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Development and Application of a Novel Eco-friendly Seed Coating Agent (DIF) for Controlling Cotton Fusarium Wilt and Improving Seedling Growth and Yield

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Abstract

Cotton Fusarium wilt, caused by *Fusarium oxysporum* f. sp. vasinfectum (Atk.), severely impairs cotton yield and quality. This study developed a novel eco-friendly seed coating agent (DIF) to replace toxic conventional formulations, with Difenoconazole as the core active ingredient.

Laboratory and field trials (2024, Anhui) evaluated DIF's effects on seed germination, seedling growth, Fusarium wilt control and cotton fibre quality, with carboxin-thiram (CT) as the positive control and uncoated seeds as blank control (CK). The 1:100 (agent: seed) DIF was optimal, boosting seedling root length (13.96 cm) and biomass, and achieving a 98.67% field control efficiency against Fusarium wilt. DIF improved seed yield and lint yield vs. CK ($P > 0.05$) and had no adverse effects on key fibre quality parameters. This eco-friendly DIF has great potential for cotton Fusarium wilt management with economic and environmental benefits.

Keywords Cotton Fusarium wilt, Cotton seed coating agent; Germination percentage; Yield; Fiber quality

1. Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most economically important crops in the world, cultivated in more than 100 countries¹. China ranks first in cotton production globally, with an annual output of 684 thousand metric tons, and it plays a significant role in the global fibre and oil production industry². As the main raw material of the textile industry, cotton provides economic income for industrial producers and farmers³. However, during cotton seed germination and seedling growth stages, insect pests, weeds and various fungal pathogens can cause considerable yield losses if not effectively controlled⁴.

In cotton-producing regions, more than sixty diseases occur every year, among which seed-borne and soil-borne diseases such as Verticillium wilt and Fusarium wilt are the two most destructive diseases threatening cotton production worldwide⁵. Cotton Fusarium wilt, caused by *Fusarium oxysporum* f. sp. vasinfectum (FOV), is a typical soil-borne disease that seriously affects cotton yield and fibre quality⁶. Moderate to severe FOV infestations can lead to significant yield reduction, poor fibre quality and huge economic losses⁷. At present, chemical control is the main measure for cotton Fusarium wilt management in China, but conventional chemical fungicides often cause seed damage and unsatisfactory control effects⁸. In addition, FOV can survive in soil for a long time in the form of microsclerotia, making this soil-borne disease extremely difficult to prevent and control^{9,10}, and there is still no ideal control method available in production.

Seed treatment is an important measure in the integrated disease management system, and seed coating technology has become a research hotspot due to its advantages of safety, easy operation and low environmental pollution¹¹. Compared with foliar spraying of chemical fungicides, seed coating agents can provide long-term protection for seeds and seedlings, and have fewer side effects on natural enemies¹². They can also improve seed emergence rate and seedling stem robustness, and this technology has been widely recommended for the production of high-value agricultural crops^{13,14}. Seed coating agents are generally composed of active ingredients (pesticides and plant growth regulators) and inert ingredients (film-forming agents, suspending agents, pigments, etc.). Recent studies have shown that seed coating technology can effectively prevent and control mould-induced diseases and insect pests, promote seedling growth and increase cotton yield^{15,16}. However, most of the seed coating agents reported in previous studies use high-toxicity fungicides such as carbofuran, thiram and triadimenol as active ingredients, which not only pose potential acute and chronic health risks to humans, but also have adverse effects on plant metabolism and morphology, ultimately affecting crop growth and productivity^{17,18}. In addition, the control effect of seed coating agents is significantly affected by active ingredient types, coating ratios and application methods. Therefore, the development of a novel, efficient, safe and eco-friendly seed coating agent has become an urgent research topic in cotton disease control and sustainable production.

To meet the production demand for safe and efficient control of cotton Fusarium wilt and protect cotton yield potential, this study first screened high-efficiency and low-toxicity fungicides through laboratory toxicity tests, then developed an optimal formula of novel seed coating agent (DIF) by orthogonal test combined with additive screening, and verified its effects on cotton seed germination index, seedling growth index, Fusarium wilt control efficiency, yield traits and fibre quality through field trials. This study aims to provide a new technical solution for the green control of cotton Fusarium wilt and lay a theoretical foundation for the rational application of eco-friendly seed coating agents in cotton production.

2. Materials and methods

2.1 Test materials

The cotton cultivar used in this study was Huimian 1, provided by the Institute of Industrial Crops, Anhui Academy of Agricultural Sciences. The seeds were delinted and strictly selected for uniformity and plumpness before seed treatment. Five categories of high-purity fungicides were used for toxicity screening, among which Difenoconazole was set with two technical grades of different purities for parallel toxicity test. The tested fungicides were as follows: Thifluzamide (99.2% purity), Difenoconazole (99.2% purity, technical grade 1), Benthiamide (99.9% purity), Difenoconazole (97.2% purity, technical grade 2), Tebuconazole (98.2% purity). All fungicide samples were purchased from Suolaibao Laboratory Technologies Inc. (Beijing, China). Carboxin-thiram (CT), a traditional cotton seed coating agent used as the positive control in this

study, was purchased from Compton Chemical Company (Shanghai, China). Emulsifier A (polyoxyethylene castor oil ether) and Emulsifier B (calcium dodecyl benzene sulfonate), common agricultural anionic and nonionic emulsifiers, were used as formulation additives; gibberellin (GA3) and indole-3-butyric acid (IBA) were added as plant growth regulators to promote seed germination and root development, with the stock solution concentration of $0.05 \text{ g}\cdot\text{L}^{-1}$ for GA3 and $0.02 \text{ g}\cdot\text{L}^{-1}$ for IBA, respectively. All solvents and other chemicals used in the study were of analytical grade.

