Development, Registration and Commercialization of a Microbial Fungicide for Controlling Cotton Verticillium Wilt in China

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About 500-550 ha cotton growing in China every year

Different culture practices in different cotton growing regions

--- Yangtze River region, nutritional bowl seedling first, then transplant to fields.
--- Yellow River region, direct sowing in fields.
--- Northwest region, direct sowing, high density and Drip Irrigation.

Cotton production regions

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Cotton verticillium wilt, caused by *Verticillium dahliae*, is the main and severe disease in every growing regions of China. It occurred in 50% cotton growing areas and caused 0.8 million tons of seed cotton loss per year.
No stable resistant cultivar, only tolerant cultivars (Pathogenic differentiation, most are defoliating pathotype)

No effective chemical pesticide (pathogen only infects roots)

Soil solarization and rotation were difficult to be used in the most regions of China (limited lands, and long-term survival of microsclerotia)

Other control measures need to be developed
Since 1995 we started to study bio-control of cotton verticillium wilt.

The target antagonistic strains were screened according to their abilities of antifungal compound production, biofilm formation and phosphate solubilization, and then were evaluated under greenhouse and field conditions.

*Bacillus subtilis* strain NCD-2 was isolated and showed effective control against cotton verticillium wilt in field trials.

A formulation of “*Bacillus subtilis*” was developed, and applied for registration in 2003 according to the regulations.
Only qualified companies can apply for a new pesticides including chemical and biological ones.

Application materials needed for the new product

1. Introduction of the new product
2. Chemical and biological characteristics of the product (e.g. shelf life)
3. Toxicology report (qualified unit)
4. Control efficacy, including bioassay in Lab, and field trials in 4 different provinces of 2 years (qualified unit)
5. Residue report (2 years) (qualified unit)
6. Environmental impact assessment (qualified unit)
7. Label of the product
A preparation of the microbial fungicide, $10^9$ spores/g *B. subtilis* WP, was formulated based on *B. subtilis* strain NCD-2. It was registered in 2006.
Formal field trials

Field trials showed that the control efficacies of the preparation against CVW were 60%-80% conducted by qualified units in 4 provinces in 2 years.

### Hebei

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<th>Pesticide</th>
<th>2004</th>
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<tbody>
<tr>
<td></td>
<td>DI</td>
<td>EC (%)</td>
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<tr>
<td>B. subtilis WP</td>
<td>10.4 f</td>
<td>80.5</td>
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<tr>
<td>30% Ethylicin EC</td>
<td>35.1 b</td>
<td>34.1</td>
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<td>Control</td>
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### Shandong

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<td>B. subtilis WP</td>
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### Henan

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### Xinjiang

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<td></td>
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<td>EC (%)</td>
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Fermentation optimization

The fermentation conditions of strain NCD-2 were optimized in flasks, 500L, 5000L and 15000L fermentation tanks by adjusting Carbon source and Nitrogen sources, respectively. The fermentation level was increased 20 times, and fermentation period was 25% shortened.
Post-processing method of strain NCD-2 was also modified by using continuous centrifugation and spray drying techniques.
Application in different cotton areas

Yellow River region

Location: Hebei, Henan and Shandong
Demo area: 200 mu, about 13.3 ha
Treatment: Seed coating 1:25 and 1:50, direct sowing
Control efficacy: 34%–71%
Application in different cotton areas

Yangtze River region

Location: Hubei, Hunan, Anhui and Jiangsu
Demo area: 5950 mu, about 396.6 ha
Treatment: Seed dressing 1:10, and transplanting
Control efficacy: 62%-79%
Application in different cotton areas

Northwest region

Location: Xinjiang
Demo area: 7566 mu, about 504.4 ha
Treatment: Drip irrigation, 9 kg/ha, 2 times
Control efficacy: 43%-61%

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Control of cotton verticillium wilt (seed dressing, 1:10 seed weight)
Control of cotton fusarium wilt (seed dressing)

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Control of eggplant verticillium wilt (root drenching with the bio-control agent, 500X, 250mL/plant, 2 times)

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Control of tomato replant disease (application in the planting hole, 0.5g/plant)

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Control of yam root rot

- Tuber treatment, control efficacy 73.7%
- Fresh root weight increased 83.5%-165.2% with treatment
Potential action mechanisms of strain NCD-2

- Production of antifungal compounds
- Destroy microsclerotia (MS) of *V. dahliae*
- Colonization on the surface of cotton root
- Growth promotion

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The preparation “$10^9$ spores/g B. subtilis WP” was the only one bio-fungicide registered in China for controlling cotton verticillium wilt, and it was used in many places of cotton production areas.

Mass production technology of the preparation was optimized, and it accelerated the commercialization of the product.

Some potential action mechanisms of strain NCD-2 were discussed.
Acknowledgements

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