Selecting formulation components and tank mixtures for optimum performance

ABIM 2023

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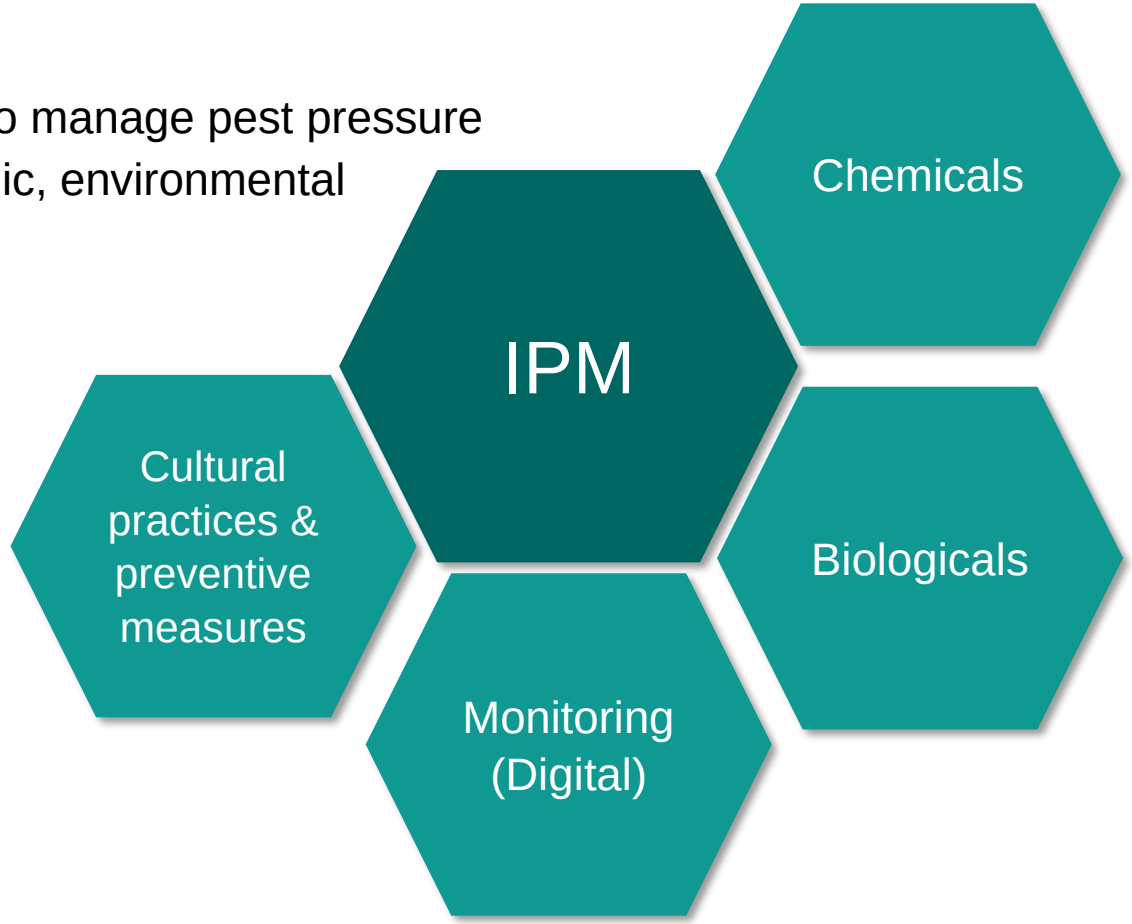
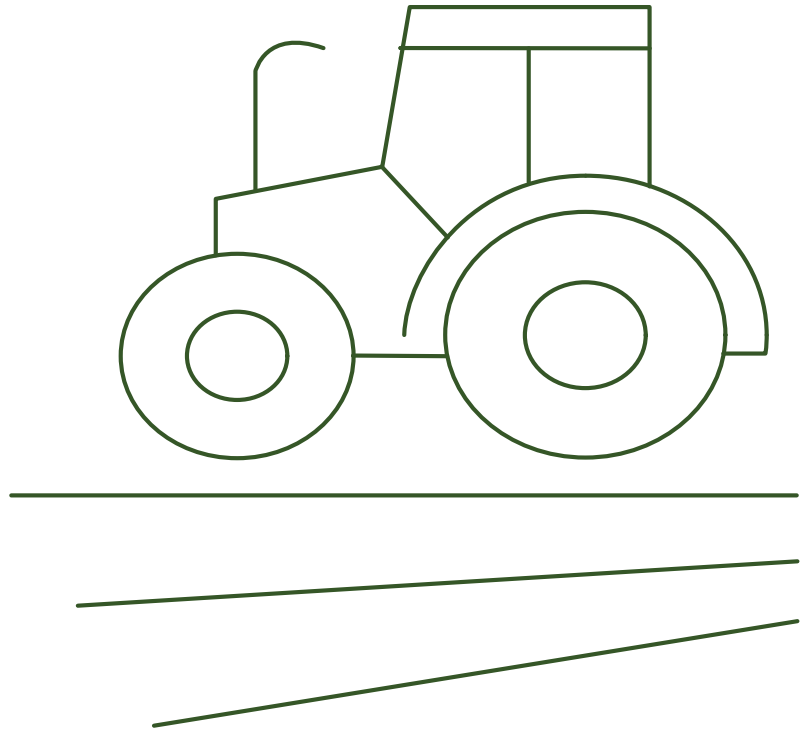
Care Chemicals
Application Development - Crop Solutions
25 October 2023

