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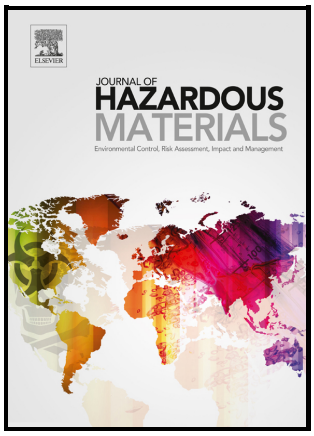

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Understanding phytotoxicity of fosthiazate on crop seedlings through uptake kinetics, ROS burst and chloroplast metabolism

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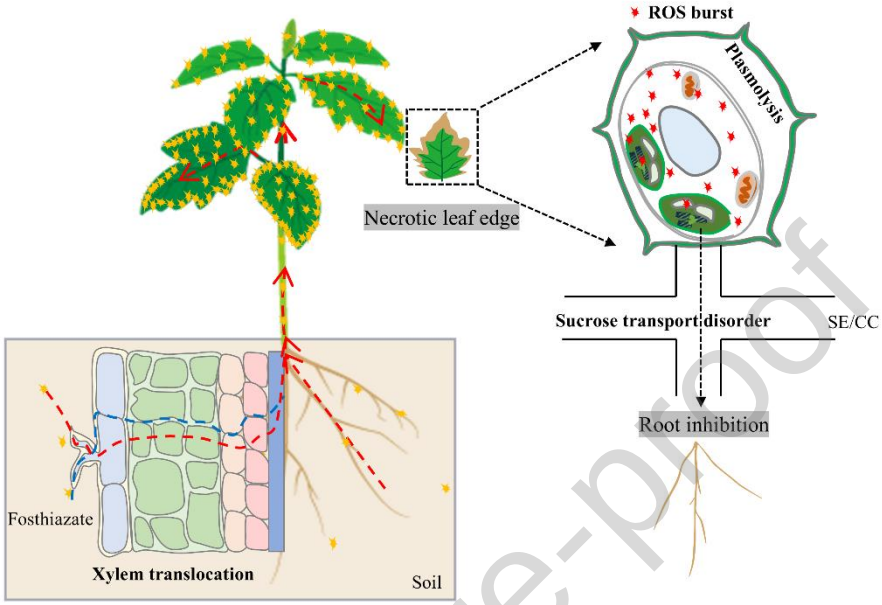
Graphical abstract



Abstract

As a crucial management strategy for crop diseases, pests and weeds, the use of pesticides can also have some adverse effects on plant health. Understanding the specific mechanisms is essential for developing effective mitigation measure. However, most studies on phytotoxicity mechanism have focused on ionic balance and biochemical responses, with little consideration given to pesticide distributions within plants. Herein, symptoms and the underlying mechanisms of fosthiazate phytotoxicity to crops represented by tomatoes were investigated. Necrotic leaf edge and the root inhibition of tomato seedlings was observed after fosthiazate soil applied at the maximum registered dose. Given its high hydrophilicity, fosthiazate dissolved in soil solution was readily absorbed by plant roots and efficiently translocated upward via the

transpiration stream, leading to varying concentrations across different organs and thus differential phytotoxicity. As fosthiazate accumulates, it induced plasmolysis, triggered reactive oxygen species (ROS) bursts, and disrupted photosynthesis, resulting in leaf wilting and necrotic. The interference of sucrose synthesis, transport and metabolism further inhibited root growth. Fosthiazate-loaded microcapsules could alleviate its phytotoxicity by slowing down the release rate. Our findings provided an important basis for the improvement of pesticide application safety and guiding the development of chemicals targets at specific organisms.

Keywords:

phytotoxicity, high hydrophilicity, uptake kinetics, photosynthate, mitigation measure.

Environmental implication

Fosthiazate is a nematicide commonly applied by soil treatment for managing root-knot nematodes. Given its high hydrophilicity, fosthiazate dissolved in soil solution was readily absorbed by plant roots and efficiently translocated upward via the transpiration stream, leading to varying concentrations across different organs and thus differential phytotoxicity. As fosthiazate enriched, it induced plasmolysis, ROS bursts and disruption of chloroplast function. Finally, fosthiazate-loaded MCs were prepared to mitigate the phytotoxicity by slow down the release rate. This study provided basis for the phytotoxicity mechanism of hydrophilic chemicals and offered guidance for the developing chemicals targeted at different organisms.

1. Introduction

With the increasing globalization of agricultural trade, food safety and crop yield have garnered significant attention, both of which are primarily contingent upon plant health. However, crops may suffer from numerous diseases, pests and weeds throughout the whole growth period, and chemical control is one of the most important protection and curation measures [1]. However, most of the applied pesticides either adversely affect non-target crops or escape into the external environment [2]. Root-knot nematodes (RKNs, *Meloidogyne* spp.) damage the root systems of almost all vascular plants worldwide by forming galls, and are considered the most destructive plant-parasitic nematodes (PPNs) [3]. Fosthiazate is efficient against RKNs and have been registered in many countries [4]. The mode of action of fosthiazate was to block nerve pulse conduction in RKNs by binding to acetylcholinesterase (AchE), making it phosphorylation. The accumulation of acetylcholine (Ach) excessively activates choline receptors, RKNs continues to be excited to death [5]. Fosthiazate exhibits high water solubility (9000 mg/L) and systemic activity. It is generally soil treated by broadcast or root-irrigation, and then takes effect through direct contact with RKNs or root absorption [5]. However, its excessive application may pose risks to non-target organisms. Especially concerning is the emergence of fosthiazate-resistant RKN population [6], which could necessitate increased application rates and thereby exacerbate phytotoxicity. Non-target phytotoxicity often occurs on the organisms sharing similar action sites, such as those non-selective herbicides, triazoles and

quinone outside inhibitors[7][8]. Undoubtedly, fosthiazate exhibited high toxicity to humans and non-target animals due to its inherent toxicological and environmental characteristics [9][10]. Although the homology between AchE in plants and animals is extremely low [11], fosthiazate has been observed to cause phytotoxicity in crops [12][15]. The underlying phytotoxicity mechanisms remain poorly understood, which hinders the development of effective scientific mitigation strategies. Therefore, exploration of the specific phytotoxicity mechanisms is essential for advancing our understanding and management of phytotoxicity.

At present, numerous researches has been conducted on the kinetics of pesticide uptake in plants; however, few studies explored the relationship between pesticide accumulation and phytotoxicity. Typically, for soil treatment, plants absorb pesticides passively from the surrounding soil solution through roots via apoplast pathway, symplast pathway or their combination, along the concentration gradient [16]. After root absorption, some pesticides enrich in the roots, while others enter the xylem through root epidermis and then transferred to the plant shoots [17]. This process is influenced by both chemical properties and plant species [18][19]. Pesticides with higher hydrophilicity tend to be more readily absorbed and translocated. Plant transpiration, which creates a series of water potential gradients, serves as the main driving force for the root absorption and shoot translocation of highly water-soluble pesticides [20]. Understanding the absorption and translocation mechanisms of pesticides in plants is crucial for evaluating their accumulation and distribution. Although the uptake kinetics of fosthiazate in wheat roots and shoots have been

determined previously under hydroponic conditions [21], its actual behavior within different parts of the plant in soil-plant system remains unclear.

