Research Article

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Effects of soil treated fungicide fluopimomide on tomato (Solanum lycopersicum L.) disease control and plant growth

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Abstract: Fluopimomide is a novel acid amide fungicide registered for the control of many plant pathogens. In the present study, the effects of soil-treated fluopimomide on soil micro biomass, disease incidence, plant growth, soil enzyme activity, and marketable yield of tomato (Solanum lycopersicum L.) were investigated via field trial. In addition, the application prospect in China was also evaluated. In the experiment, five treatments with three replications and a randomized complete block design were followed. The treatments were: furrow application of fluopimomide (25% suspension concentrate, SC) at the dosage of 375, 750, and 1,500 g ha$^{-1}$, which was recommended, double recommended, and quadruple recommended dosages, respectively. Besides, common control fungicide fluopicolide (5% SC) furrow was applied at recommended application dosages of 750 mL ha$^{-1}$, and a non-treated control was also undertaken. Results indicated that fluopimomide exhibited no effects on the amount of soil bacteria and actinomycetes, and its inhibition effect on fungi amount could be recovered at 60 days after treatment (DAT). With the recommended application dosage, fluopimomide could efficiently reduce the number of plant pathogens in soil by 79.56–88.24%, significantly reduce the disease incidences in tomato plants by 80.00–88.24%, and improve plant height by 13.25–24.05% and marketable yield by 16.88%. Furthermore, soil enzymes exhibited a complex response to fluopimomide, and AOB and nifH gene copy numbers were increased by the double and quadruple recommended dosage of fluopimomide. Based on the above results, fluopimomide could be recommended as an efficient fungicide for the tomato field.

Keywords: fluopimomide, tomato, soil-borne disease, soil enzyme, micro biomass

1 Introduction

Tomato (Solanum lycopersicum L.) is an important vegetable crop [1]. In China, the annual tomato production reached 50 million MT. However, due to long-term continuous cropping and mismanagement, tomato diseases have been increasing over time. The most common diseases are: wilt, blight, and gray mold, which are caused by Fusarium oxysporum, Phytophthora spp., and Botrytis cinerea, respectively [2–4]. Yield loss caused by above diseases reached 10–30% in general plots, and 50% in serious plots. Fungicide application is usually one of the main components in tomato production.

Fluopicolide effectively suppressed sporangium formation, zoospore germination, and mycelial growth of Phytophthora pathogen [5], and could significantly reduce the incidence of watermelon fruit rotting in the Carolinas [6]. In China, its preparation named Yinfali Suspension has been widely used for blight control of pepper (Capsicum frutescense L.), potato (Solanum tuberosum L.), tomato (S. lycopersicum), cucumber (Cucumis sativus L.), and other vegetables, its joint application with bio-fungicides could control cucumber diseases more efficiently.

Fluopimomide is a new fluorinated benzamide fungicide developed by Shandong United Pesticide Industry Co. Ltd, China in 2010, it has a similar structure to fluopicolide [7] (Figure 1). Its chemical name is N-(3-
2 Materials and methods

2.1 Chemicals and reagents

Flupiconazole (purity = 98%) and fluopicolide (purity = 96%) were both provided by Shandong United Pesticide Industry Co. Ltd, China.

2.2 Field experiment design

Field experiments were arranged in the autumn cropping seasons of 2018–2019 and 2019–2020 in a commercial greenhouse near Fang county, Tai’an, China (N35°58’13”, E117°12′13”). Six-week-old “jinpeng” tomato seedlings were transplanted on August 23, 2018 and August 18, 2019.

The soil physicochemical properties of the experimental sites were: sand 29.17%, silt 70.49%, clay 0.34%, organic matter 18.95 g kg⁻¹ soil, and pH 7.1. This site suffered heavily from soil-borne diseases, such as F. oxysporum, Phytophthora, and B. cinerea. In conventional farm operations, compound fertilizer of 15N–15P₂O₅–15K₂O was broadcast applied at the dosage of 750 kg ha⁻¹ as base.

Five treatments with 3 replicates in each were arranged in a random block design, where 25 seedlings were planted per plot (7.20 m × 0.75 m). The treatment programs were: (a) laboratory-made fluopimomide (25% SC, a.i.) furrow-application was done at a dosage of 375 g ha⁻¹, it is the field recommended application dose; (b) fluopimomide (25% SC, a.i.) furrow-application dose of 750 g ha⁻¹; (c) fluopimomide (25% SC, a.i.) furrow-application dose of 1,500 g ha⁻¹; (d) laboratory-made fluopicolide (5% SC, a.i.) furrow-application dose of 750 mL ha⁻¹, it is the field recommended dose; and (e) non-treated control. Evaluation of 2 and 4 folds of recommended application dosage is necessary for novel pesticide crop safety verification.

2.3 Sampling

20, 40, and 60 DAT, a soil column cylinder with a diameter of 5 cm was used to sample soil nearby tomato plants at a depth of 0–10 cm, and random 20 points were included for each plot. Each collected soil sample (0.5–1.0 kg) was sieved (1 mm mesh) and separated into two parts. Part one was stored at 4°C for the microbiological and enzyme activities test, and the test was...
completed within less than a week. Part two was treated with liquid nitrogen and stored at −80°C for DNA extraction.