2.2 Experimental conditions

Fusarium oxysporum f. sp. vasinfectum (Atk., FOV) race 4 strain was provided by the Cotton Disease Control Team, Institute of Cash Crops, Anhui Academy of Agricultural Sciences. The race identity of the strain was validated as race 4 (the dominant physiological race in cotton-growing areas of Anhui Province) through morphological identification and race-specific PCR detection, following standard identification protocols for FOV race 4. The initial inoculum level was adjusted to 1×10^6 conidia mL^{-1} for all experiments. Fungal strain activation and preservation were conducted on potato dextrose agar (PDA) medium at 25°C in the dark, and the strains were stored on PDA slants at 4°C for short-term use; a light and temperature-controlled incubator was used for all laboratory culture experiments.

2.3 Laboratory toxicity evaluation

In this experiment, *Fusarium oxysporum* f. sp. vasinfectum was used as the target pathogen to determine the toxicity of the tested

fungicides. The mycelial radial growth inhibition method was used to evaluate fungicide toxicity¹⁹. A series of gradient concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, 10 mg L⁻¹) were set for each fungicide; appropriate volumes of pesticide stock solutions prepared with acetone were added to the molten PDA medium before pouring into petri dishes, with each concentration tested in quintuplicate. A 0.6 cm diameter fungal plug from a 7-day-old PDA culture was placed in the center of a 9 cm diameter PDA plate. The plates were incubated at 28°C in the dark for 6 days, and the colony radius was measured to calculate the mycelial growth inhibition rate^{15,16}. Acetone-treated PDA medium was used as the negative control, and sterile water-treated PDA medium was used as the blank control. *Growth inhibition rate (%) = [(colony radius of control - colony radius of treatment) / colony radius of control] × 100%*²⁰. EC₅₀ (half-maximal effective concentration) values were calculated using the probit analysis method based on the linear regression between the log₁₀ of fungicide concentration and the probit of growth inhibition rate, and the fitting degree of the regression equation was evaluated by R².

2.4 Preparation of the seed coating agent

The wet sand processing superfine grinding method was adopted for the preparation of the seed coating agent¹¹, and an L9(3⁴) orthogonal test was designed with four factors (active ingredient dosage, emulsifier ratio, wetting dispersant dosage, film-forming agent dosage) and three levels to screen the optimal formula of DIF. The basic preparation conditions were determined based on previous studies: water and surfactant ratios were fixed according to pre-experiments, and a rotor-

stator homogenizer was used to make the pesticide active ingredients form a stable dispersion system. Then, plant growth regulators (GA3 and IBA), film-forming auxiliaries and other additives were added to the aqueous solution according to the optimal ratio, and the solution was continuously stirred at 25°C for 4-5 h until completely dissolved²⁰. The finished DIF seed coating agent was obtained after homogenization and filtration, and its physical and chemical properties were determined according to the recommended Collaborative International Pesticides Analytical Council (CIPAC) method, all of which met the national pesticide preparation standards. DIF is defined as the novel eco-friendly cotton seed coating agent with Difenconazole as the main active ingredient, compounded with plant growth regulators, environmentally friendly emulsifiers and film-forming agents.

2.5 Laboratory experiments

Cotton seeds were surface-sterilized by immersion in 2% sodium hypochlorite for 5 min, followed by five rinses with autoclaved distilled water²¹. Five treatment groups were set up: uncoated cotton seeds as blank control (CK), and seeds coated with DIF at ratios of 1:50, 1:100, 1:200, 1:500 (agent:seed, w/w). The seed coating was conducted by manual stirring at 30°C for 24 h, and the coated seeds were air-dried at room temperature for 30 min before sowing. Fifty cotton seeds were sown per pot, with three biological replicates per treatment; the pots were placed in an artificial climate chamber (28°C/25°C day/night, 12 h light/12 h dark, relative humidity 75%), and irrigated every two days as required. The germination potential (3 days) and germination percentage

(7 days) were calculated according to the International Rules for Seed Testing (ISTA, 2006)²¹. After 7 days of culture, the seedlings were carefully uprooted, and the roots were rinsed with distilled water to determine seedling growth indices including plant height, root length, fresh weight and dry weight of root and shoot. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($P < 0.05$) was used to compare significant differences among treatments, and the optimal DIF coating ratio was selected for subsequent field trials based on comprehensive seed germination and seedling growth performance.

2.6 Field trial and fibre quality measurements

A field trial was conducted from 25 May to 12 November 2024 in Wangjiang, Anhui Province, China. The experimental field was a sandy loam soil with pH 7.2, organic matter content 1.8%, and a 5-year continuous cotton cropping history; the natural disease pressure of cotton Fusarium wilt in the previous year was 35.2%, and no artificial inoculation was conducted in this trial. Three treatment groups were set up: CK (uncoated seeds), DIF (coated at the optimal ratio of 1:100), CT (traditional seed coating agent, positive control). The experiment was designed with a randomized complete block design, each treatment had three biological replicates, and each plot was 2.0 m × 3.0 m (6 m²) with a 20 cm wide isolation row between adjacent plots. All test seeds were coated manually at a rate of 1 mL per 100 g of seeds, and approximately 300 cotton seeds were sown per plot.