Most pesticides, once accumulated in plants, can disrupt physiological and biochemical process as well as metabolism pathways, thereby affecting the plant growth and development. At present, knowledge about the non-target phytotoxicity mechanism of fosthiazate is almost blank, with only limited literature documenting its phenotype [14]. Fosthiazate belongs to the organophosphate (OPP) class of pesticides, which includes nematicides, herbicides, insecticides, and fungicides. Studies on the OPP phytotoxicity have mainly focused on adverse effects on plants, including photosynthesis, photochemical reactions, oxidative stress, carbon metabolism, and nitrogen metabolism [22]-[23]. To determine whether these mechanisms are applicable to fosthiazate, a preliminary comparison of phytotoxicity phenotypes of several highly water-soluble OPPs (i.e., glyphosate, glufosinate-ammonium, fosetyl-al, dichlorvos dimethoate, acephate and fosthiazate) was conducted. Besides the dichlorvos and fosetyl-al, the tested OPPs exhibited a common symptom of necrotic leaf edges (Fig. S1). Notably, the phytotoxicity phenotypes of dimethote and acephate were the most similar to those of fosthiazate. Dimethote was reported to decrease the photosynthetic pigments and efficiency, destroy the homeostasis of reactive oxygen species (ROS) in plant seedlings [24][25]. Generally, it is assumed that phytotoxicity of soil-applied agrochemical is resulted from direct root contact, causing root damage, affecting nutrient absorption, and then impacting aboveground plant parts [26][27]. For instance, the phytotoxicity of soil treated ammoniacal fertilizers initiates from the root apex and

subsequently extends to the entire seedling [28]. In contrast, fosthiazate-induced phytotoxicity is a continuous process, where the necrotic area expands from the leaf edge towards the center. We hypothesize that if the root was initially injured by fosthiazate, the phytotoxicity observed on the leaf would develop more slowly, and the necrotic areas should be the entire leaf. It is suggested that the root is not the main injury site, although the development of the plant's root was significantly inhibited. The interrelationship between root and shoot in this context requires further investigation.

The objectives of this study were to i) assess the phytotoxicity phenotype of fosthiazate on tomato seedlings; ii) examine the accumulation and translocation behaviors of fosthiazate in tomato plants after soil treatment; iii) evaluate the influence of fosthiazate on ROS production and scavenging; iv) determine the impact of fosthiazate on photosynthetic efficiency; v) investigate the phytotoxicity alleviating effect of slow-release fosthiazate formulations.

2. Materials and methods

2.1 Test materials

Chemicals and regents were detailed in Text S1 of the Supporting Information (SI). Crop variety, seed disinfection, germination and cultivation methods were detailed in Text S2.

2.2 Fosthiazate phytotoxicity

A series of fosthiazate dosage (0, 2, 4, 8, 17, 33, 67, 133 and 267 mg/plant) were designed to test the phytotoxic effects on crop seedlings, including tobacco, pepper, cucumber, cabbage, carrot and tomato. These crops were selected due to their high

susceptibility to RKN infections. The dosage series were set based on the maximum recommended dosage of 67 mg/plant for RKN control (China Pesticide Information Network, ICAMA, http://www.chinapesticide.org.cn/). Uniform-sized crop seedlings were transplanted into 10 cm × 10 cm pot containing a mixture of nutrient soil and fosthiazate for root treatment. Water consumption was 200 mL/plant, ensuring thorough mixing of the soil and fosthiazate. Phytotoxicity symptoms on different crops were observed, and the necrotic ratio were calculated. Tomato was served as a representative crop for in-depth studies on phytotoxicity and its underlying mechanisms. Parameters including stem length, leaf necrotic area ratio, root system architecture, net assimilation rate, root shoot ratio and relative water content were measured (Text S3). The leaf cell viability and root activity were determined using evans blue staining method [29] and triphenyltetrazolium chloride (TTC) method [30], respectively. Additionally, the influence of soil type, cultivation pH, soil mixing ratio on fosthiazate phytotoxicity towards crops were examined (Text S4). The experiments were conducted three times with three replicate seedlings.

2.3 Split root

Split root tests were performed to investigate the shoot-root interactions in tomato plants [31]. Roots were divided into two approximately equal parts and subjected to two treatments: fosthiazate soil (treatment group) and control soil (response group). The dosages of fosthiazate were 0, 2, 4, 8, 17, 33, 67, 133 and 267 mg/plant. Root lengths and activities in both the treatment and response groups were measured as described. After 7 d, roots from the response group were sampled for detection of

fosthiazate content. The experiments were conducted three times with three replicates.

2.4 Fosthiazate residue

The former results indicated that the seedlings treated with 17, 67 and 267 mg/plant fosthiazate exhibited mild (slightly necrotic leaf edges), moderate (inhibited plant growth) and severe (dead seedlings) phytotoxicity, respectively. Therefore, to explore the accumulation behavior of fosthiazate in tomato plants, the three initial dosages were selected. Soil, root, stem, leaf edge and leaf center samples were collected at 0.25, 1, 2, 3, 5, 7, 14 and 21 d after treatment. Root concentration factor (RCF), bioconcentration factor (BCF), and transfer factor (TF_{stem} for root to stem, TF_{leaf} for root to leaf) values were calculated (Text S17). To investigate the translocation pattern of fosthiazate within leaves across different spatial dimensions, upper, middle, and lower leaves from seedlings treated with 67 mg/plant fosthiazate were collected. To examine the subcellular distribution of fosthiazate, root, stem and leaf samples were collected from seedlings applied with 67 mg/plant fosthiazate on day 1 (no visible phytotoxicity), day 3 (wilted leaf edges) and day 5 (necrotic leaf edges). Apoplasts, cell walls, organelles and soluble fractions were separated (Text S5). All samples were kept at -20℃ until analysis. The experiments were performed in three replicates.

Fosthiazate in the samples was extracted using a QuEChERs method (Text S6), and subsequently analyzed by UPLC-MS/MS (Text S7). Details of quality control (QC) and quality assurance (QA) procedures are provided in Text S8.

2.5 The effect of transpiration on the phytotoxicity of fosthiazate

The transpiration rate was regulated by altering culture conditions (i.e., temperature

and illuminance) and addition of abscisic acid (ABA) (Text S9), and the relevant fosthiazate phytotoxicity was observed. The temperature settings were 18, 25, 30 and 37℃, the illuminance levels were set at 5000, 10000 and 15000 lux. Additionally, the transpiration rates and phytotoxicities on tobacco, pepper, cucumber, cabbage and carrot were evaluated. Transpiration measurements were taken after 48 h as detailed in Text S9, with leaf area recorded accordingly. The experiments were performed three times with three replicates.

