

Risk assessment of *Ralstonia solanacearum* to fluazinam and evaluation of combinatory fungicides

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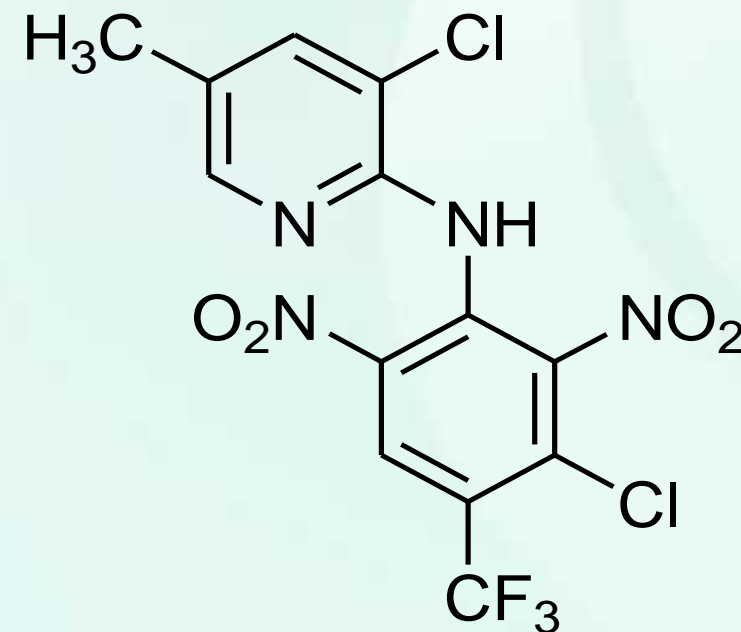
Tobacco Bacterial Wilt

- **Destructive soil-borne vascular disease**
- *Ralstonia solanacearum*
- **Chemical control: Antibiotics, Copper fungicides, Sulfur fungicides**



Fluazinam

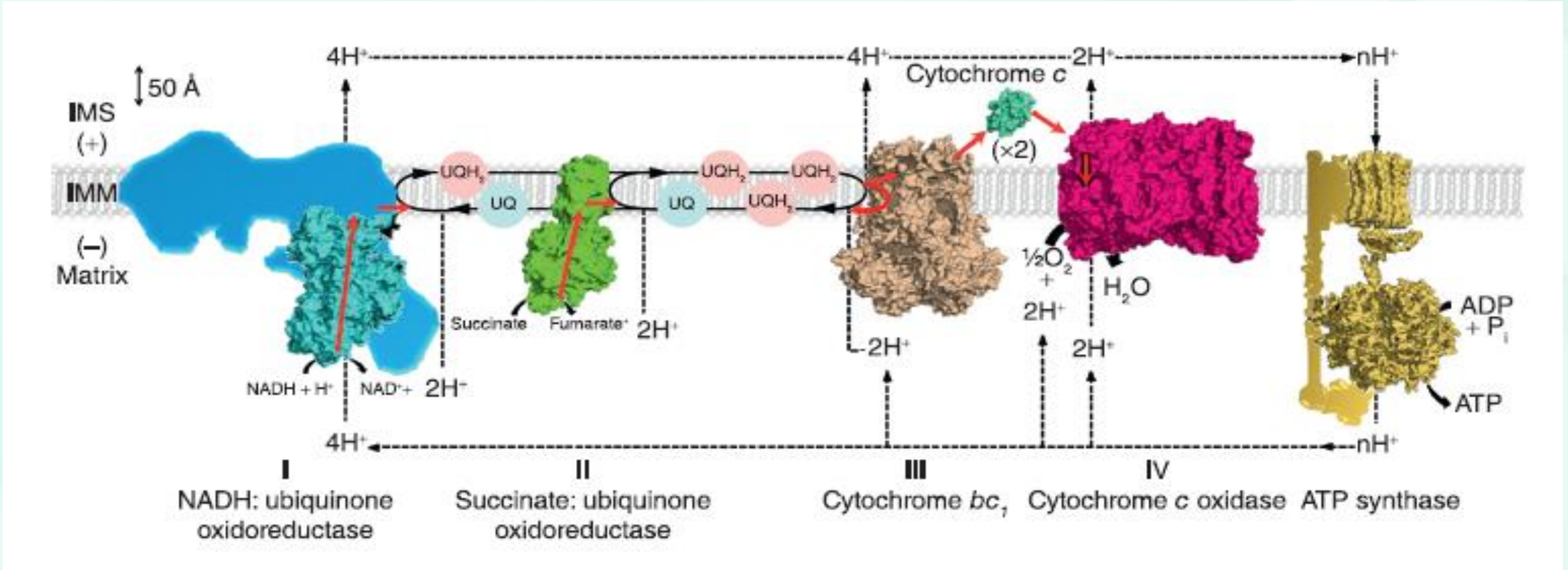
Fungicide	Fluazinam
Chemical group	2,6-dinitro-Anilines
Company	Ishihara Co., LTD
Registration object	Pepper Blight, Potato late blight, Chinese cabbage clubroot
Mode of action	Protective fungicide
Toxicity	Low