During the field growth period, conventional field management measures (fertilization, irrigation, insect pest control) were carried out

uniformly without any fungicide application for Fusarium wilt control. At the seedling stage, 10 representative plants were randomly selected from each plot to determine fresh weight, dry weight and plant height. At the peak of cotton Fusarium wilt occurrence (flowering and boll-forming stage), the diagonal 5-point sampling method was used to investigate disease occurrence: the interval between two adjacent sampling points was 10 m, 100 consecutive plants were investigated at each point, and the disease incidence and disease index were calculated according to the 5-level classification standard of Wei et al.²². Control efficiency (%) = [(disease index of CK - disease index of treatment) / disease index of CK] × 100%. At harvest, the yield traits including number of bolls per plant, average boll weight and ginning outturn (GOT) were measured for each plot. Mature fibres were collected from all treatment groups for quality determination (the description of drought stress in the original text was a clerical error and was removed), and fibre quality parameters (micronaire, upper half mean length, breaking strength, uniformity index and fibre elongation) were determined using the USTER HVI 1000 High Volume Instrument at the Institute of Industrial Crops²³.

2.7 Data analysis

Microsoft Excel 2010 and SPSS 26.0 software were used for statistical data analysis (the SPSS v23 in the original text was a clerical error and was removed), and all data were expressed as mean ± standard deviation (SD) with the significance level set at $P < 0.05$. One-way ANOVA followed by Duncan's multiple range test was used to compare significant differences among different treatments; Pearson

correlation analysis was performed to analyze the correlation between laboratory and field seedling growth indices. GraphPad Prism 7 (GraphPad Software, Inc., San Diego, USA) was used for figure drawing, and all figures were marked with statistical significance labels (different lowercase letters indicate significant differences at $P < 0.05$) and error bars representing the standard deviation (SD) of three replicates.

3. Results

3.1 Laboratory screening of fungicides for the control of cotton

Fusarium oxysporum f. sp. vasinfectum

Table 1 shows the antifungal activity of five tested fungicides against *Fusarium oxysporum f. sp. vasinfectum*, and significant differences in toxicity were observed among the different fungicides ($P < 0.05$). Difenoconazole (99.2% purity, technical grade 1) had the strongest antifungal activity with the lowest EC_{50} value of 0.0829 mg L^{-1} , followed by Tebuconazole (0.3197 mg L^{-1}) and Difenoconazole (97.2% purity, technical grade 2) with an EC_{50} value of 1.2063 mg L^{-1} ; Thifluzamide had the weakest antifungal activity with an EC_{50} value of $49.7046 \text{ mg L}^{-1}$. The difference in EC_{50} values between the two Difenoconazole samples was mainly attributed to the difference in active ingredient purity of the technical grades. Probit analysis showed that all fungicides had a good linear relationship between concentration and growth inhibition rate ($R^2 > 0.90$), indicating the reliability of the toxicity test results.

Difenoconazole, as a high-efficiency and broad-spectrum systemic fungicide, showed significant potential for the control of cotton *Fusarium*

wilt and was selected as the main active ingredient of the novel seed coating agent DIF.

Table 1. Half-maximal effective concentration (EC₅₀) of tested fungicides against *Fusarium oxysporum* f.sp. *vasinfectum* in cotton

| Fungicides | <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> | | |
|--|---|---------------------|----------------|
| | EC ₅₀ (mg L ⁻¹) 1) | Regression equation | R ² |
| Thi flu zamide | 49.7046 | 3.8536+0.4664x | 0.9796 |
| Difenoconazole (99.2% purity, technical grade 1) | 0.0829 | 5.4662+0.2910x | 0.9018 |
| Benthamide | 16.5048 | 4.2410+0.6233x | 0.9658 |
| Difenoconazole (97.2% purity, technical grade 2) | 1.2063 | 4.9248+0.9237x | 0.9888 |
| Tebuconazole | 0.3197 | 5.4660+0.9411x | 0.9482 |

Note: X² in the original text was a clerical error, corrected to R² (coefficient of determination).

3.2 Preparation of the DIF seed coating agent and its effect on cotton seed germination and seedling growth

The optimal formula of DIF was screened by L9(3⁴) orthogonal test, and the main components and physical and chemical properties of the prepared DIF seed coating agent are shown in Fig. 1. The seed coating

agent was prepared by the wet sand processing superfine grinding method²⁴, and all main performance indexes (pH, particle size, viscosity, film-forming time, suspensibility, etc.) met the national pesticide preparation standards, with good stability and coating performance (Fig. 1B).