2.6 ROS content and oxidative damage

2.6.1 H_2O_2 and $O_2^{\cdot-}$ content

Leaf tissue samples (0.1 g) was collected after treated with fosthiazate at the dosage of 0, 17, 67 and 267 mg/plant for 0.25, 1, 2, 3 and 5 d. The H_2O_2 contents and $O_2^{\cdot-}$ release rates were detected using the detection kits provided by Sangon Biotech (Shanghai) Co., Ltd. and Beijing Solarbio Science & Technology Co., Ltd., respectively, strictly following the manufacturer's instructions. The experiments were conducted three times with three replicates.

2.6.2 The activity of antioxidant enzyme

Fresh leaf tissue (0.1 g) was homogenized in 4 ml of precooled PBS (phosphate buffer solution, 50 mM, pH 7.0) containing0.1 mM EDTA (ethylene diamine tetraacetic acid) and 1% (w/v) PVP (polyvinylpyrrolidone). The homogenate was then centrifuged at 15000 rpm for 10 min at 4℃. The supernatant was collected for the determination of SOD (superoxide dismutase, EC 1.15.1.1), POD (peroxidase, EC 1.11.1.7), CAT (catalase, EC 1.16.1.6) and APX (ascorbate peroxidase, EC 1.11.1.11) activities (Text

S10). The experiments were conducted three times with three replicates.

2.6.3 Membrane lipid peroxidation and permeability

MDA (malondialdehyde) content was quantified using the supernatant obtained from the Section 2.6.2 (Text S10). Membrane permeability was determined via relative conductivity (Text S11).

2.7 Plant photosynthetic carbon assimilation analysis

2.7.1 TEM observation

A transmission electron microscope (TEM, JEM-1200EX, Joel Co. Ltd., Japan) was employed to investigate the influence of fosthiazate on the ultrastructure of tomato cells and chloroplasts. Seedings were treated with fosthiazate at the dosage of 0 and 67 mg/plant for 3 d. The junction of health and wilted regions of leaves from the fosthiazate-treated group, as well as the corresponding areas from the control group were excised into thin sections by a scalpel. The thin strips were disposed normally as described [32].

2.7.2 Photosynthetic parameters

A portable photosynthesis apparatus (LC pro-SD, ADC BioScientific Ltd., UK) was used to measure the net photosynthetic rate (Pn). The maximum photochemical efficiency (Fv/Fm) and the overall functional activities of PSI, PSII and electron transport chain (PI total) were assessed by a plant efficiency analyzer (Handy PEA, Hansatech Instruments Ltd., UK). The experiments were performed three times with three replicates.

2.6.3 Photosynthetic pigments, Rubisco activity, ATP and photosynthate content

The photosynthetic pigments, including Chla, Chlb and Ct, were extracted by 80% (v/v) acetone following the methos described by Lichtenthaler et al. [33]. Rubisco activity was assayed using a detection kit (BC0440, Solarbio life sciences). The ATP content was determined using a detection kit (ATP-2-Y, Suzhou Comin biotechnology Co., Ltd., China). Sucrose was extracted with 80% ethyl alcohol (v/v) and then tested using the resorcinol colourimetric method [34]. Starch content was measured with the anthracenone-H_2SO_4 method [35].

2.8 Gene expression

Tomato seedlings were treated with fosthiazate at the dosage of 0, 17, 67 and 267 mg/plant for 0.25, 0.5, 1, 2 and 3 d to analyze the dynamic changes in the expression of *Slrbohs* genes, as well as genes encoding antioxidant enzymes and sugar metabolism enzymes. The detailed methodology is described in Text S12.

2.9 Plant hormone levels and osmotic adjustment substances content

The root hormone levels of ethylene (MM-088802), cytokinin (MM-069202), gibberellin (MM-012502), auxin (MM-095302), and abscisic acid (MM-118502) were determined using the ELISA kit (Jiangsu Meimian Industrial Co., Ltd., China) after treated with fosthiazate at the dosage of 0, 17, 67 and 267 mg/plant. The experiments were performed three times with four replicates.

The influence of fosthiazate on proline and soluble protein content of crops were tested (Text 13).

2.10 The influence of the release profile of fosthiazate on the phytotoxicity

2.10.1 Phytotoxicity of fosthiazate-loaded microcapsules (MCs)

According to the results from the aforementioned experiments, the release rate of fosthiazate was modulated to mitigate its phytotoxicity. Consequently, three MCs with different a.i. (10%, 20% and 30%) were prepared using interfacial polymerization method (Text S14). The particle size, morphology, encapsulation efficiency and release kinetics were characterized (Text S15). Phytotoxicity of both technical-grade fosthiazate and formulated products on tomato seedlings were assessed at the dosage of 67 mg/plant, as detailed in Section 2.2. Healthy and necrotic leaf areas were measured daily for 7 d using imageJ software. The experiments were repeated three times with three replications.

2.10.2 The control efficacy of fosthiazate-loaded MCs against RKNs

Field trials were carried out in Apr. 2023 and Sep. 2024 to determine the control efficacy of both the technical grade and formulated fosthiazate against RKNs (Text 16).

2.11 Data analysis

ANOVA (one-way analysis of variance) followed by Turkey's tests was performed to analyze the significant difference among treatments at a significance level of $P<0.05$ (SPSS statistics 26, IBM Corporation, USA).

3. Results and discussion

3.1 Fosthiazate application caused severe phytotoxicity on plants

Fosthiazate, a frequently used nematicides, had been observed to induce varying degrees of phytotoxicity in crop seedlings (Fig. S2), and the specific influence were evaluated under pot experiments. Notably, necrotic leaf edges were the most prominent phytotoxicity symptom, occurring even at the dosage of 2 mg/plant (Fig. 1B). After the

initial 7-d treatment, stem growth was significantly inhibited starting from 8 mg/plant, while root system architecture was notably affected from 17 mg/plant (Fig. 1C-D, S3). Furthermore, fosthiazate's impact on relative water content and activity of leaf/root also differed significantly (Fig. 1E-G). Leaf cell viability under a 267 mg/plant treatment decreased significantly after just 1 d, whereas root activity showed a marked decline from day 5 at the same dosage (Fig. 1F-G). As the application dosage increased, both the net assimilation rate and dry matter accumulation of the plants decreased (Fig. 1H-I). These results suggested that fosthiazate affected the plant roots more slightly and later than leaves, despite being applied via soil treatment; although the root shoot ratio was not significantly different among treatments (Fig. 1I). This performance is commonly observed among highly hydrophilic substances (Fig. S1), including ammonium fertilizers and salinity as well. However, this type of phytotoxicity has predominantly been explained through ionic balance and biochemical responses [36][37]. While it is well established that highly water-soluble chemicals absorbed by roots can migrate to leaves [38], limited research has directly linked chemical distribution patterns to the mechanisms of phytotoxicity.