Biologicals part of the Integrated Pest Management of the future

A sustainable model to manage pest pressure and minimize economic, environmental and health exposure.





Clariant at a Glance – a Globally Leading Company in Specialty Chemicals

Crop Solutions – Solutions for the agriculture industry

Full range portfolio for Crop Solutions

ADJUVANTS	BIOCIDES *	EMULSIFIERS	SOLVENTS	WETTING AND DISPERSING AGENTS
Synergen®	Nipacide®	Emulsogen®	Genagen®	Dispersogen® Genapol®

Setup for testing products for biologicals available at Clariant

Biocompatibility tests

- CFU counting
- Cell viability assays

Formulation stability

- Physical and chemical testing

Laboratory application tests

- Contact angle measurements
- Rainfastness testing
- UV absorbance

Greenhouse and field trials

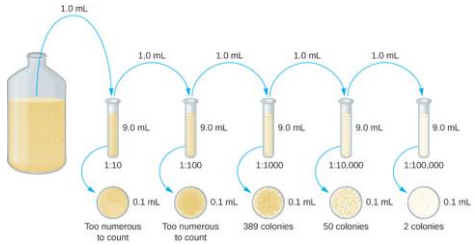
- Wide array of advanced devices and methods for studying the behaviour of actives and plants

Screening method at Clariant: biocompatibility

DIRECT

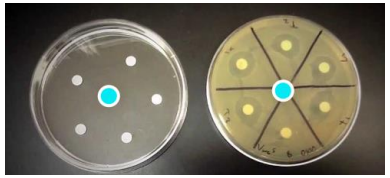
CFU counting

- **Standard** equipment available at every microbiology lab
- Established quality control method for viability



Disk-diffusion method

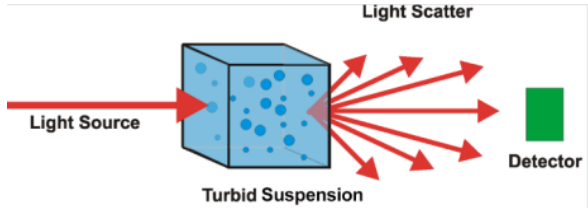
- Allows for testing of higher amount of samples
- Established method for **antibiotic testing**



INDIRECT

Turbidity (OD600)

- Easy to implement
- Established method to monitor fermentation processes
- Allows automation and high throughput

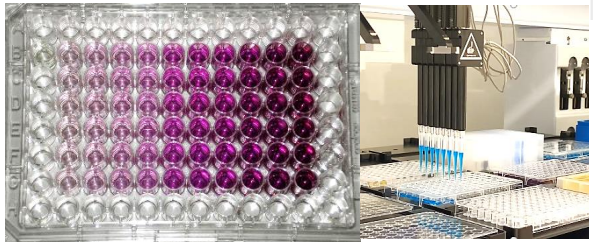


Colorimetric assays (Tetrazolium salts)

- Allows automation and high throughput

Fluorometric assays (Resazurin)

- Relatively easy to implement
- Several output signals
- Allows **automation and high throughput**



Fluorometric method as High-throughput choice

Resazurin

- water-soluble redox dye
- stable in buffered culture medium,
- non-toxic and permeable through cell membrane suitable for aerobic microorganisms.

Resorufin

- highly fluorescent
- might suffer quenching and further reduction to colorless dihydroresorufin.

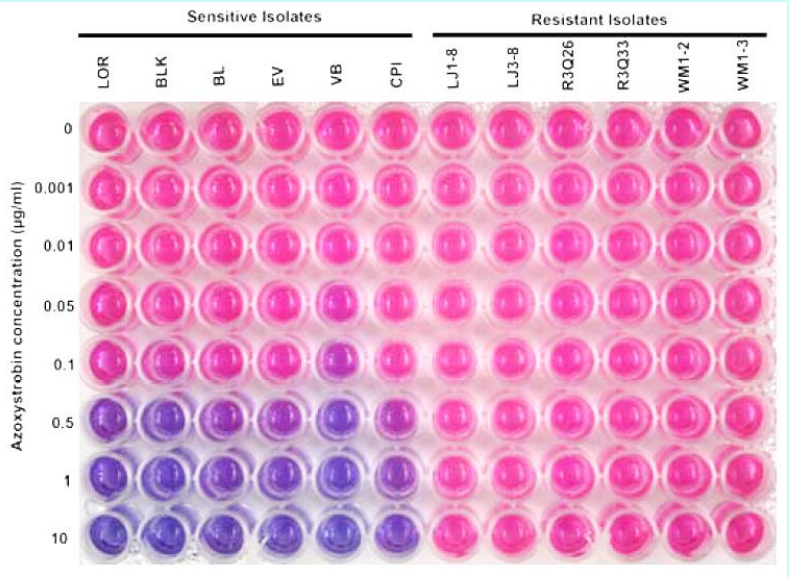
Excessively **long incubation times are NOT recommended.