The optimal preparation process of DIF is as follows: 5.0 g of Difenoconazole, 2.0 g of Emulsifier A and 2.0 g of Emulsifier B were mixed first; 1.50 g of 2-naphthalenesulfonic acid (NNO, wetting dispersant) and an appropriate amount of distilled water were added slowly to the mixture and gently stirred; the mixture was stirred at 2000 r/min for 2-3 h at room temperature using an electronic constant speed mixer until completely dissolved; then other additives (film-forming auxiliaries, GA3, IBA), and 3 mL of 5% sodium lignosulfonate solution were added dropwise to the aqueous solution; finally, the mixture was stirred at 500 r/min for 4-5 h at 25°C under normal pressure, and distilled water was added to make the total mass 100 g to obtain the finished DIF seed coating agent. The final mass concentration of GA3 and IBA in the finished DIF formulation was 0.005% (w/w) and 0.002% (w/w), respectively. Under the optimal coating ratio of 1:100 (agent:seed, w/w), the final effective dose of GA3 and IBA on cotton seeds was 0.5 mg/kg seed and 0.2 mg/kg seed, respectively.

The effects of different DIF coating ratios on cotton seed germination and seedling growth were investigated in the laboratory, and the results showed that different DIF dosages had significant effects on cotton seedling growth ($P < 0.05$), but no significant effect on seed

germination percentage ($P > 0.05$), with the germination percentage of all treatments close to 100% (Fig. 2). The optimal coating ratio was 1:100 (agent:seed), at which the relative germination rate was $>90.00\%$ after 7 days of culture, and the seedling growth indices were the best: seedling height 11.48 cm, root length 13.96 cm, fresh weight 5.96 g, dry weight 1.42 g (Fig. 3). The DIF treatment at 1:50 (high dosage) showed a slight inhibitory effect on seedling growth, while the 1:500 (low dosage) treatment had no significant promotion effect on root length ($P > 0.05$) compared with CK, so these two ratios were not suitable for field application. Based on comprehensive seed germination and seedling growth parameters, the 1:100 coating ratio was selected for subsequent field trials.

| A | | | B | |
|-----------------------------|----------------|--------------------|---|-----------|
| Components | Content %(g/g) | Property | Test index | |
| Difenoconazole | 5.00 | Active ingredient | pH | 6.36 |
| Sodium lignosulfonate | 0.8 | Wetting dispersant | Particle size (D90) / μm | 3.91 |
| Emulsifiers A | 2.00 | Emulsifiers | Viscosity ($\text{mPa}\cdot\text{s}$) | 34.3 |
| Emulsifiers B | 2.00 | Emulsifiers | Film forming time (min) | 2.7 (+) |
| XG | 0.15 | Thickening agent | Suspensibility (%) | 97.9 |
| Magnesium aluminum silicate | 1.50 | Thickening agent | Failure rate (%) | 0.3 |
| Octanol | 0.30 | Anti-foaming agent | Uniformity of coating (%) | 95 |
| Ethylene glycol | 3.00 | Antifreeze agent | Cold storage stability | Qualified |
| Pigment red | 6.00 | Dye agent | Heat storage stability | Qualified |
| Film-forming agent NKY | 4.00 | Film-forming agent | | |
| Deionized water | 75.25 | -- | | |

Fig. 1. The main components (A) and physicochemical properties (B) of the novel cotton seed coating agent DIF. Note: All physical and chemical property indexes meet the CIPAC pesticide preparation standards.

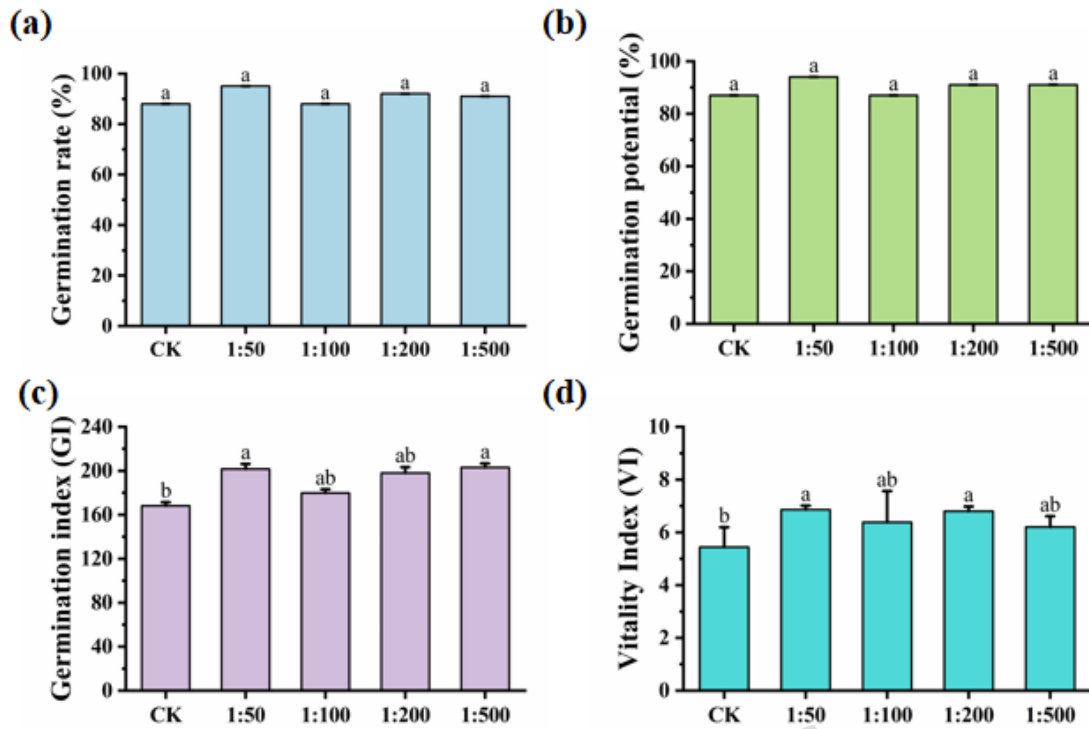


Fig. 2. Effects of different DIF coating ratios on cotton seed germination rate (a), germination potential (b), germination index (c) and vitality index (d). Different lowercase letters indicate significant differences at $P < 0.05$; error bars represent SD of three replicates.