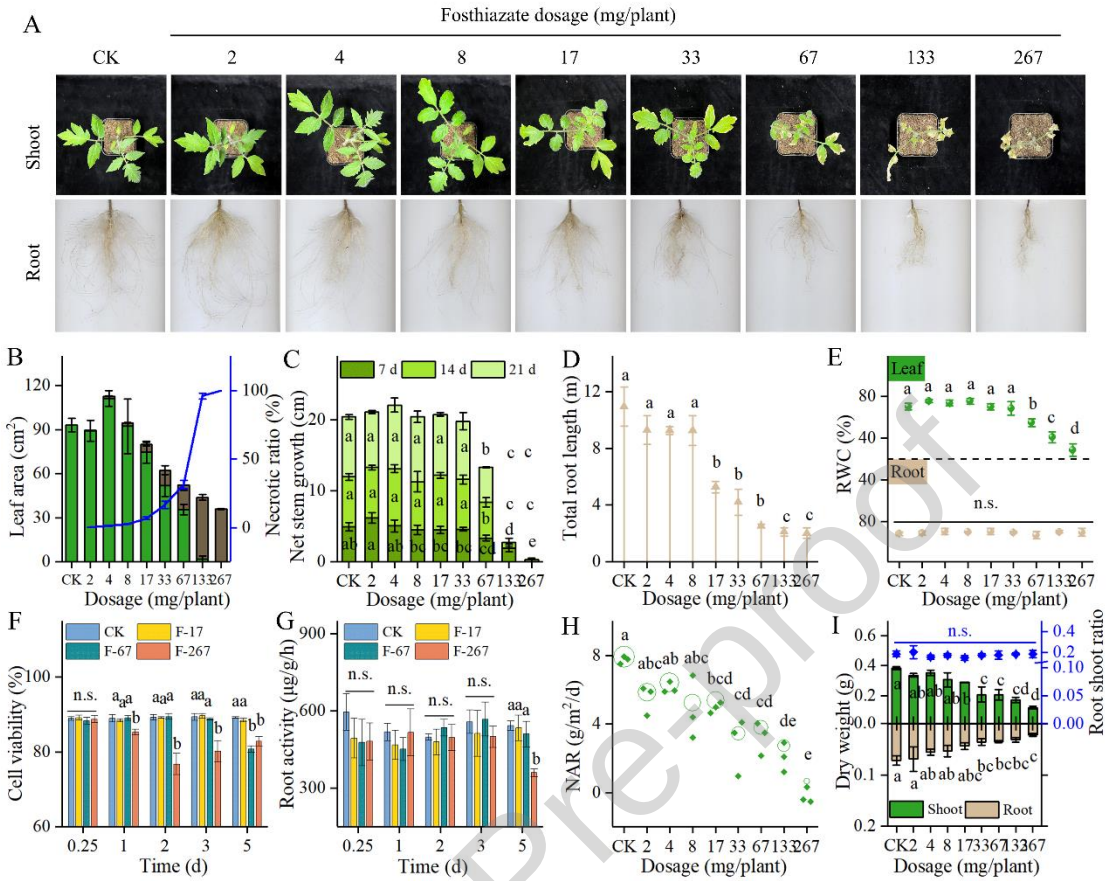


Fig. 1 The phytotoxicity of fosthiazate on tomato seedlings. A, phytotoxicity symptoms on tomato shoots and roots; B, ratio of necrotic and healthy leaf area; C, net stem growth measured every 7 d; D, total root length; E, relative water content in leaves and roots; F, cell viability of tomato leaves assessed using evans blue; G, root activity determined by the TTC reduction method; H, net assimilation rate (NAR); I, root shoot ratio calculated based on dry weights of roots and shoots.

3.2 Fosthiazate accumulated to the leaf by transpiration pull

The absorption, uptake and translocation behaviors of pesticides in plants are influenced by multiple factors, including physicochemical properties, crop species, cultivation environments and subcellular distributions [18][19] [39]. Briefly,

fosthiazate dissolved in the soil solution is absorbed into root cell sap and subsequently transferred to xylem via both symplast and apoplast pathways. It is then translocated acropetally by transpirational pull to leaves. As water evaporates, the involatile fosthiazate remains, accumulates and induce phytotoxicity (Fig. 2A). The volatilization of dichlorvos likely explains the absence of phytotoxicity symptoms at the leaf edges (Fig. S1).

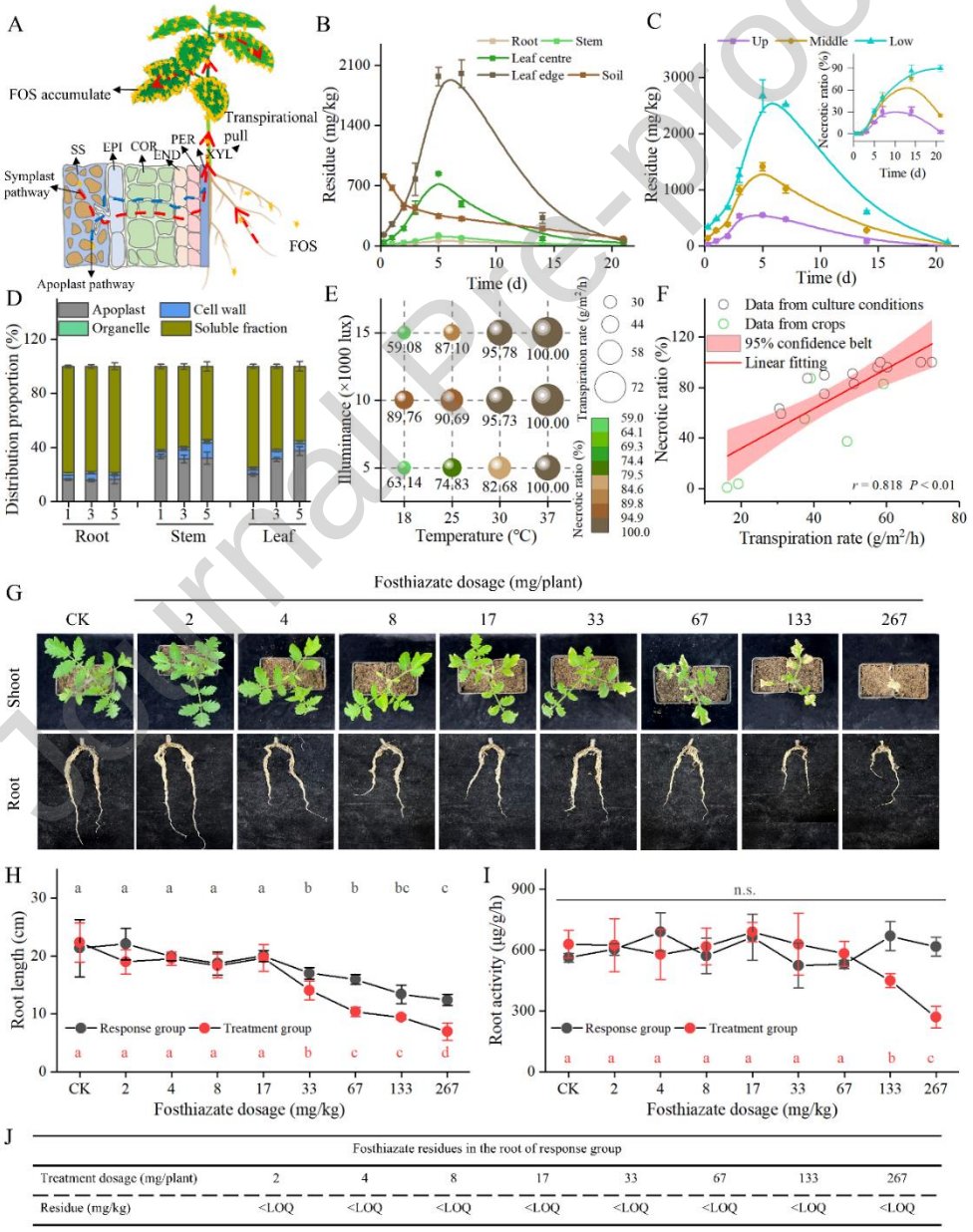


Fig. 2 Schematic representation of tomato seedlings absorbing fosthiazate via symplast

and apoplast pathway, followed by translocation to the leaf edge driven by transpirational pull (A); B, enrichment kinetics of fosthiazate at a dosage of 67 mg/plant; C, fosthiazate residues and associated phytotoxicity across different spatial dimensions; D, subcellular distribution of fosthiazate; E-F, correlation between plant transpiration and fosthiazate phytotoxicity; G, fosthiazate phytotoxicity following split root treatment; H-I, root length and root activity in response group (right) and treatment group (left); J, fosthiazate residues in roots of the response group, LOQ was 0.005 mg/kg.