Mechanism of action

Uncoupling of oxidative phosphorylation



Objective

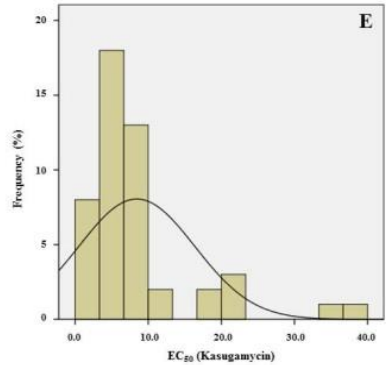
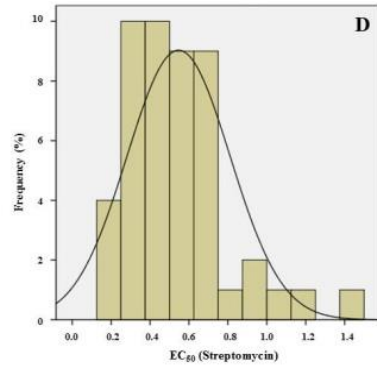
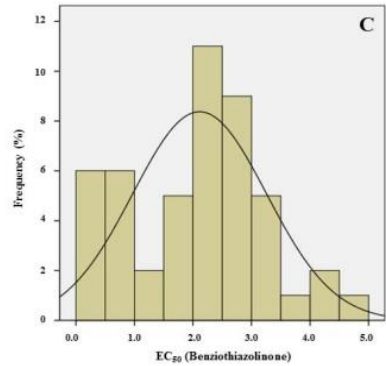
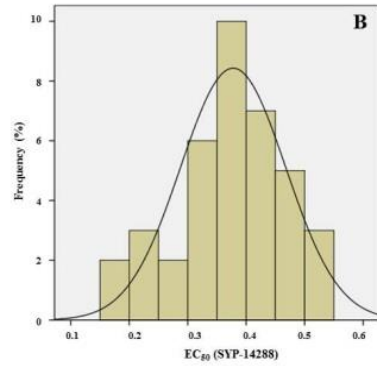
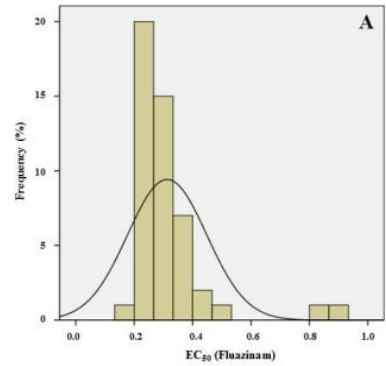
I. Risk Assessment of *Ralstonia solanacearum* to fluazinam