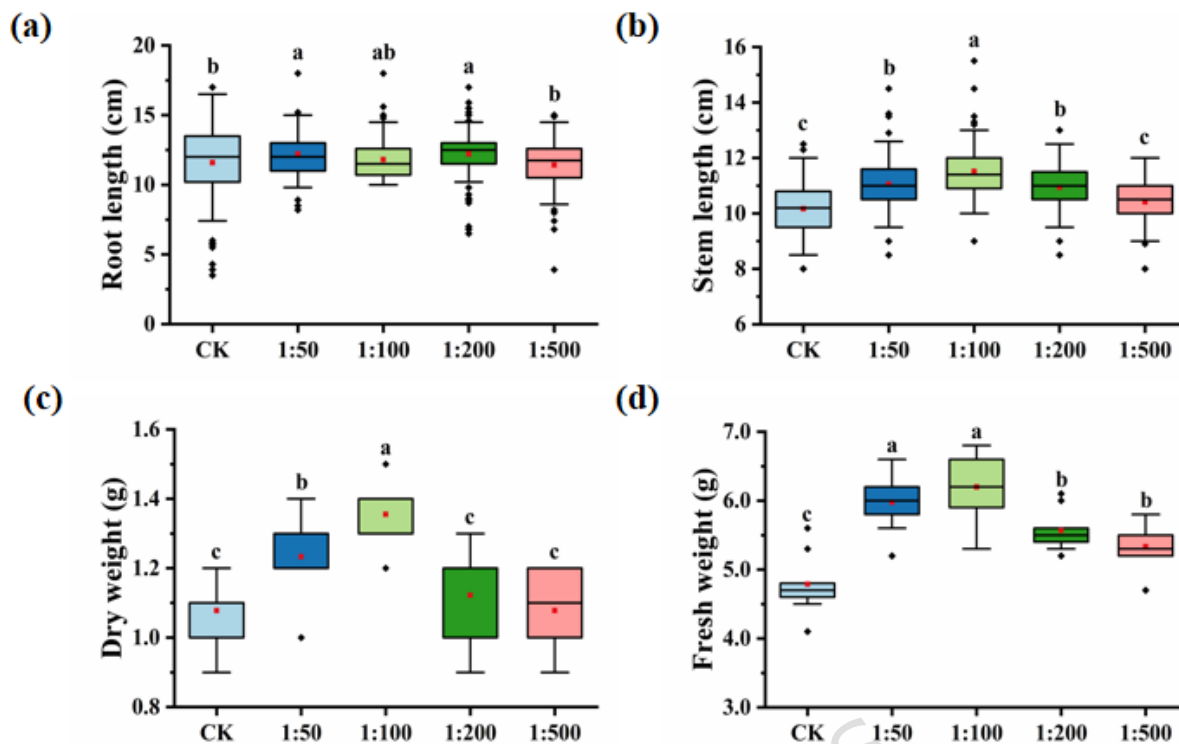


Fig. 3. Effects of different DIF coating ratios on cotton seedling root length (a), stem length (b), dry weight (c) and fresh weight (d). Different lowercase letters indicate significant differences at $P < 0.05$; error bars represent SD of three replicates.

3.3 Field trial results of DIF seed coating agent

3.3.1 Effects on cotton seedling growth and *Fusarium wilt* control efficiency

Field trial results showed that DIF (1:100) significantly improved cotton seed germination and seedling biomass compared with CT and CK ($P < 0.05$), and had a significant positive promotion effect on seedling root length, stem height, fresh weight and dry weight (Fig. 4). Pearson correlation analysis showed a significant positive correlation between

laboratory and field seedling growth indices ($r > 0.85$, $P < 0.01$), indicating the reliability of the laboratory screening results.

Compared with the traditional seed coating agent CT, DIF had a more significant promotion effect on cotton seedling growth and a better control effect on Fusarium wilt during the whole seedling stage (Fig. 4). The seedling height and root length of CT-treated cotton were 6.54 cm and 4.25 cm, respectively, while those of DIF-treated cotton reached 9.23 cm and 6.19 cm, with increases of 41.1% and 45.6%, respectively; the fresh weight and dry weight of DIF-treated seedlings were 15.74 g and 1.58 g, respectively, which were significantly higher than those of CT-treated seedlings (10.52 g and 0.82 g, $P < 0.05$). DIF had an excellent control effect on cotton Fusarium wilt in the field, with a control efficiency of 98.67%, which was significantly higher than that of CT (76.32%, $P < 0.05$).