3.2.1 Fosthiazate enrichment level correlated to the phytotoxicity symptom

Three dosages of fosthiazate (17, 67 and 267 mg/plant), corresponding to mild, moderate, and severe phytotoxicity, were selected to evaluate its behavior in tomato plants (Fig. S4A). The concentrations of fosthiazate in tomato plants were found to be dose-dependence, consistent with the phytotoxicity degrees. Within a specific period, the residue levels in different plant parts were nearly proportional to the applied fosthiazate dosage (Fig. 2B, S4B). Higher exposure dosage led to higher accumulation within the plant-soil system. For instance, after 6 h of treatment, the residues in the leaf edges were 34.24 ± 2.46, 130.04 ± 7.73 and 499.87 ± 52.39 mg/kg for the three dosages, respectively, with no significant difference in BCF and TF_{leaf} values (Fig. S5). However, after 5 d, the residues in leaf edges were 498.80 ± 9.52, 1969.58 ± 106.17 and 2991.03 ± 193.59 mg/kg, respectively, with a reduced BCF and TF_{leaf} values in F-267 group. The BCF values for F-267 group began to decrease from day 3, due to necrotic leaves

obstructing fosthiazate conduction [40]. The distribution and conduction of fosthiazate in tomato plants followed a consistent pattern: leaf edge > leaf centre > stem > root, which aligns with the observed phytotoxicity symptoms (Fig. 2C-E). As illustrated in Fig. S5, no significant difference was observed among the RCF values of the three treatments, indicating that fosthiazate concentration in plant roots was relatively stable across different treatment. Correspondingly, the root activity was not influenced, allowing for continuous absorption of fosthiazate, which might explain why the split root treatment did not relieve phytotoxicity (Fig. 2G). The low molecular weight (283.40 g/mol) and lipophilic nature of fosthiazate facilitate its passage through the Casparian strip into xylem, then translocating upwardly [40]. Fosthiazate accumulation patterns in leaves varied with spatial dimensions, following the trend: upper leaves < middle leaves < lower leaves (Fig. 2C). This distribution may be due to the fact that water is primarily supplied to the lower mature leaves to maintain carbon supply, xylem flow in the upper young leaves was relatively smaller. Accordingly, the necrotic areas were most pronounced in the lower leaves. These findings indicated that fosthiazate content is a critical factor influencing phytotoxicity differences among application dosages, plant organs, and leaf canopies. Similar to fosthiazate, the upward translocation of other chemicals with high hydrophilicity also plays a fundamental role in their phytotoxic effects; for instance, salt stress causes necrosis and salting out at the leaf edges.

3.2.2 Roots absorbed fosthiazate through apoplast and symplast pathway

After being absorbed into the roots, pesticide may distribute into different component

due to their varying interactions with subcellular fractions. Research on fosthiazate's subcellular distribution contributed to comprehending its mechanisms of translocation, accumulation and metabolism in tomato seedlings [41]. Under a 67 mg/plant treatment, leaves appeared healthy on day 1, wilted on day 3, and become necrotic by day 5. Thereafter, these three time points were selected to monitor the dynamic distribution of fosthiazate in the apoplasts, cell walls, organelles and soluble fractions. The apoplasts are the extracellular zone; cell walls are primarily composed of lipids, polysaccharides, and fibers; organelles consist mainly of proteins and organic substances; and soluble fractions contains vacuole solution and matrix [42]. Fosthiazate was distributed across these four components (Fig. 2D), suggesting it could transport across membranes via aquaporins, with cells (vacuoles) serving as the main storage sites. In tomato roots, stems, and leaves, the proportion of fosthiazate in the apoplast (15.76-37.39%) was lower than that in the symplast (62.61-84.24%) (Fig. S6A), in agreement with the subcellular distribution of hydrophilic neonicotinoids [16][43]. Although the specific distribution proportions varied among different plant tissues, fosthiazate generally exhibited the lowest distribution ratio in organelles (1.43-1.99%) and the highest in soluble fractions (54.49-78.63%). Importantly, it should be noted that the presence of concentration does not necessarily imply accumulation, fosthiazate was taken up through symplast pathway mainly, as the apoplast pathway was the primary route for water transport. Just like fosthiazate was undoubtedly absorbed by roots, yet its residues were detected at relatively lower levels. The high efficiency and low resistance of the apoplast pathway contribute to its lower residue levels. Conversely, the symplast

pathway, characterized by slower transmission, exhibited a higher retention rate of fosthiazate. Previous studies have indicated that chemicals with higher hydrophilicity mainly resided in cell sap and demonstrate stronger translocation abilities [44], similar to our results (Fig. S6C). However, the dynamic distribution ratios of the four components in roots, stems, and leaves become more complex when phytotoxicity occurs. In stem, fosthiazate distributed more in cell wall. Meanwhile, as the leaf cell membrane broken down, the pesticide content increased in the apoplasts (Fig. 2D).

3.2.3 Transpiration flow was the key driver for fosthiazate enrichment

Except for physicochemical properties of chemical, transpirational flow is a critical factor influencing plant uptake and translocation (Fig. S7), since the entry of most chemicals into plants is a passive process [45]. The effect of plant transpiration on phytotoxicity was evaluated through different methods. Exogenous ABA application delayed and mitigated fosthiazate phytotoxicity by closing stomata and reducing plant transpiration (Fig. S8). The transpiration rate of tomato seedlings was passively reduced due to leaf wilting after treated with fosthiazate for 30 h. This contrasts with previous results suggesting that transpiration reduced actively upon exposure to organic chemicals to limit the entry of exogenous toxicants [46]. It could be inferred that fosthiazate might disturb stomatal regulation [47], that needs further exploration. Artificially regulating transpiration significantly altered fosthiazate phytotoxicity (P<0.01, Fig. 2E-F). These results demonstrated a positive correlation between fosthiazate phytotoxicity, accumulation, and transpiration. However, stomata are distributed throughout the leaf, while fosthiazate accumulates predominantly at the leaf

edges, suggesting that other factors contribute to this accumulation behavior. Hydathodes, which are directly correlated with guttation, are mainly located at the leaf apexs and edges, consistent with observed phytotoxicity symptoms. Thus, pesticide accumulation in leaf was influenced by transpiration, whereas the higher concentration at the leaf edges could be attributed to guttation, a hypothesis that requires further investigation. Split-root experiments showed that fosthiazate does not translocate downward but still inhibits root growth to some extent (Fig. 2G-J). This inhibition may be attributed to carbon deficiency caused by cellular dysfunction.