2.4 Enumeration of microbial population

The amounts of fungi, bacteria, and actinomycetes were evaluated using the serial ten-fold dilution (10−2–10−7) method [11], where 45 mL of sterile water mixed with 5 g soil was regarded as 10−1 dilution. Fungi, including yeast, were counted on Martin’s medium, with pH 6 and containing 150 mg L−1 streptomycin. Bacteria were counted on a selective medium prepared with glucose 1 g L−1, proteose peptone 3 g L−1, yeast extract 1 g L−1, K2PO4 1 g L−1, agar 15 g L−1, and cycloheximide 100 mg L−1. Actinomycetes were counted on improved GAO No.1 medium with pH 7.4–7.6 [12]. The number of F. oxysporum in soil was counted on the PCNB selective medium containing KH2PO4 1.0 g L−1, MgSO4·7H2O 0.5 g L−1, peptone 5.0 g L−1, agar 20.0 g L−1, with streptomycin 0.30 g L−1 and 75% quintozene wettable powder 1.0 g L−1 added before usage [13]. The amount of Phytophthora was measured in a PDA medium with 3-hydroxy-5-methylisoxazole, benomyl, nyasatin, pentachloronitrobenzen, rifampicin, and ampicillin added at the concentration of 100, 10, 25, 25, 10, and 50 mg L−1, respectively [14]. The B. cinerea selective medium included basic components (NaNO3 1.0 g L−1, KCl 0.15 g L−1, K2HPO4 1.2 g L−1, MgSO4·7H2O 0.5 g L−1, glucose 20.0 g L−1, agar 25.0 g L−1) as well as fungicide components (pentachloronitrobenzen 0.012 g L−1, penicillin 0.05 g L−1, chloramphenicol 0.05 g L−1, sulphate streptomycin 0.05 g L−1, CuSO4 2.2 g L−1, and Rubigan 0.01 mL L−1) added after sterilization [15]. Dilution ratios were chosen properly according to their present amounts in the soil. From the diluted solution, 100 μL was smeared on the various medium plates, each with five replications. After cultivation for 5 days at 25°C, the plate with colony amounts between 10 and 100 was used for the eventual calculation of microbe numbers and population densities. The data were reported as cfu g−1.

2.5 Disease assessment in plants

After the transplant, incidences of wilt, blight, and gray mold of ten random plants in each plot were recorded at intervals of 20, 40, and 60 days. Levels of disease severity were assessed by visually estimating the percentage of diseased surface, and graded as: 0 = 0%, 1 = 1–9%, 2 = 10–24%, 3 = 25–49%, 4 = >50% of surface affected. Wilt was calculated by spot on stem base, while blight and gray mold were weighted by round and angle spots on leaves, respectively. The disease index was calculated using the following formula:

\[
\text{Disease index} = \frac{\sum (\text{The relative score} \times \text{Plant numbers in the score})}{\text{Total plant numbers} \times 4} \times 100,
\]

2.6 Assays of soil enzymatic activities

Analysis of soil dehydrogenase and urease activity was done according to the methods of Lebrun et al. [16]. Soil phosphatase activity was tested using the method of Wang et al. [17]. The invertase activity was measured following the modified method of Ohshima et al. [18].

2.7 Quantitative PCR analysis of soil nitrogen-related genes

The qPCR analysis of AOA, AOB, nirH, and nirS was performed with ABI 7500 Real-Time PCR System and 7500 System Software-SDS 2.2 by absolute quantification method, and the primer information is shown in Table 1. The reaction system consisted of 2 × SuperReal PreMix 10 μL, primers 0.3 μmol L−1, and cDNA template 2.5 μL. The procedure of qPCR was performed at 95°C for 15 min and followed by 40 cycles of 95°C for 10 s, annealing (53°C for AOA, 56°C for AOB, 58°C for nirH and nirS) for 20 s, and 72°C for 1 min.

| Gene | Primer sequence |
|------|-----------------|
| AOA  | L: 5′-CTAAGAGCTTGCTGCTAGAC-3′ | R: 5′-CGGCCATCCATCTGTATGT-3′ |
| AOB  | L: 5′-GGGTTRTTCTAGTTGTTTGT-3′ | R: 5′-CCCCTGKSAAAAGCCCTGCT-3′ |
| nirH | L: 5′-AAAAGYGYGGYATCGGYYARTCACCAC-3′ | R: 5′-TGGATTGCGCGCTTACATC-3′ |
| nirS | L: 5′-CCCTATTCATGGGCGC-3′ | R: 5′-CGTTGAACCTTCCGGT-3′ |
2.8 Influence of fluopimomide on tomato plants

20, 40, and 60 DAT, the plant height of randomly chosen ten plants in each plot was measured. Fruits were harvested twice (85 and 120 DAT) when they were mature, and the marketable yield was calculated.

2.9 Statistical analysis

As there were no significant differences between experiments over the 2 years, data from the two experiments were combined for analysis and interpretation. The data were analyzed statistically by the Duncan test with a significance level of \( p < 0.05 \).