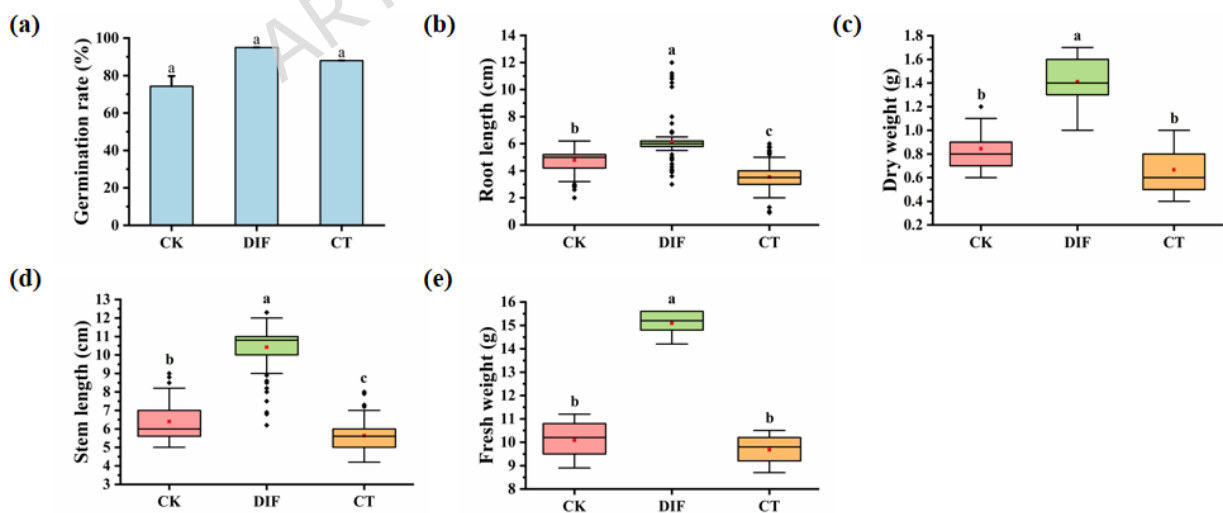


Fig. 4. Effects of CK, DIF and CT treatments on cotton seedling root length (a), stem length (b), dry weight (c) and fresh weight (d) in the

field. Different lowercase letters indicate significant differences at $P < 0.05$; error bars represent SD of three replicates.

3.3.2 Effects on cotton yield traits

DIF had a significant effect on cotton yield traits, and the number of bolls per plant of DIF-treated cotton was significantly increased compared with CK and CT ($P < 0.05$) (Fig. 5). The plant height of DIF-treated cotton was 16.6% higher than that of CK, while there was no significant difference in plant height between CT and CK ($P > 0.05$). The average boll weight of all treatments was about 5.2 g, with no significant difference ($P > 0.05$) (Table 2).

The seed cotton yield and lint yield of different treatments showed no significant statistical difference ($P > 0.05$, Table 2): the seed yield of CK, DIF and CT was $170.4 \text{ kg}\cdot\text{hm}^{-2}$, $178.4 \text{ kg}\cdot\text{hm}^{-2}$ and $185.0 \text{ kg}\cdot\text{hm}^{-2}$, respectively, and the lint yield was $68.8 \text{ kg}\cdot\text{hm}^{-2}$, $73.0 \text{ kg}\cdot\text{hm}^{-2}$ and $74.3 \text{ kg}\cdot\text{hm}^{-2}$, respectively. The higher plot yield of CT was due to the influence of field microclimate factors such as local temperature and precipitation during the trial period, while the per plant lint yield of DIF-treated cotton increased by 15.6% compared with CT under natural disease pressure, which was a direct reflection of improved seedling growth and reduced disease incidence. The ginning outturn of DIF-treated cotton was 40.9%, which was slightly higher than that of CK (40.4%) and CT (40.2%), with no significant difference ($P > 0.05$). Additionally, such as temperature, pH, salinity or planting depth, which may also determine the later ability for plant establishment^{25,26}.

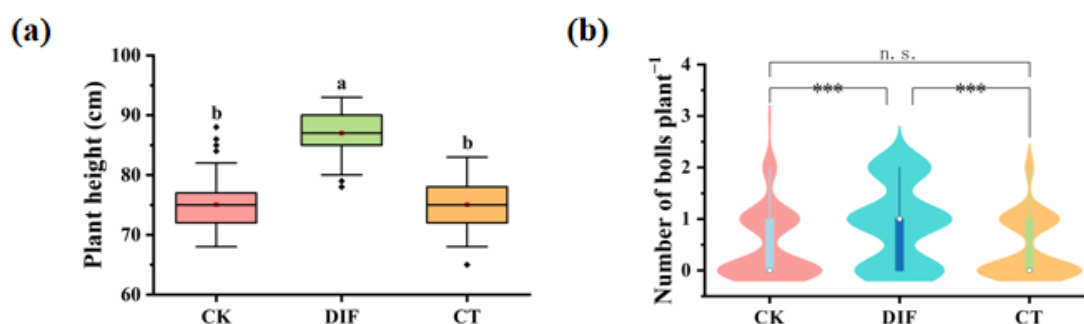


Fig. 5. Effects of CK, DIF and CT treatments on cotton plant height (a) and number of bolls per plant (b) in the field. Different lowercase letters indicate significant differences at $P < 0.05$; error bars represent SD of three replicates.