3.3 Fosthiazate induced a disruption of ROS homeostasis

As pesticide enriched, they affect various physiological and biochemical processes as well as metabolic pathways. The turbulence in ROS production and the antioxidant enzyme system led to a burst of ROS (Fig. 3A), attacking the membrane structure of cell and organelle from fluid to rigid, and destroying the cell integrity and function [48]. Necrosis and apoptosis are common forms of cell death. As expected, with increased fosthiazate exposure dosage and time, the H_2O_2, $O_2^{\cdot-}$, MDA content, and the membrane permeability increased (Fig. 3B-F). ROS was primarily generated from chloroplasts (photosynthetic electron transport chain), mitochondria (respiratory chain), peroxisomes (e.g., acyl-coenzyme), plasmalemma [e.g., respiratory burst oxidase homologues (RBOHs)] and apoplast (e.g., amine oxidase) [49]. In this study, overexpression of *SlrbohB* might have promoted ROS production (Fig. 3D). Following fosthiazate treatment, the activities of key antioxidant enzyme (i.e., SOD, POD, CAT and APX) exhibited complex patterns. POD was the primary enzyme responding to

fosthiazate accumulation, while APX was the most sensitive (Fig. 3G-K). SOD in F-17 and F-67 group initially activated but then stabilized at the normal level, whereas in the F-267 group, it was inhibited throughout the entire process (Fig. 3G). The results were consistent with the relative gene expression levels. The expression of *Slpod5* and the *Sltpx2* (a POD precursor) was up regulated (Fig. 3K). Therefore, ROS accumulation might be attributed to high ROS production and low efficiency of enzymatic scavenging system.

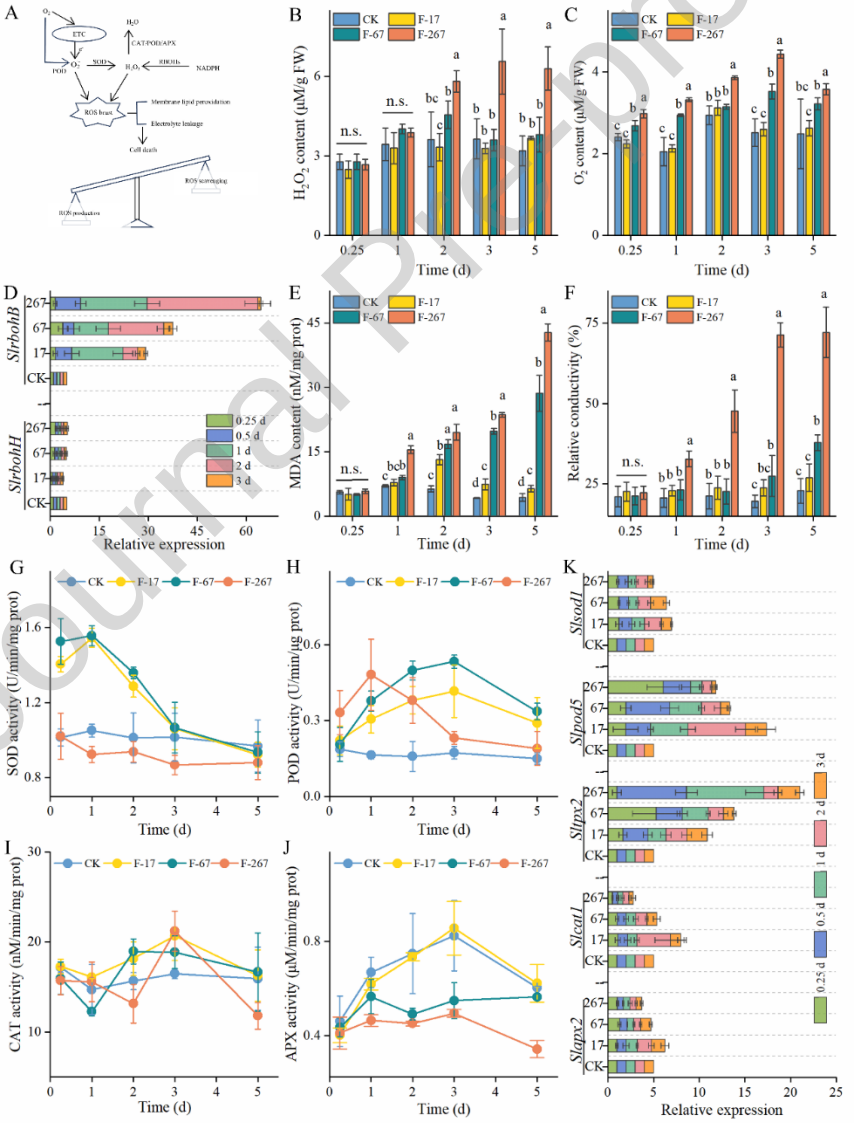


Fig. 3 Schematic diagram illustrating the influence of fosthiazate on ROS production

and scavenging (A). B, ROS content; C, relative expression of ROS production gene of *SlrbohB* and *SlrbohH*; E-F, MDA content and relative conductivity; G-J, activities of SOD (superoxide dismutase), POD (peroxidase), CAT (catalase) and APX (ascorbate peroxidase), K, the relative gene expression induced by fosthiazate. Values were mean ± SD, with letters indicating significant differences among treatments ($P < 0.05$).

3.4 Fosthiazate affected plant photosynthesis

Photosynthesis is the central metabolic pathway for generating ATP and carbohydrates, both of which are necessary for plant growth; however, this process is highly sensitive to multiple stresses [50]. The ROS burst could also attack the chloroplast metabolism. After fosthiazate accumulation, the process of photosynthetic carbon assimilation was broken, and the capacity for sucrose transport from source tissues to sink tissues (root) was decreased, then inhibiting the root growth (Fig. 4A). At the early stage, osmotic adjustment substances of proline, soluble proteins and sucrose content increased to equilibrate water potential, and plasmolysis was occurred (Fig. 4B, Fig. S9). It suggested a higher concentration of fosthiazate in extracellular fluid, consistent with the former point of view in Section 3.2.2. The relative expression of *Slsps1* was increased after exposure to fosthiazate at 267 mg/plant for 6 h, and subsequently decreased (Fig. 4C). The down-regulation of the sucrose transport gene *Slsut4* and *Slsweet11α*, and metabolism gene (*Slinvan4* and *Slinvvr1*) might also contribute to sucrose accumulation (Fig. 4C, S10). As enzyme activities were generally inhibited, sucrose content was decreased and stabilized (Fig. 4B-C). Sucrose not only

functions as a photosynthate but also acts as a signaling substance. Its increase gives negative feedback to decrease photosynthesis [51]. The interference with the sucrose transport to roots (the main sink during vegetative growth of plant seedlings) would inhibit root growth and development (Fig. S11). Moreover, the lower mature leaves served as the primary carbon supply source, their high necrotic ratio may exacerbate carbon starvation in the roots (Fig. 2C). Besides, disturbances in endogenous plant hormone disturbance might also be a critical factor contributing to the root inhibition (Fig. S12).