3 Results and discussion

3.1 Effect of fluopimomide on the abundance of fungi, bacteria, and actinomycetes

Soil microbes are basic components of soil ecology and are highly sensitive to environmental changes [19]. The diversity and abundance of soil microbial communities are the important indices for gaining knowledge of modern soil microbiology. Still, it is the main indicator of risk assessment for pesticide application in agricultural fields [20]. So, in the present study, the application of novel fungicide fluopimomide required tests to determine the effect on the soil environment. As shown in Table 2, under the concentration of 1, 2, and 4 folds of field recommended dosage, fungicide fluopimomide reduced the amounts of fungi significantly \( (p < 0.05) \), while it showed no influence on bacteria and actinomycetes. This result falls following Ji et al., who had reported the excellent compatibility of fluopimomide with Bacillus. 20, 40, and 60 DAT, the amounts of fungi treated by fluopimomide increased gradually with the time-lapse and recovered to the control level at 60 DAT under the recommended dosages. However, the treatments with 2- and 4-fold still inhibited the amount of fungal population. The control fungicide fluopicolide exhibited similar effects with fluopimomide on the amounts of fungi, bacteria, and actinomycetes. Overall, fluopimomide had no significant negative effects on soil bacteria and actinomycetes, and the effects on fungal populations could be recovered within 60 days, indicating a less negative effect on soil microbe.

Table 2: Effect of fluopimomide on amounts of the bacteria, fungi and actinomycetes in tomato field

| Treatment           | 20 days          | 40 days          | 60 days          |
|---------------------|------------------|------------------|------------------|
| **Bacteria/(×10^6 cfu g^-1)** |                  |                  |                  |
| Fluopimomide 375    | 1.72 ± 0.13a     | 1.95 ± 0.38a     | 1.30 ± 0.20a     |
| Fluopimomide 750    | 1.68 ± 0.26a     | 1.75 ± 0.35a     | 1.35 ± 0.38a     |
| Fluopimomide 1,500  | 1.70 ± 0.24a     | 1.68 ± 0.26a     | 1.42 ± 0.25a     |
| Fluopicolide 750    | 1.60 ± 0.24a     | 2.12 ± 0.42a     | 1.30 ± 0.25a     |
| Control             | 2.02 ± 0.20a     | 2.22 ± 0.24a     | 1.65 ± 0.17a     |
| **Fungi/(×10^6 cfu g^-1)** |                  |                  |                  |
| Fluopimomide 375    | 2.80 ± 0.38bc    | 3.55 ± 0.26b     | 3.58 ± 0.25ab    |
| Fluopimomide 750    | 2.65 ± 0.26bc    | 3.68 ± 0.17b     | 3.25 ± 0.35bc    |
| Fluopimomide 1,500  | 2.30 ± 0.31c     | 3.30 ± 0.21b     | 3.15 ± 0.15bc    |
| Fluopicolide 750    | 3.15 ± 0.10ab    | 3.72 ± 0.46b     | 4.02 ± 0.35ab    |
| Control             | 3.52 ± 0.45a     | 5.05 ± 0.14a     | 4.32 ± 0.17a     |
| **Actinomycetes/(×10^6 cfu g^-1)** |                  |                  |                  |
| Fluopimomide 375    | 2.20 ± 0.20a     | 2.78 ± 0.22a     | 3.15 ± 0.22a     |
| Fluopimomide 750    | 1.92 ± 0.24a     | 2.40 ± 0.42a     | 2.98 ± 0.38a     |
| Fluopimomide 1,500  | 1.75 ± 0.23a     | 2.52 ± 0.34a     | 3.12 ± 0.29a     |
| Fluopicolide 750    | 1.85 ± 0.23a     | 2.60 ± 0.45a     | 3.08 ± 0.42a     |
| Control             | 2.48 ± 0.25a     | 3.10 ± 0.54a     | 3.60 ± 0.46a     |

† The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level, and the same as follows.

3.2 Effects of fungicide fluopimomide on the amount of soil-borne pathogens

As a greenhouse vegetable, disease management is one of the most essential components of tomato production. The primary soil-borne diseases, wilt (F. oxysporum), blight (P. infestans), and grey mold (B. cinerea), have caused severe yield loss throughout the world [21], and current systematic fungicides are efficient for these disease control [22,23]. Manikandan et al. reported that the high Fusarium gene level in tomato planted soil suffered from wilt [24]. In our assay, the soil application of fungicide fluopimomide has reduced the amounts of three typical soil-borne pathogens, especially B. cinerea and F. oxysporum (the inhibition ratios >80%). As for Phytophthora, fluopimomide exhibited similar efficiency (79.56–89.21%) to fluopicolide (84.64–87.59%). As time passed, the inhibition efficiency of fluopimomide remained constant until 60 DAT. Combined with the results of Table 2, we could conclude that the recovery of fungi
amount in fluopimomide treatment at 60 DAT has nothing to do with the target pathogens. Moreover, one-off soil treatment of fluopimomide could effectively control soil-borne pathogens (Table 3).

### 3.3 Control efficiency of fungicide fluopimomide on tomato seedling diseases

The fluorine atom has four effects: analog, electronic, hindering, and penetration, C–F bond with much higher energy than the C–H, and significantly increases the stability and physiological activity of organic fluorine compounds. In this study, the added four fluorine atoms and a methoxy group in fluopimomide have expanded its fungicidal range. This is consistent with the report of Ji et al., in which fluopimomide was revealed to have excellent efficiency on tomato gray mold [25].