II. Integrated fluazinam and five fungicides to control tobacco bacterial wilt

Baseline sensitivity



中国烟草
CHINA TOBACCO



Fungicide	EC ₅₀ Range (μg/mL)	Average EC ₅₀ (μg/mL)
Fluazinam	0.147 1~0.890 4	0.305 0
SYP-14288	0.018 7~0.053 4	0.037 8
Benziothiazolinone	0.099 5~4.825 3	2.429 9
Streptomycin	0.187 6~1.407 3	0.546 3
Kasugamycin	4.157 7~37.628 2	9.927 3

Resistance factors and stability of fluazinam-resistant mutants

Resistance type	Isolate	EC ₅₀ (μg/mL)					Resistance factor				
		1 st	10 th	20 th	30 th	40 th	1 st	10 th	20 th	30 th	40 th
Sensitive isolate	29	0.3592	0.3358	0.3197	0.3871	0.2597	--	--	--	--	--
Low resistant mutants	FD50-29	6.4840	5.9539	6.8459	8.2492	4.4045	18.05	17.73	21.41	21.31	16.96
	FD100-29	3.9274	10.4765	5.1848	5.2311	5.5589	10.94	31.20	16.22	13.51	21.41
Sensitive isolate	32	0.3466	0.2707	0.3135	0.3278	0.2365	--	--	--	--	--
Low resistant mutants	FD50-32	2.4571	2.1791	4.0109	5.6468	5.4145	7.09	8.05	12.80	17.23	22.89
	FD100-32	2.8071	3.8017	3.3328	3.5648	5.7056	8.10	14.04	10.63	10.87	24.13
Sensitive isolate	33	0.3754	0.2730	0.3067	0.3753	0.2657	--	--	--	--	--
Highly resistant mutants	FD50-33	0.0986	8.9827	15.7132	13.2747	61.4739	0.26	32.90	51.23	35.37	231.38
	FD100-33	2.5375	10.4937	9.8682	11.7950	39.3120	6.76	38.43	32.17	31.43	147.97
Sensitive isolate	35	0.3699	0.2642	0.3204	0.3651	0.2308	--	--	--	--	--
Highly resistant mutants	FD50-35	2.3881	5.6855	20.2363	5.8987	42.3486	6.46	21.52	63.16	16.16	183.47
Intermediate resistant mutants	FD100-35	6.9595	31.9028	20.4330	11.1337	9.3372	18.81	120.73	63.78	30.50	40.45
Sensitive isolate	42	0.2545	0.2374	0.3587	0.3133	0.2187	--	--	--	--	--
Intermediate resistant mutants	FD50-42	9.3449	14.6329	21.0938	9.7931	11.5797	36.72	61.64	58.80	31.25	52.95
Sensitive isolate	43	0.3715	0.2389	0.2769	0.3067	0.1864	--	--	--	--	--
Intermediate resistant mutants	FD50-43	13.7871	7.3017	16.2606	12.0975	16.6456	37.11	30.56	58.72	39.44	89.28
Highly resistant mutants	FD100-43	21.2261	16.4888	27.8214	63.2640	28.3705	57.14	69.02	100.46	206.25	152.17
Sensitive isolate	49	0.3158	0.1888	0.2719	0.2309	0.2025	--	--	--	--	--
Highly resistant mutants	FD50-49	6.8679	17.3231	3.4932	2.5184	36.9461	21.75	91.77	12.85	10.90	182.45
Intermediate resistant mutants	FD100-49	6.5564	4.4432	4.2747	4.3616	19.8554	20.76	23.54	15.72	18.89	98.05
Sensitive isolate	57	0.3489	0.2177	0.2610	0.1989	0.1687	--	--	--	--	--
Low resistant mutants	FD50-57	2.9812	3.5638	3.7102	2.6552	3.4524	8.54	16.37	14.22	13.35	20.46
Highly resistant mutants	FD100-57	3.3337	4.8145	7.1825	10.6284	34.9184	9.56	22.12	27.52	53.43	206.93