Fosthiazate enrichment caused wilted leaf edges, the damage of chloroplast at the connection of healthy and wilted part was characterized by irregular envelope (EN), swollen starch grain (SG), disorganized grana (GR) and dissolved plastoglobules (P) (Fig. 4D). Photosynthetic parameters, such as net photosynthetic rate (Pn), maximum photochemical efficiency (Fv/Fm), overall functional activity of PSI, PSII and the electron transport chain (PI total), as well as photosynthetic pigments, were significantly decreased in F-67 and F-267 treatment compared to the control (Fig. 4E-F). Additionally, Rubisco activity was negatively influenced by fosthiazate, and there was no doubt about the decreased ATP content (Fig. 4G-H). Sucrose and starch were the main photosynthates. The results shown in Fig. 4I suggested that starch content was also reduced, although the relative expression of *SlagpL3* that encoding starch synthesis enzyme was up-regulated. It can be explained by the decrease of the number of chloroplasts (Fig. S9A).

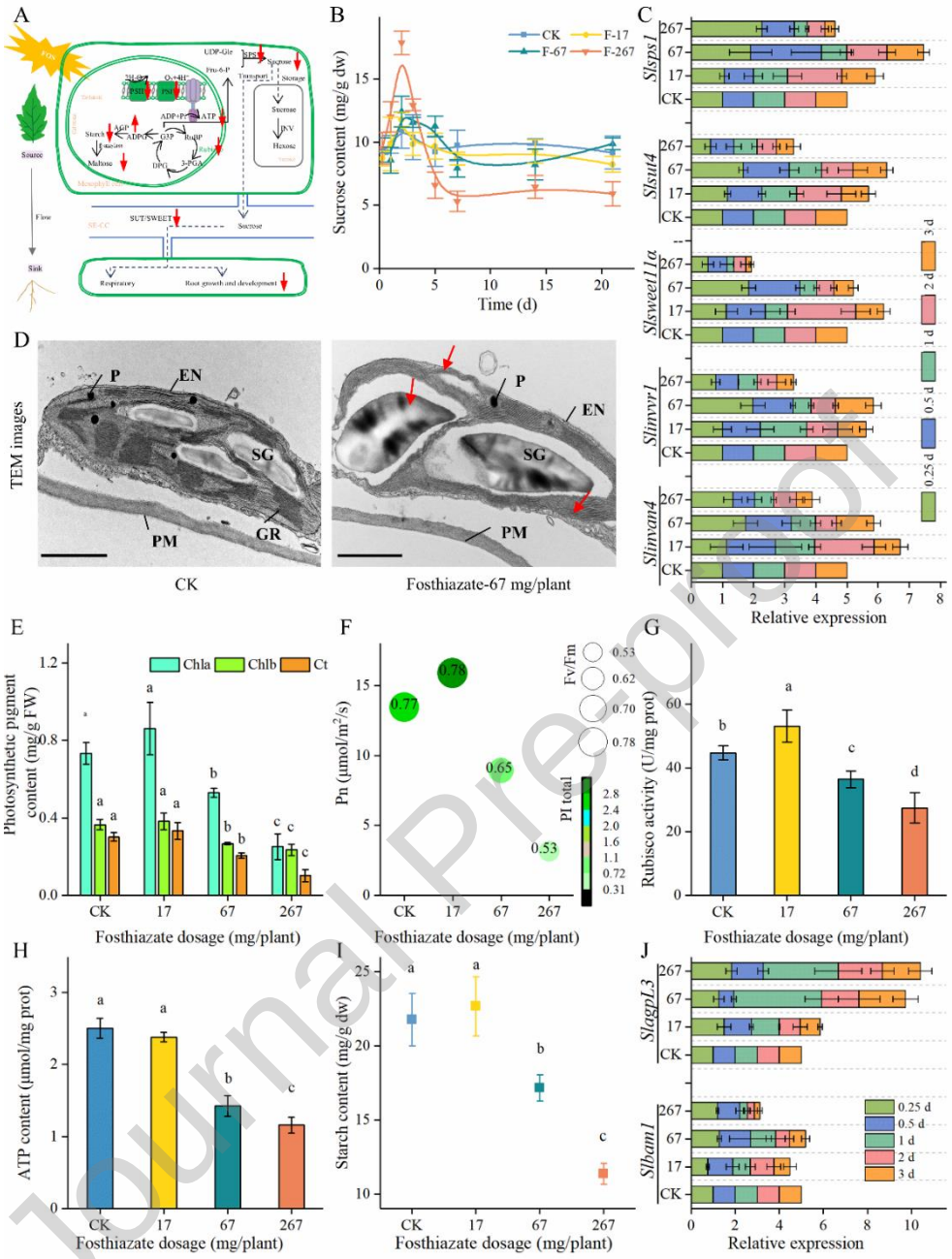


Fig. 4 Schematic of the response of plant photosynthesis to fosthiazate (A). B-C, dynamic change in sucrose content and the relative expression levels of genes related to sucrose synthesis, transport, and metabolism; D, the observation of chloroplast structure by TEM at 30000× magnification. The difference was marked by red arrowheads; E, photosynthetic pigments F, photosynthetic parameters including net photosynthetic rate (Pn), maximum photochemical efficiency (Fv/Fm), overall

functional activity of PSI, PSII and electron transport chain (PI total); the inhibition on Rubisco activity (G) and ATP content (H); I-J, the starch content and relative expression of its synthesis and degradation genes. PSII, photosystem II; PSI, photosystem I; ADP, adenosine diphosphate; ATP, adenosine triphosphate; G3P, glyceraldehyde-3-phosphate; RuBP, ribulose 1,5-bisphosphate; 3-PGA, 3-phosphoglyceric acid; DPG, 1,3-diphosphoglycerate; ADPG, adenosine diphosphate glucose; AGP, glucose-1-phosphate adenylyltransferase; UDP-Gle, uridine diphosphate-glucose; Fru-6-P, fructose-6-phosphate; SPS, sucrose phosphate synthase; INV, invertase; SUT, sucrose transport protein; SE-CC, sieve element-companion cell complex; PM, plasma membrane; P, plastoglobules; EN, envelope; SG, starch grain; GR, granum. Values were mean ± SD. The letters above indicated the significant difference at different treatment ($P < 0.05$).