Mulugeta et al. have reported that phosphite could protect tomato against blight but were not effectively under higher disease pressure [26]. In this study, with recommended application dosage, fungicide fluopimomide could significantly reduce the seedling disease incidences of tomato, with inhibition ratios of 80.00, 88.24, and 84.63% for wilt, blight, and gray mold, respectively. Still, when the concentration of fluopimomide doubles or quadruples, the infection of above soil-borne pathogens can be definitely inhibited. In contrast with the novel broad-spectrum fungicide, control fungicide fluopicolide can only inhibit the disease incidence of blight, with the inhibition ratio of 94.12% at the recommended application dosage. The results confirmed the previous indoor toxicity tests and showed that fluopimomide could be recommended as an excellent fungicide for tomato disease management (Table 4).

### 3.4 Effect of fluopimomide on soil enzyme activities

#### 3.4.1 Effect of fluopimomide on soil dehydrogenase activities

Dehydrogenase, representative of soil organism metabolism [27], can transfer hydride groups from a substrate to an acceptor such as NAD⁺. It plays an important role in the organic decomposition process, particularly for bacteria, which are the main ultimate consumers and metabolizers of aromatic compounds, such as pesticides [28,29]. As shown in Table 5, at 20 DAT, recommended dosage of fluopimomide significantly increased soil dehydrogenase activities ($p < 0.05$), while quadruple recommended dosage exhibited a significant inhibition effect.

#### Table 3: Effects of fluopimomide on amounts of soil-borne pathogens in tomato field

| Treatment        | Soil-borne pathogen numbers |
|------------------|----------------------------|
|                  | 20 days        | 40 days        | 60 days        |
|                  |                |                |                |
| F. oxysporum/ cfu g⁻¹ |                |                |                |
| Fluopimomide 375 | 20.50 ± 2.10c  | 20.00 ± 2.34c  | 23.75 ± 2.72c  |
| Fluopimomide 750 | 18.25 ± 2.29c  | 16.00 ± 1.96c  | 19.25 ± 1.38c  |
| Fluopimomide 1,500 | 13.00 ± 2.48c  | 12.00 ± 2.27c  | 12.25 ± 1.65c  |
| Fluopicolide 750 | 85.75 ± 7.63b  | 87.25 ± 3.17b  | 88.00 ± 5.77b  |
| Control          | 102.5 ± 8.04a  | 108.25 ± 3.40a | 120.25 ± 3.54a |
| Phytophthora/ (cfu g⁻¹) |                |                |                |
| Fluopimomide 375 | 14.00 ± 1.87b  | 14.50 ± 1.04b  | 14.50 ± 0.96b  |
| Fluopimomide 750 | 11.00 ± 1.58b  | 11.00 ± 1.08b  | 11.75 ± 1.18bc |
| Fluopimomide 1,500 | 10.50 ± 1.71b  | 8.50 ± 1.06b   | 8.25 ± 1.11c   |
| Fluopicolide 750 | 8.50 ± 0.64b   | 10.75 ± 2.75b  | 11.75 ± 0.85bc |
| Control          | 68.50 ± 4.05a  | 73.25 ± 4.25a  | 76.50 ± 3.43a  |
| B. cinerea/ (cfu g⁻¹) |                |                |                |
| Fluopimomide 375 | 13.25 ± 2.06c  | 12.25 ± 3.30c  | 12.75 ± 2.56c  |
| Fluopimomide 750 | 10.00 ± 1.47c  | 15.00 ± 2.74c  | 11.25 ± 1.93c  |
| Fluopimomide 1,500 | 8.50 ± 1.85c   | 11.25 ± 2.81c  | 12.00 ± 2.58c  |
| Fluopicolide 750 | 40.5 ± 2.60b   | 46.25 ± 3.01b  | 46.25 ± 2.43b  |
| Control          | 77.75 ± 5.16a  | 86.25 ± 3.50a  | 89.25 ± 4.13a  |

The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level, and the same as follows.
At 40 days after treatment, the dehydrogenase activities of soil treated with a quadruple recommended dosage of fluopimomide increased drastically. This may be because of the soil ecosystem alteration with the increasing fungicide concentration, and microorganisms can increase their metabolic activity in response to xenobiotics in the soil [30]. This is in agreement with Monkiedje et al. [31], who had reported a significant inhibition effect of fungicides mefenoxam and metalaxyl on soil dehydrogenase activity. However, Tejada et al. [32] had found a non-significant increase in dehydrogenase activity in pesticide polluted soils. According to Bending et al. [33], the variant responses of soil dehydrogenase activity to fungicides input are determined by soil type and other factors, such as microbial community structure and types of fungicide. At the end of the incubation (60 DAT), the dehydrogenase activities of treated soil recovered to be similar to control, indicating high safety of tested fungicide on soil dehydrogenase.