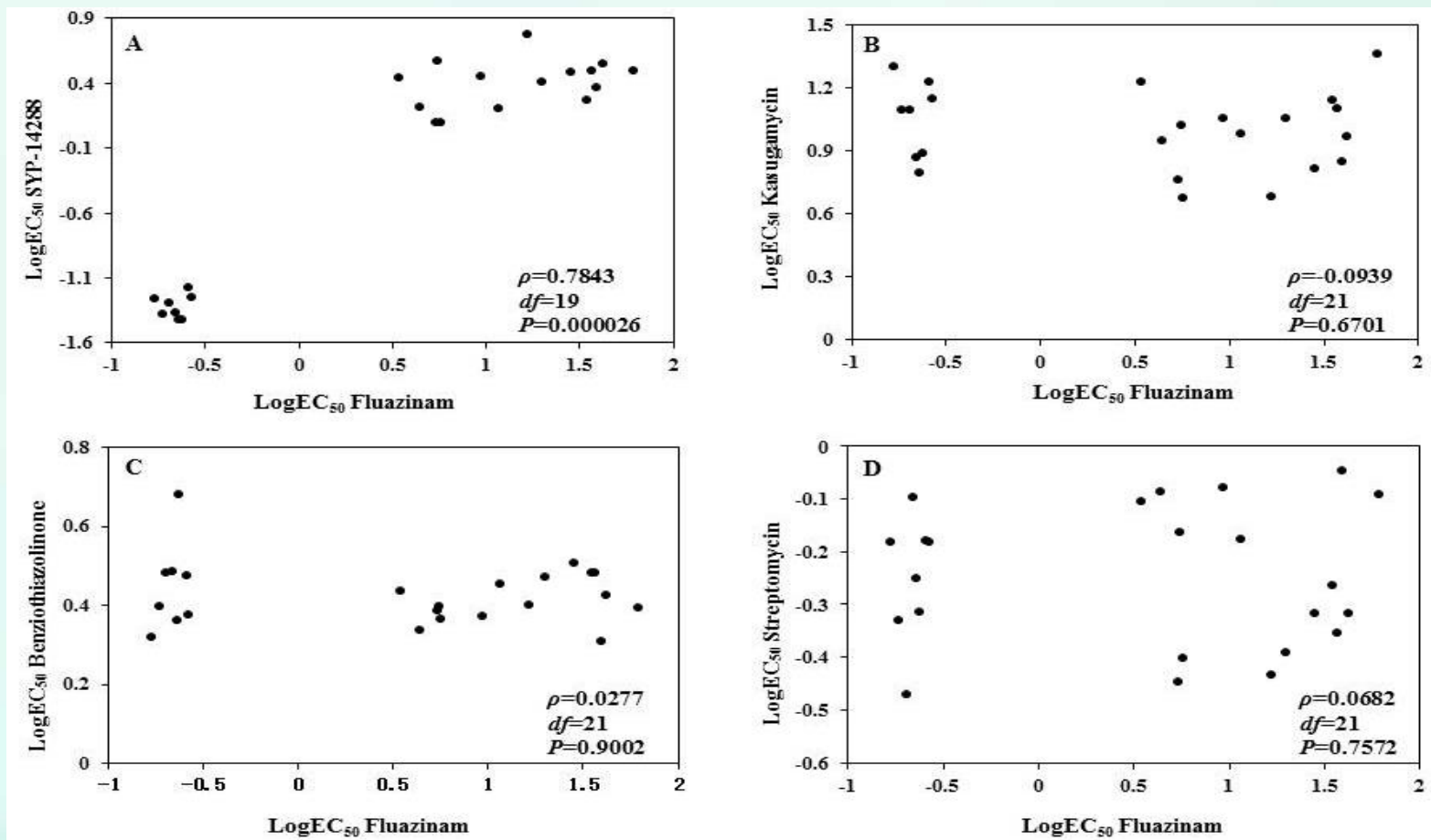
Fitness of fluazinam-resistant mutants



Resistance type	Isolate	Temperature sensitivity					Incidence (%)	Disease index (%)	Lesion length (cm)
		7 °C	15 °C	25 °C	30 °C	37 °C			
Sensitive isolate	29	0.097a	0.120b	0.358a	0.500a	0.167b	100.00	33.33	2.1 a
Low resistant mutants	FD50-29	0.100a	0.246a	0.289b	0.588a	0.382a	100.00	33.33	1.8 a
	FD100-29	0.086b	0.083c	0.154c	0.493a	0.233b	100.00	88.89	0.6 a
Sensitive isolate	32	0.102a	0.121a	0.412b	0.565c	0.304b	50.00	16.67	2.2 a
Low resistant mutants	FD50-32	0.096b	0.108ab	0.495a	0.781a	0.372a	100.00	50.00	0.6 a
	FD100-32	0.091c	0.094b	0.250c	0.742b	0.299b	100.00	50.00	1.6 a
Sensitive isolate	33	0.099b	0.112a	0.420b	0.451c	0.137c	100.00	25.93	2.4 a
Highly resistant mutants	FD50-33	0.099b	0.115a	0.490a	0.602a	0.368a	100.00	33.33	1.4 b
	FD100-33	0.104a	0.105a	0.264c	0.580b	0.194b	83.33	50.00	0.6 c
Sensitive isolate	35	0.108a	0.183b	0.474b	0.889a	0.228b	0.00	0.00	0.0 b
Highly resistant mutants	FD50-35	0.100a	0.219a	0.588a	0.910a	0.502a	100.00	48.15	1.1 a
Intermediate resistant mutants	FD100-35	0.105a	0.115c	0.372c	0.865a	0.530a	16.67	16.67	0.0 b