3.3.3 Effects on cotton fibre quality

The seed coating agent treatments had no significant practical impact on cotton fibre quality, and most fibre quality parameters showed no significant difference among CK, DIF and CT ($P > 0.05$) (Table 2). The upper half mean length of cotton fibre in all treatments was about 28-30 mm, the breaking strength was about 28.5-30.8 cN·tex⁻¹, the uniformity index was about 84.6-85.7%, and the micronaire was about 5.2-5.3, all of which met the national standard of high-quality cotton fibre. Although the fibre elongation rate showed a slight but significant difference among treatments ($P < 0.05$), the variation range was only 6.4%-6.6%, which is within the acceptable range of industrial cotton fibre quality standards and has no practical impact on textile production.

Table 2. Effects of CK, DIF and CT treatments on cotton agronomic characters, yield and fibre quality

| Index | Treatment | | |
|--|-------------|-------------|-------------|
| | CK | DIF | CT |
| Boll weight /g | 5.2±0.2a | 5.2±0.1a | 5.1±0.1a |
| Lint percent (%) | 40.4±0.1ab | 40.9±0.3a | 40.2±0.3ab |
| Seed yield (kg·hm ⁻²) | 170.4±13.7a | 178.4±14.1a | 185.0±12.6a |
| Lint yield (kg·hm ⁻²) | 68.8±5.5a | 73.0±5.3a | 74.3±4.9a |
| Fiber length (mm) | 29.6±0.4a | 28.4±0.3a | 29±0.4a |
| Break strength (cN·tex ⁻¹) | 30.8±0.2a | 29.0±1.0a | 28.5±0.8a |
| Uniformity index (%) | 85.7±0.8a | 84.6±0.8a | 85.6±1.2a |
| Micronaire | 5.2±0.0a | 5.3±0.1a | 5.2±0.2a |
| Elongation rate (%) | 6.6±0.0a | 6.5±0.0b | 6.4±0.0b |

Note: Different lowercase letters in the same row indicate significant differences at $P < 0.05$; all data are mean \pm SD of three replicates.

4. Discussion

This study successfully developed a novel eco-friendly cotton seed coating agent (DIF) with Difenoconazole as the main active ingredient by screening high-efficiency fungicides and optimizing the formulation through orthogonal tests. Laboratory and field trials confirmed that DIF has the dual functions of promoting cotton seedling growth and efficiently controlling Fusarium wilt, and has no negative impact on cotton fibre quality, which is an ideal alternative to traditional toxic seed coating agents for cotton Fusarium wilt control.

4.1 Mechanism of DIF in promoting cotton root growth and seedling development

DIF can significantly promote cotton seedling root growth and improve seedling vigor, which is the result of the combined effect of its active ingredients and plant growth regulators. First, the main active ingredient Difenoconazole has a high-efficiency inhibitory effect on *Fusarium oxysporum* f. sp. *vasinfectum* ($EC_{50}=0.0829 \text{ mg L}^{-1}$), which can effectively inhibit the pathogen from infecting the seed and root system, reduce root damage caused by Fusarium wilt, and ensure the normal growth and development of the root system. Second, DIF is added with plant growth regulators GA3 and IBA: GA3 can break seed dormancy, promote seed germination and hypocotyl elongation, while IBA can induce root primordium formation, promote root meristematic activity and increase root absorption area for water and nutrients. This result is consistent with the research of Qiu et al. (2005), who reported that seed coating with plant growth regulators could significantly improve crop seedling growth and stress resistance¹⁷. In addition, the film-forming agent of DIF forms a uniform and stable physical barrier on the seed surface, which can lock the seed moisture and nutrients, improve seed emergence rate and seedling uniformity, and lay a good foundation for subsequent seedling growth.

4.2 Mechanism of DIF in controlling cotton Fusarium wilt

DIF has an extremely high control efficiency (98.67%) on cotton Fusarium wilt in the field, which is attributed to the combination of chemical inhibition and physical barrier effects. On the one hand,

Difenoconazole, as a broad-spectrum systemic fungicide, can be absorbed by cotton seeds and seedlings during germination and growth, and translocated to the root system with the xylem sap, which can effectively inhibit the mycelial growth and spore germination of *Fusarium oxysporum* f. sp. *vasinfectum* in the rhizosphere soil and root tissue, and prevent the pathogen from spreading in the plant body. On the other hand, the film-forming agent of DIF forms a non-toxic and breathable physical film on the seed surface, which can effectively prevent the pathogen in the soil from invading the seed and young seedling through the seed coat and hypocotyl, and reduce the initial infection rate of the disease. In contrast, the traditional seed coating agent CT only has a contact fungicidal effect, and its active ingredients cannot be translocated in the plant body, so it can only control the surface pathogen of the seed, and the control effect on the soil-borne pathogen *Fusarium oxysporum* f. sp. *vasinfectum* is limited (control efficiency 76.32%). This result is consistent with the research of Zeng et al. (2010) on eco-friendly wheat seed coating agents, who found that systemic fungicides combined with film-forming agents had a better control effect on soil-borne diseases than traditional contact fungicides¹¹.