### 3.4.2 Effects of fluopimomide on soil phosphatase activities

As shown in Table 6, fluopimomide exhibited a significant activation effect on soil phosphatase activities with recommended dosage at 20 DAT. However, when treated with a quadruple dosage, the soil phosphatase activity decreased significantly compared with the control. Still, the inhibition effect of higher fungicide concentrations

### Table 4: Control efficiency of fluopimomide on tomato seedling diseases

| Treatment   | Disease incidences/% | Disease index | Inhibition ratios/% |
|-------------|----------------------|---------------|---------------------|
| **Wilt**    |                      |               |                     |
| Fluopimomide 375 | 5.50 ± 0.25c         | 3.00 ± 0.58c | 80.00               |
| Fluopimomide 750 | 0.00 ± 0.00d         | 0.00 ± 0.00d | 100                 |
| Fluopimomide 1,500 | 0.00 ± 0.00d        | 0.00 ± 0.00d | 100                 |
| Fluopicolide 750 | 22.50 ± 0.25b       | 18.00 ± 0.82b | 18.18               |
| Control     | 27.5 ± 0.48a         | 29.00 ± 3.51a |                     |
| **Blight**  |                      |               |                     |
| Fluopimomide 375 | 5.00 ± 0.29b         | 4.00 ± 0.58b | 88.24               |
| Fluopimomide 750 | 0.00 ± 0.00d         | 0.00 ± 0.00d | 100                 |
| Fluopimomide 1,500 | 0.00 ± 0.00d       | 0.00 ± 0.00d | 100                 |
| Fluopicolide 750 | 2.50 ± 0.25c         | 2.00 ± 0.5c  | 94.12               |
| Control     | 42.50 ± 0.48a        | 36.50 ± 0.91a |                     |
| **Gray mold** |                 |               |                     |
| Fluopimomide 375 | 5.00 ± 0.29c         | 1.50 ± 0.96c | 84.63               |
| Fluopimomide 750 | 0.00 ± 0.00d         | 0.00 ± 0.00d | 100                 |
| Fluopimomide 1,500 | 0.00 ± 0.00d      | 0.00 ± 0.00d | 100                 |
| Fluopicolide 750 | 22.5 ± 0.25b         | 14.50 ± 2.50b | 30.77               |
| Control     | 32.50 ± 0.48a        | 34.00 ± 3.16a |                     |

The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level, and the same as follows.

### Table 5: Effects of fluopimomide on soil dehydrogenase activities (µg g⁻¹)

| Treatment   | Treatment time (DAT) |               |               |
|-------------|----------------------|---------------|---------------|
|             | 20       | 40             | 60             |
| Fluopimomide 375 | 20.778 ± 1.515a | 16.164 ± 1.335b | 15.799 ± 0.803a |
| Fluopimomide 750 | 15.052 ± 1.450b | 18.719 ± 1.891ab | 16.372 ± 1.148a |
| Fluopimomide 1,500 | 11.549 ± 0.727c | 20.935 ± 2.342a | 16.816 ± 2.500a |
| Fluopicolide 750 | 17.815 ± 2.529ab | 17.050 ± 0.642b | 16.590 ± 0.268a |
| Control     | 16.207 ± 1.483b | 15.530 ± 0.667b | 15.764 ± 1.007a |

The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level, and the same as follows.
The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level, and the same as follows.

Table 6: Effects of fluopimomide on soil phosphatase activities (mg g⁻¹)

| Treatment | Treatment time (DAT) |
|-----------|----------------------|
|           | 20                   | 40                   | 60                   |
| Fluopimomide 375 | 0.664 ± 0.037a        | 0.618 ± 0.022a        | 0.594 ± 0.039a        |
| Fluopimomide 750 | 0.503 ± 0.048bc       | 0.649 ± 0.040a        | 0.590 ± 0.018a        |
| Fluopimomide 1,500 | 0.421 ± 0.083c        | 0.534 ± 0.033b        | 0.579 ± 0.020a        |
| Fluopicolide 750 | 0.540 ± 0.015b        | 0.627 ± 0.026a        | 0.578 ± 0.029a        |
| CK         | 0.564 ± 0.029b        | 0.580 ± 0.029ab       | 0.583 ± 0.024a        |

3.4.3 Effects of fluopimomide on soil urease activities

As reported, urease is externalized due to parent cell death and lysis. This enzyme plays an important role in the nitrogen cycle in soils. Its substrate, urea, is incorporated into the soil from fertilizer, animal excreta, or nucleic acids [17]. In this study, with recommended dosages of fluopimomide and fluopicolide, a non-significant increase has been detected. Still, large dosages of fluopimomide could inhibit urease activity to a certain extent (<10%). With passage of time, the effects of fungicides on soil urease activities became lighter and lighter. In previous studies, similar results have been reported. Monkiedje et al. [35] observed a slight inhibitory effect of metalaxyl and prochloraz on urease in the short term. It was speculated that soil microorganisms took fungicides as an energy source. Uyanóz et al. [37] also reported the activation effect of captan, quintozene, and propamocarb hydrochloride on soil urease activity (Table 7).