Sensitive isolate	42	0.099a	0.142a	0.302b	0.626a	0.459a	60.00	42.22	1.0 a
Intermediate resistant mutants	FD50-42	0.106a	0.135b	0.506a	0.603b	0.443b	100.00	51.11	1.8 a
Sensitive isolate	43	0.101a	0.101a	0.345a	0.778a	0.612a	0.00	0.00	0.0 a
Intermediate resistant mutants	FD50-43	0.105a	0.116a	0.318b	0.749b	0.535b	20.00	6.67	0.4 a
Highly resistant mutants	FD100-43	0.102a	0.103a	0.223c	0.714c	0.510b	0.00	0.00	0.0 a
Sensitive isolate	49	0.107a	0.208a	0.614a	1.157a	0.591b	100.00	74.07	5.9 a
Highly resistant mutants	FD50-49	0.103ab	0.179b	0.464b	0.739b	0.774a	100.00	77.78	6.2 a
Intermediate resistant mutants	FD100-49	0.094b	0.104c	0.287c	0.643c	0.333c	100.00	74.07	6.6 a
Sensitive isolate	57	0.112a	0.227a	0.571a	1.036a	0.445a	100.00	100.00	6.8 a
Low resistant mutants	FD50-57	0.096b	0.093b	0.270b	0.913b	0.126b	100.00	91.11	4.0 a
Highly resistant mutants	FD100-57	0.089b	0.095b	0.360b	0.724c	0.563a	100.00	66.67	3.7 a

Cross-resistance

Cross-resistance was detected between fluazinam and the uncoupler fungicide SYP-14288, but not between fluazinam and kasugamycin, benziothiazolinone, streptomycin.