3.5 The delay of fosthiazate release rate alleviated the phytotoxicity

Pesticide MCs are slow- and controlled-release formulations characterized by a core-shell structure, produced by encapsulating pesticides using natural or synthetic high-molecular materials as the capsule wall through physical, chemical, biological or physicochemical methods [52]. As we discussed above, the ultra-high concentration enrichment of fosthiazate at leaf edge was the main mechanism responsible for the phytotoxicity. Then, the release rate of fosthiazate was considered to be delayed to alleviate the phytotoxicity (Fig. 5A). The particle sizes of the three fosthiazate-loaded MCs were similar (Fig. S13), that helped eliminate the influence of particle sizes on the

MCs performance. As the active ingredient concentration of MCs decreased, the rigidity and encapsulation rate of the samples increased (Fig. S14-15). The step-down release rate alleviated the phytotoxicity development (Fig. 5B-D), but not affected the excellent control efficacy against RKNs. The control efficacy of the TC and three MCs were not significantly different ($P < 0.05$, Fig. 5E, S16). The MC process utilizes simple and convenient technologies and is relatively lower costing than MC granules or powder, making it suitable for the development of water-based formulations [52]. Compared with traditional preparations, the release rate of chemicals within the capsule shell can be precisely controlled, thereby reducing the risk of excessive exposure to non-target organisms, extending the persistence period, decreasing the frequency and dosage of application, and significantly improving pesticide utilization rates [53]. This is particularly important for fosthiazate, which exhibits strong photolysis, high ecotoxicity risks, and non-persistent soil degradation (PPDB database). Currently, the number of MC registrations for RKN disease control has been increasing annually, reaching an 8.63% registration proportion based on ICAMA 2024 data. Thus, MCs hold significant potential for large-scale agricultural production. Except for the MCs, fosthiazate might also be prepared with the gel that lock the chemical, thereby regulating the release rate and reducing its concentration in soil solution [54].

According to the results based on the phytotoxicity influence factor, the necrotic leaf ratio was positively correlated with silt and organic content (Fig. S17A) [55], likely due to the enhanced adsorption capacity of organic matter. Alkaline condition notably decreased phytotoxicity by inhibiting root biological functions and accelerating

fosthiazate degradation (Fig. S17B) [56]. However, relying on increased chemical adsorption or degradation to mitigate phytotoxicity could compromise disease control efficacy. As the mixed soil amount increases, fosthiazate concentration in the soil solution and the phytotoxicity degree decrease (Fig. S17C). Since RKNs are mainly distributed in the 0-20 cm soil layer, applying fosthiazate through soil mixing can alleviate phytotoxicity while saving labor costs. Nevertheless, decrease of pesticide concentration per unit soil volume may reduce its efficacy. Increasing fosthiazate dosage to ensure effective control may pose threats to non-target soil organisms [10], necessitating further research to achieve an optimal balance.

Additionally, compounding fosthiazate with other highly effective nematicides were put forward as a mitigation measures, such as fluopyram. On one hand, fluopyram was also an excellent nematicide but has phytotoxic potential and a high cost [57]. On the other hand, the recommended fosthiazate dosage (67 mg/plant) was relatively high, compared with fluopyram of 15 mg/plant. Thereafter, combination of fosthiazate and fluopyram perhaps could alleviate phytotoxicity, save application costs, and lower the fosthiazate application dosage. However, since fosthiazate take actions by AchR activation, further tests are required to determine whether the mixture exhibits additive or synergistic effects. Of course, most fosthiazate is wasted by translocating to plant shoots, therefore, structural modification of fosthiazate for decreasing its hydrophilicity while maintaining the nematocidal activity as possible could be the optimal approach.

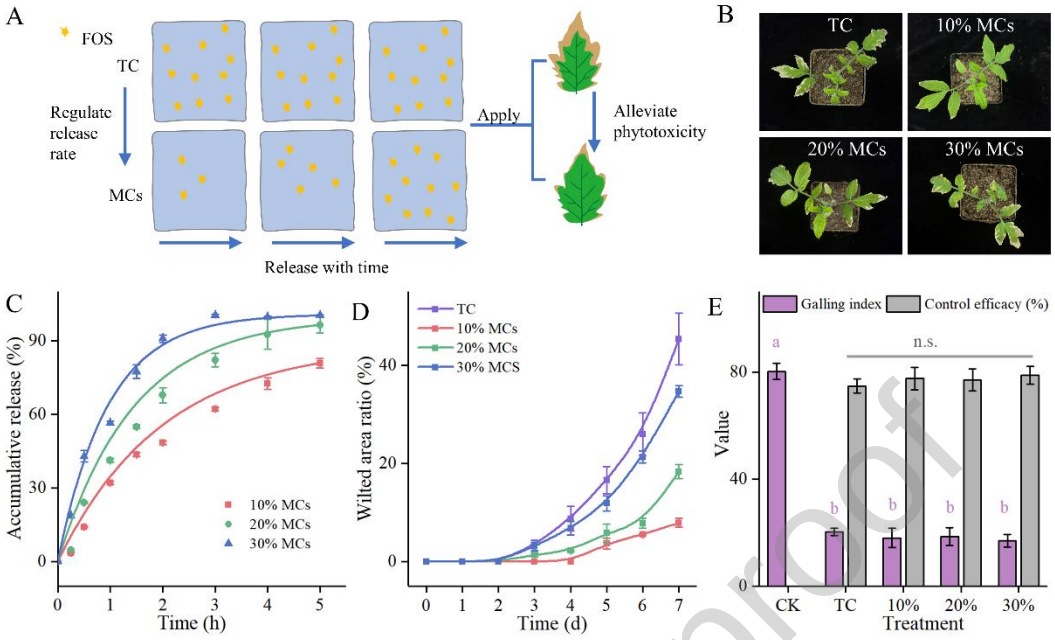


Fig. 5 Schematic of the fosthiazate phytotoxicity alleviation by release rate regulation (A). B, the phytotoxicity of TC (technical grade fosthiazate), 10%, 20% and 30% MCs; C, the accumulative release of the three MCs; D, the phytotoxicity development after applied with TC and the three MCs; E, the gilling index and control efficacy of TC, 10%, 20% and 30% MCs under the field trials conducted in Apr. 2023.

4. Conclusion

In conclusion, fosthiazate, due to its high hydrophilicity, can be absorbed by both apoplast and symplast pathway, and translocated readily within plants. The ultra-high fosthiazate accumulation at leaf edge make it wilted and necrotic. Along with the enrichment, the imbalance ROS production and scavenging ultimately caused photosynthetic damage. The impaired transport of photosynthates reduced the carbon source available for root growth and development. Consequently, the overall growth of crop seedlings was inhibited. The preparation of controlled-release particles was proved

to alleviate this type of phytotoxicity efficiently. This study offers novel insights into the integrated research on the environmental behavior and toxic effects of pesticides, enriches the theory of source-sink interaction under pesticide stress, provides a scientific solution to significant challenge of balancing disease control and phytotoxicity of hydrophilic pesticides such as fosthiazate, thereby establishing a robust theoretical foundation for advancing sustainable agricultural development. Despite fosthiazate being registered to control soil nematodes, most of chemicals transferred to plant shoots, leading to inefficient application. Therefore, future pesticide formulations targeting soil nematodes, pests, or pathogens should focus on modulating their hydrophilicity to enhance application efficiency.

Acknowledgements

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- Fosthiazate caused severe phytotoxicity against plant seedlings.
- Fosthiazate accumulation and distribution led to phytotoxicity difference.
- Fosthiazate caused plasmolysis, ROS balance and chloroplast metabolism.
- The regulation of fosthiazate release rate alleviated the phytotoxicity.