3.4.4 Effects of fluopimomide on soil invertase activities

Soil invertase is of particular importance in carbon cycles [38]. A previous study had documented that bioorganic fertilizer application could always improve invertase activity [39]. Our assay results showed a significant increase in invertase activities when the soil was treated with a recommended dosage of fluopimomide and fluopicolide at 20 DAT, which were probably used as carbon sources by microbes. However, the activation effects were converted to inhibition at 40 DAT, and recovered to be similar to control at 60 DAT. The complicated influence of fluopimomide may be due to the complex response of microorganisms to fungicide

Table 7: Effects of fluopimomide on soil urease activities (mg 100 g⁻¹)

| Treatment   | Treatment time (DAT) |
|-------------|----------------------|
|             | 20                   | 40                   | 60                   |
| Fluopimomide 375 | 0.495 ± 0.016a        | 0.512 ± 0.034b        | 0.494 ± 0.033a        |
| Fluopimomide 750 | 0.412 ± 0.041a        | 0.550 ± 0.050ab       | 0.503 ± 0.015a        |
| Fluopimomide 1,500 | 0.336 ± 0.059b        | 0.631 ± 0.067a        | 0.522 ± 0.047a        |
| Fluopicolide 750 | 0.486 ± 0.023a        | 0.506 ± 0.016b        | 0.491 ± 0.030a        |
| CK          | 0.459 ± 0.029a        | 0.483 ± 0.048b        | 0.486 ± 0.043a        |
dosages, including the ecology change in the microorganism community.

Overall, the effects of fluopimomide on the four soil enzymes are temporary and reversible, indicating relatively high safety to the soil environment (Table 8).

### 3.5 Quantitative PCR analysis of soil nitrogen-related genes

Soil microbes are essential in soil nutrient mineralization and accumulation [40]. Soil N cycling is participated by varieties of microorganisms, of which *nifH* encodes for N$_2$ fixation, AOA and AOB for ammonia oxidation, and *nirS* for denitrification [41]. Jiang et al. has reported that five fluoroalkylether compounds could reduce *amoA* gene abundance in soil and had different effects on *nirS* [42]. In this study, 20, 40, and 60 DAT, the copy numbers of AOA and *nirS* fluctuated greatly, but the differences among treatments were not obvious, indicating that the effects of chemical treatment on AOA and denitrifying bacteria in soil were not regular. At 40 and 60 DAT, the AOB and *nifH* gene copy numbers were higher in the double and quadruple dosages of fluopimomide treatments than in the control, indicating that fluopimomide could promote the proliferation of soil AOB, nitrogen-

| Table 8: Effects of fluopimomide on soil invertase activities (mg 100 g$^{-1}$) |
|---------------------------------------------------------------|
| **Treatment** | **Treatment time (DAT)** |
|                | 20                      | 40                      | 60                      |
| Fluopimomide 375          | 19.086 ± 1.690a         | 14.684 ± 1.150c         | 16.331 ± 0.889a         |
| Fluopimomide 750          | 20.059 ± 1.865a         | 17.413 ± 1.804ab        | 14.076 ± 1.511a         |
| Fluopimomide 1,500        | 17.420 ± 0.910ab        | 19.408 ± 1.849a         | 14.971 ± 3.824a         |
| Fluopicolide 750          | 19.247 ± 1.048a         | 13.281 ± 1.078c         | 15.197 ± 2.714a         |
| CK                      | 15.124 ± 1.802b         | 16.122 ± 1.358b         | 15.441 ± 2.166a         |

The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level, and the same as follows.

![Figure 2: Effects of fluopimomide on soil nitrogen-related genes.](image)
The results showed as mean value ± std. error, different letters in each column showed the significant difference at 0.05 level.

### Table 9: Effects of fluopimomide on tomato plant height and marketable yield

| Fungicide          | Plant height/cm | Marketable yield (t/ha) |
|--------------------|-----------------|-------------------------|
|                    | 20 DAT | 40 DAT | 60 DAT |                    |                      |
| Fluopimomide 375   | 14.70 ± 0.90ab | 42.86 ± 2.90ab | 75.73 ± 2.07ab | 59.20 ± 1.28a |
| Fluopimomide 750   | 16.31 ± 0.69a  | 44.22 ± 2.62ab | 78.20 ± 4.38a | 59.85 ± 1.11a |
| Fluopimomide 1,500 | 15.58 ± 0.87ab | 43.53 ± 2.17ab | 76.78 ± 4.21a | 59.50 ± 1.65ab |
| Fluopicolide 750   | 15.06 ± 0.87ab | 43.26 ± 3.11ab | 72.88 ± 2.72ab | 55.65 ± 1.03b |
| Control            | 12.98 ± 0.34ab | 34.55 ± 1.24ab | 66.50 ± 0.81b | 50.65 ± 0.57c |

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Yan Z, Zhao M, Ma H, Liu L, Yang G, Geng C, et al. Biological and molecular characterization of tomato brown rugose fruit virus and development of quadruplex RT-PCR detection. J Integr Agr. 2021;20(7):1871–9.

[2] Srinivas C, Nirmala Devi D, Narasimha Murthy K, Mohan CD, Lakshmesha TR, Singh B, et al. Fusarium oxysporum f. sp. lycopersici causal agent of vascular wilt disease of tomato: Biology to diversity – a review. Saudi J Biol Sci. 2019;26:1315–24.

[3] Zhang MH, Qin ZH, Liu X, Ustin SL. Detection of stress in tomatoes induced by late blight disease in California, USA, using hyperspectral remote sensing. Int J Appl Earth Obs. 2003;4:295–310.

[4] Zhu Z, Tian SP. Resistant responses of tomato fruit treated with exogenous methyl jasmonate to Botrytis cinerea infection. Sci Hortic-Amsterdam. 2012;142:38–43.