Synergistic activity test between fluazinam and nano fungicides

Fluazinam: Nano sulfur	EC ₅₀ (µg/mL)	Synergy Ratio	Synergistic Interaction	Fluazinam: Nano-copper	EC ₅₀ (µg/mL)	Synergy Ratio	Synergistic Interaction	Fluazinam: Nano-silver	EC ₅₀ (µg/mL)	Synergy Ratio	Synergistic Interaction
1: 0	0.207 3			1: 0	0.166 7			1: 0	0.309 3		
0: 1	17.399 8			0: 1	21.479 7			0: 1	15.420 4		
80: 1	0.281 2	0.746 5	Additive	80: 1	0.142 2	1.186 7	Additive	80: 1	0.387 3	0.808 2	Additive
40: 1	0.304 2	0.698 4	Additive	40: 1	0.236 8	0.721 4	Additive	40: 1	0.497 1	0.637 4	Additive
20: 1	0.330 5	0.658 3	Additive	20: 1	0.220 3	0.794 1	Additive	20: 1	1.789 5	0.181 3	Antagonism
4: 1	0.433 6	0.596 0	Additive	4: 1	0.269 8	0.770 9	Additive	4: 1	1.179 3	0.326 2	Antagonism
1: 1	1.514 3	0.270 6	Antagonism	1: 1	0.894 0	0.370 1	Antagonism	1: 1	1.859 2	0.326 1	Antagonism
1: 4	1.766 9	0.560 0	Additive	1: 4	2.047 7	0.394 8	Antagonism	1: 4	3.060 4	0.467 7	Antagonism
1: 20	3.023 8	1.162 7	Additive	1: 20	2.680 2	1.130 7	Additive	1: 20	3.295 2	1.406 7	Additive
1: 40	3.608 0	1.595 5	Synergism	1: 40	4.909 8	1.062 3	Additive	1: 40	5.212 6	1.349 7	Additive
1: 80	6.040 6	1.423 3	Additive	1: 80	7.203 3	1.156 5	Additive	1: 80	9.459 6	1.016 8	Additive

Synergistic activity test between fluazinam and kasugamycin, benziothiazolinone

Fluazinam: Kasugamycin	EC₅₀ (µg/mL)	Synergy Ratio	Synergistic Interaction	Fluazinam: Benziothiazolinone	EC₅₀ (µg/mL)	Synergy Ratio	Synergistic Interaction
1: 0	0.1150	--	--	1: 0	0.1168	--	--
0: 1	3.6844	--	--	0: 1	1.4918	--	--
80: 1	0.0967	1.2031	Additive	80: 1	0.1097	1.0772	Additive
40: 1	0.0923	1.2762	Additive	40: 1	0.0874	1.3677	Additive
20: 1	0.1015	1.1875	Additive	20: 1	0.0865	1.4129	Additive
4: 1	0.1263	1.1293	Additive	4: 1	0.0888	1.6135	Synergism
1: 1	0.2081	1.0716	Additive	1: 1	0.1972	1.0990	Additive
1: 4	0.4693	1.0893	Additive	1: 4	0.2784	1.5978	Synergism
1: 20	1.3266	1.1207	Additive	1: 20	0.2562	3.7314	Synergism
1: 40	1.5499	1.3529	Additive	1: 40	0.2283	5.0773	Synergism
1: 80	1.7244	1.5447	Synergism	1: 80	0.2378	5.4777	Synergism

Conclusion

- **The sensitivity baselines of the five fungicides to tobacco bacterial wilt were normal. The 15 mutants with different resistance levels had strong temperature adaptability and pathogenicity. The results of comprehensive evaluation showed that the resistance risk of tobacco bacterial wilt to fluazinam was moderate to high.**
- **The mixtures of fluazinam with benziothiazolinone, kasugamycin, nano sulfur at the ratio of 1:80, 1:80, 1:40 respectively had synergistic effects. The additive effect of fluazinam and nano-copper, nano-silver at the ratio of 80:1, 1:20 was the most obvious.**

Thank you for your attention !