Al Nasir, 2008). Edongali and Ferris (1981) stated that both hatching and infectivity of M. incognita J2s were suppressed sig- nificantly when the egg masses were exposed to either sodium chloride or calcium chloride at 3.5 mmohs/cm; the suppressive effect increased as salinity increased to 5 mmohs/cm. In our in vitro study the exposure of J2s to 0.5% potassium phosphonate with an EC of 4.32 mS/cm did not reduce egg hatching or J2 survival of both RKN species. On the contrary, materials containing calcium phosphonate and magnesium phosphonate with an EC value of 5.63 and 3.99 mS/cm, respectively, in- hibited hatching and caused 100% J2 mortality of both RKN species. Therefore, the nematicidal effect might be due to a combined effect of the salts content of the Calphos and Magphos , in addition to their low pH. Loewenberg et al. (1960) indicated that solutions with different minerals and concentrations but the same pH values varied on their effects on egg hatching and survival of M. incognita J2s. Our in vitro experiments showed that materials containing calcium phosphonate and magne- sium phosphonate were as effective as Vydate L in egg hatching inhibition and causing 100% J2 mortality. Moreover, these two phosphonate fertilizers were nema- ticidal on J2s and eggs while Vydate L was nematicidal on J2s but nematostatic on eggs; once Vydate L was re- moved from the egg masses, egg hatching resumed and the hatched J2s were active.

TheJ2 intestine of both RKN species might have deteriorated due to fertilizer exposure containing calcium phosphonate or magnesium phosphonate; the fertilizers might have increased the metabolism of the J2s causing them to use up their lipids faster than the untreated controls. This would result in reduction of energy resources needed for the nematode to search and invade host plants and

eventually would lead to death. Atkinson et al. (2001) reported that the decline in lipid content of dormant Globodera rostochiensis J2s com- promised infectivity. The presence of large vacuoles in the intestine of treated J2s and the role of these vacuoles is not understood and need further investigation; it is possible, that the vacuoles were filled up with lipids prior to the treatments the lead to lipid depletion.

The pot experiments indicated that numbers of RKN galls on tomato roots in all treatments infected with M. incognita were higher than those infested with M. javanica which might be due to the possibility that GS12 tomato cultivar is more susceptible to M. incognita than the M. javanica. Reproduction, in terms of egg masses and RF, of the two RKN species was significantly reduced when soil was drenched with phosphonate fertilizers; J2s inocula will be reduced which will result in less root galling formation. In contrast to the in vitro experiments, fertilizer containing potassium phospho- nate was as effective as materials containing calcium phosphonate in the pot experiments. Moreover, ma- terials containing potassium phosphonate caused the highest foliage weights compared to the other fertilizers. It is possible that materials containing potassium phos- phonate might induce resistance in tomato plants against the RKN. Several reports showed that the addition of potassium phosphate and phosphonate salts played a role in the systemic acquired resistance (Gottstein and Ku, 1989; Zainuri et al. 2001). Moreover, Phosphoros has 3% nitrogen in addition to potassium phosphonate which might be responsible for the increase of foliage weight.

Magphos was the most effective phosphonate fertil- izer in reducing root galling and egg mass production, and its effectiveness might be related to its components, magnesium phosphonate and potassium phosphonates. Magnesium phosphonate lowered the soil pH and in- creased the salinity of the soil, and thus, reduced both egg hatching and survival of J2s which reduced the penetration of J2s into the plant and eventually to less galling. On the other hand, potassium phosphonate might induce a systemic acquired resistance in tomato plants as mentioned earlier. Further large scale field studies will be conducted to confirm our findings.

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