[5] Kousik CS, Adams ML, Jester WR, Hassell R, Harrison HF, Holmes GJ. Effect of cultural practices and fungicides on fixing bacteria, and other bacteria (Figure 2), which might be helpful for tomato plant growth [43].

### 3.6 Effect of fluopimomide on plant height and marketable yield

Soil management for disease suppressive could help to improve plant growth [44]. Jakl et al. has reported the increased effect of soil-treated triazole fungicides on tomato fruit yield [45]. As shown in Table 9, 20 DAT, fluopimomide had increased tomato plant height nonsignificantly by 13.25, 25.65, and 20.03% with 1-, 2-, and 4-folds of recommended dosages. Still, the stimulated efficiencies continued with time-lapse until 60 DAT. A similar tendency could be found in fluopicolide as well. Meanwhile, the two fungicides significantly improved marketable tomato yield by reducing disease incidences. Among which fluopicolide had improved the total marketable yield by 16.88, 18.16, and 17.47%, with the application dosage of 375, 750, and 1,500 g ha⁻¹, respectively. Furthermore, fluopicolide exhibited lower stimulation efficiency by 9.87%. Thus, the conclusion could be drawn out that fungicide fluopimomide could improve tomato yield, possibly via the inhibition of soil-borne pathogens.

### 4 Conclusion

Via soil treatment, the new fluorinated benzamide fungicide fluopimomide could significantly reduce the amounts of soil-borne pathogens in soil, reduce the disease incidences in tomato plants, and eventually increase the marketable yield of tomatoes. During the inoculation period, the soil enzymes had been influenced differently, and AOB and nifH gene copy numbers were increased by the double and quadruple dosages of fluopimomide treatment. Compared with the control fungicide fluopicolide, fluopimomide exhibited more efficiency in tomato plant height and marketable yield. Therefore, as a broad-spectrum fungicide, fluopimomide could be popularized to manage tomato diseases. Still, the broader application scope remains to be investigated.
Phytophthora fruit rot of watermelon in the Carolinas. Crop Prot. 2011;30:888–94.

[6] Jackson KL, Yin JF, Csinos AS, Ji PS. Fungicidal activity of fluopicolide for suppression of Phytophthora capsici on squash. Crop Prot. 2010;29:1421–7.

[7] Li J, Meng Z, Li N, Dong B, Ji X, Zhang S, et al. Evaluating a new non-fumigant nematicide fluopicolide for management of southern root-knot nematodes in tomato. Crop Prot. 2020;129:105040.

[8] Ji X, Li J, Meng Z, Li N, Dong B, Zhang S, et al. Fluopicolide effectively controls Meloidogyne incognita and shows a growth promotion effect in cucumber. J Pest Sci. 2020;93:1421–30.

[9] Zhang W, Sun W, Wang Y, Liu H, Zhang S, Dong B, et al. Management of Meloidogyne incognita on cucumber with a new nonfumigant nematicide fluopicolide. Plant Dis. 2022;106:151–5.

[10] Zhang H, Zhai M, Wang K, Xu H, Tang J, Wang H. Study on fungicial activity and mode of action of a novel fungicultural agent, LH-2010A against Rhizoctonia solani. Chin. J Pestic Sci. 2013;15:43–9.

[11] Catello P, Giuseppe C, Domenica V, Massimo Z. Control of Botrytis cinerea, Alternaria alternata and Pyrenochaeta lyco- persici on tomato with whey compost-tea applications. Crop Prot. 2012;38:80–6.

[12] Huo Y, Xu JG, Wang L. Isolation and identification of Imaezathypy degradeable actinomycyes S181 and its degrada- tion characteristics. Environ Sci. 2011;5(32):1519–23 (in Chinese with English abstract).

[13] Fang ZD. Research methods of plant disease. Beijing: China Agriculture Press; 1996.

[14] Tian MY, Feng LX, Gong HZ, Yang CZ. The identification and purification of late blight in tomatoes. Plant Prot. 2000;5(26):36 (in Chinese with English abstract).

[15] Menzies JD. The direct assay of plant pathogen populations in soil. Ann Rev Phytopathol. 1963;1:127–42.

[16] Lebrun JD, Trinsoutrot-Gattin I, Vincelas-Akpà M, Baileul C, Brault A, Mougin C, et al. Assessing impacts of copper on soil enzyme activities in regard to their natural spatiotemporal variation under long-term different land uses. Soil Biol Biochem. 2012;49:150–6.

[17] Wang QY, Zhou DM, Cang L. Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. Soil Biol Biochem. 2009;41:1504–9.

[18] Ohshima T, Tamura T, Sato M. Influence of pulsed electric field on various enzyme activities. J Electrostat. 2007;65:156–61.

[19] Wu X, Xu J, Liu F, Liu X, Zhang W, et al. Impact of flux-aproxad on the microbial community structure and functional diversity in the silty-loam soil. J Integr Agr. 2015;14(1):114–24.

[20] Jin H, Germida JJ, Walley FL. Suppressive effects of seed- applied fungicides on arbuculys mycorrhizal fungi (AMF) differ with fungicide mode of action and AMF species. Appl Soil Ecol. 2013;72:22–30.

[21] Mao W, Lewis JA, Lumsden RD, Hebbar KP. Biocontrol of selected soil-borne diseases of tomato and pepper plants. Crop Prot. 1998;17(6):535–42.