4.3 Effects of DIF on cotton yield and fibre quality

DIF can significantly improve cotton per plant yield traits (number of bolls per plant, plant height), and the per plant lint yield increased by 15.6% compared with CT, but the total plot yield showed no significant statistical difference with CT ($P > 0.05$), which is mainly affected by field environmental factors. The field trial was conducted in a single location

with natural disease pressure, and the local temperature and precipitation were uneven during the cotton boll development stage, which led to the uneven growth of cotton plants in different plots and offset the yield promotion effect of DIF at the plot level. In addition, the traditional seed coating agent CT has a faster initial fungicidal effect, which makes the seedling emergence rate of CT-treated cotton slightly higher in the early stage, and the final plot yield is slightly higher under the influence of microclimate factors. However, DIF can improve the plant type and stress resistance of cotton by promoting root growth and reducing disease incidence, which is more conducive to the formation of per plant yield, and has a greater potential for yield increase in multi-location and multi-season trials with uniform environmental conditions. The 36.8% cost reduction of DIF is based on the raw material cost calculation of the formulation (Difenoconazole has a lower dosage and the additives are environmentally friendly and low-cost), and detailed economic benefit analysis will be presented in subsequent multi-location field trials.

DIF has no practical negative impact on cotton fibre quality, which is one of its important advantages. Although the fibre elongation rate showed a slight significant difference among treatments ($P < 0.05$), the variation range was only 0.1%-0.2%, which is far within the acceptable range of the textile industry and has no impact on the spinning performance and product quality of cotton fibre. All other fibre quality parameters (fibre length, breaking strength, micronaire, uniformity index) showed no significant difference among treatments ($P > 0.05$),

indicating that DIF can promote cotton growth and yield without sacrificing fibre quality. This is because the active ingredients and additives of DIF are all low-toxic and have no adverse effects on cotton cell development and fibre formation, while the traditional seed coating agent CT contains high-toxicity thiram, which has a slight inhibitory effect on cotton fibre cell elongation, resulting in a slight decrease in fibre elongation rate.

4.4 Novelty and environmental benefits of DIF

Compared with the traditional toxic seed coating agents reported in previous studies (e.g., carbofuran, thiram, triadimenol)^{17,18}, DIF has obvious novelty and environmental benefits: first, DIF uses Difenoconazole, a high-efficiency and low-toxicity systemic fungicide, as the main active ingredient, with a low dosage (5.0% in the formulation) and no toxic residues in the soil and plant body, which avoids the health risks to humans and animals and the pollution to the soil and water environment; second, DIF uses environmentally friendly emulsifiers, film-forming agents and wetting dispersants, all of which are biodegradable and have no cumulative effect in the environment; third, DIF combines disease control and seedling growth promotion in one, which can reduce the application of other chemical fertilizers and pesticides in the field, and further reduce environmental pollution. Therefore, DIF has important application value for the green and sustainable development of the cotton industry.

4.5 Limitations and future research directions

This study has certain limitations that need to be improved in subsequent research: first, the field trial was conducted in a single location (Wangjiang, Anhui) and a single growing season (2024), with only one cotton cultivar (Huimian 1) tested, and the adaptability of DIF to different cotton cultivars, different ecological regions and different disease pressures needs to be further verified; second, the trial was conducted under natural disease pressure, and the control effect of DIF on different races of *Fusarium oxysporum* f. sp. *vasinfectum* (e.g., race 1, race 2, race 4) needs to be further studied through artificial inoculation experiments; third, the long-term environmental effects of DIF (e.g., on soil microbial community structure, earthworms and other soil animals) need to be further evaluated through long-term positioning trials. In the future, we will carry out multi-location, multi-season and multi-cultivar field trials, study the control effect of DIF on cotton Fusarium wilt under different artificial inoculation pressures, and evaluate its long-term environmental and economic benefits, so as to provide a more complete technical support for its large-scale application in cotton production.

5. Conclusions

This study screened Difenoconazole as the high-efficiency and low-toxicity main active ingredient through laboratory toxicity tests, and successfully developed a novel eco-friendly cotton seed coating agent (DIF) by orthogonal test formulation optimization. The optimal coating ratio of DIF is 1:100 (agent:seed), which can significantly promote cotton seed germination and seedling growth, with seedling root length and

stem height increased by 45.6% and 41.1% compared with the traditional seed coating agent CT, respectively.

Field trials confirmed that DIF has an excellent control effect on cotton Fusarium wilt with a control efficiency of 98.67%, which is significantly higher than that of CT (76.32%). DIF can significantly improve cotton per plant yield traits, with the per plant lint yield increased by 15.6% compared with CT, and has no practical negative impact on cotton fibre quality (the slight variation in fibre elongation rate is within the industrial acceptable range).

Compared with traditional toxic seed coating agents, DIF has the advantages of high disease control efficiency, promotion of seedling growth, environmental friendliness and low cost, and can be used as an ideal alternative for the green control of cotton Fusarium wilt in Anhui and other cotton-producing areas in China. This study provides a new technical solution for the integrated control of cotton soil-borne diseases and the sustainable development of the cotton industry.

Statement

Author Contributions: Conceptualization, C.C., and D.X. (Daoqing Xu); Methodology, C.C., and H.K. (Huachun Kan); Data curation and visualization, C.C. and S.L. (Shuying Li); Funding acquisition, C.C. All authors have read and agreed to the published version of the manuscript.

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