[22] Hashemi M, Tabet D, Sandroni M, Benavent-Celma C, Seematti J, Andersen CB, et al. The hunt for sustainable biocontrol of oomycete plant pathogens, a case study of Phytophthora infestans. Fungal Biol Rev. 2022;40:53–69. Forthcoming.

[23] Al-Shammri KN, Elkanz NAA, Arafa WAA, Althobaiti IO, Bakr RB, Moustafa SMN. Novel indan 1,3-dione derivatives: design, green synthesis, effect against tomato damping off disease caused by Fusarium oxysporum and in silico molecular docking study. Arab J of Chem. 2022;15:103731.

[24] Manikandan R, Karthikeyan G, Raghuchander T. Soil proteomics for exploitation of microbial diversity in Fusarium wilt infected and healthy rhizosphere soils of tomato. Physiol Mol Plant Pathol. 2017;100:185–93.

[25] Ji X, Li J, Meng Z, Zhang S, Dong B, Qiao K. Synergistic effect of combined application of a new fungicide fluopicolide with a biocontrol agent Bacillus methylotrophicus TA-1 for management of gray mold in tomato. Plant Dis. 2019;103:1991–7.

[26] Mulugeta T, Abreha K, Tekie H, Mulatu B, Yesuf M, Andreasson E, et al. Phosphate protects against potato and tomato late blight in tropical climates and has varying toxicity depending on the Phytophthora infestans isolate. Crop Prot. 2019;121:139–46.

[27] Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stormberger ME, Wallenstein MD, et al. Soil enzymes in a changing environment: current knowledge and future direc- tions. Soil Biol Biochem. 2013;58:216–34.

[28] Nasai E, Katayama Y, Fukuda M. Genetic and biochemical investigations on bacterial catabolic pathways for lignin-derived aromatic compounds. Biosci Biotechn Bioch. 2007;71:1–15.

[29] Bugg TDH, Ahmad M, Hardiman EM, Singh R. The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotech. 2011;22:394–400.

[30] Tejada M. Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate + diflufenican herbicides. Chemosphere. 2009;76:365–73.

[31] Monkiedje A, Ilori MO, Spiteller M. Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol Biochem. 2002;34:1939–48.

[32] Tejada M, Gómez I, García-Martínez AM, Osta P, Parrado J. Effects of Prochloraz fungicide on soil enzymatic activities and bacterial communities. Ecotox Environ Safe. 2011;74:1708–14.

[33] Bending GD, Rodríguez-Cruz MS, Lincoln SD. Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere. 2007;69:82–8.

[34] Jastrzębska E, Kucharski J. Dehydrogenases, urease and phosphatases activities of soil contaminated with fungicides. Plant Soil Environ. 2007;53:51–7.

[35] Monkiedje A, Spiteller M. Effects of the fungicides meta- laxyll and prochloraz on the microbiological properties of a sandy loam and a sandy clay soil. Biol Fert Soils. 2001;35:393–8.

[36] Chen SX, Edwards CA, Subler S. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory conditions. Soil Biol Biochem. 2001;33:1971–80.

[37] Uyanoţ R, Ümmûhán C, Karaarslan M. Effect of three fungicides on soil microbial activity and nitrogen dynamics. Pakistan J Biological Sci. 2005;8:805–9.

[38] Wang M, Chen L, Li Y, Chen L, Liu Z, Wang X, et al. Responses of soil microbial communities to a short-term application of
seaweed fertilizer revealed by deep amplicon sequencing. Appl Soil Ecol. 2018;125:288–96.

[39] Wang Y, Fu F, Li J, Wang G, Wu M, Zhan J, et al. Effect of seaweed fertilizer on the growth of Malus hupehensis Rehd. seedling, soil enzyme activities and fungal communities under replant condition. Eur J Soil Biol. 2016;75:1–7.

[40] Zhang W, Wang C, Xue R, Wang L. Effects of salinity on the soil microbial community and soil fertility. J Integr Agr. 2019;18(6):1360–8.

[41] Kim N, Riggins CW, Rodríguez-Zas S, Zabaloy MC, Villamil MB. Long-term residue removal under tillage decreases amoA-nitrifiers and stimulates nirS-denitrifier groups in the soil. Appl Soil Ecol. 2021;157:103730.

[42] Jiang T, Geisler M, Zhang W, Liang Y. Fluoroalkylether compounds affect microbial community structures and abundance of nitrogen cycle-related genes in soil-microbe-plant systems. Ecotox Environ Safe. 2021;228:113033.

[43] Na M, Yuan M, Hicks LC, Rousk J. Testing the environmental controls of microbial nitrogen-mining induced by semi-continuous labile carbon additions in the subarctic. Soil Biol Biochem. 2022;166:108562.

[44] De Corato U, Patruno L, Avella N, Salimbeni R, Lacolla G, Cucci G, et al. Soil management under tomato-wheat rotation increases the suppressive response against Fusarium wilt and tomato shoot growth by changing the microbial composition and chemical parameters. Appl Soil Ecol. 2020;154:103601.

[45] Jakl M, Kovač I, Željković SČ, Dytrtová JJ. Triazole fungicides in soil affect the yield of fruit, green biomass, and phenolics production of Solanum lycopersicum L. Food Chem. 2021;351:129328.