Action:

intermediate electron acceptor in the electron transport chain and can be **reduced to fluorescent Resorufin** by NADPH, FADH, FMNH, NADH.

Suitable for **end point** determinations AND for **time resolved** analysis.

Extensively used for testing **microbial sensitivity**.

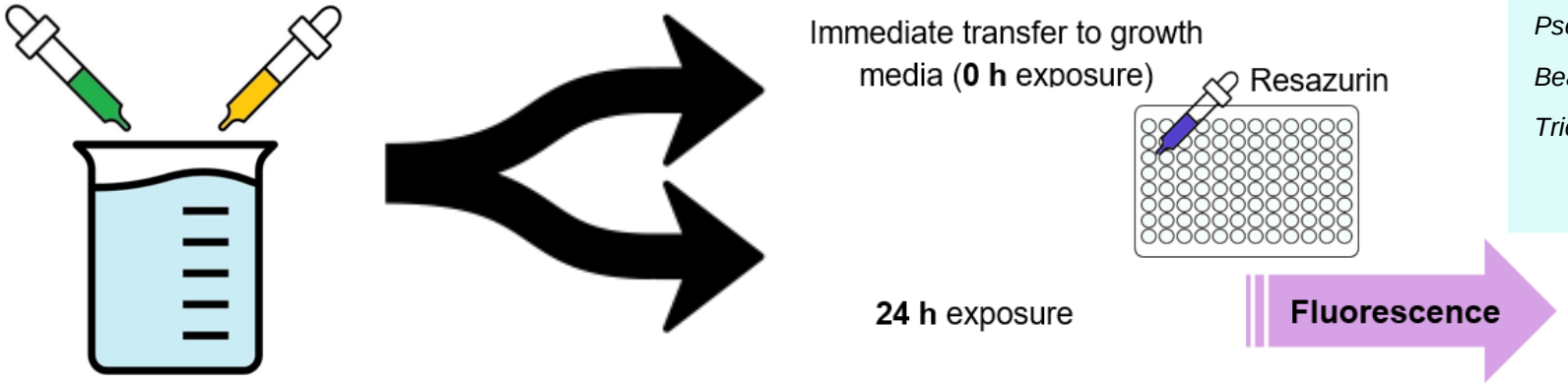


Dalecki, A.G. et al., 2016. Targeting biofilm associated *Staphylococcus aureus* using Resazurin based drug-susceptibility assay. J. Vis. Exp. 1–6.

O'Brien, J. et al., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur J Biochem. 267, 5421-5426.

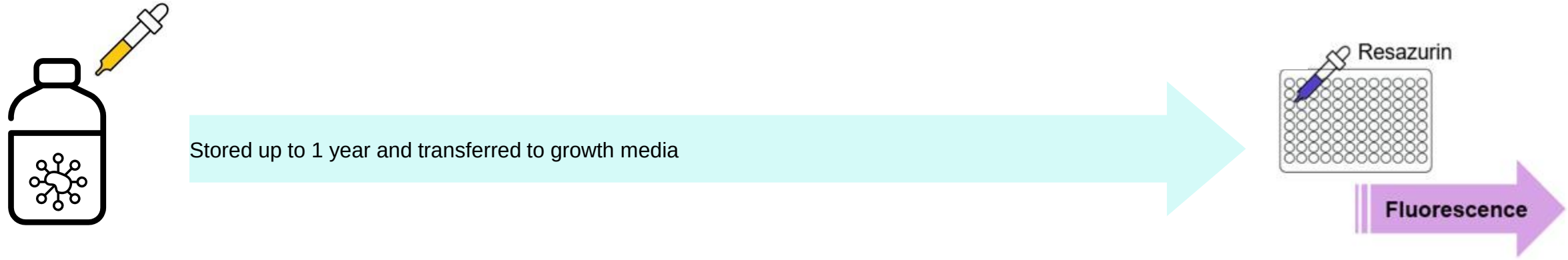
http://dx.doi.org/10.1094/PDIS-12-11-1037-RE

Biocompatibility with microorganisms in Spray Tank



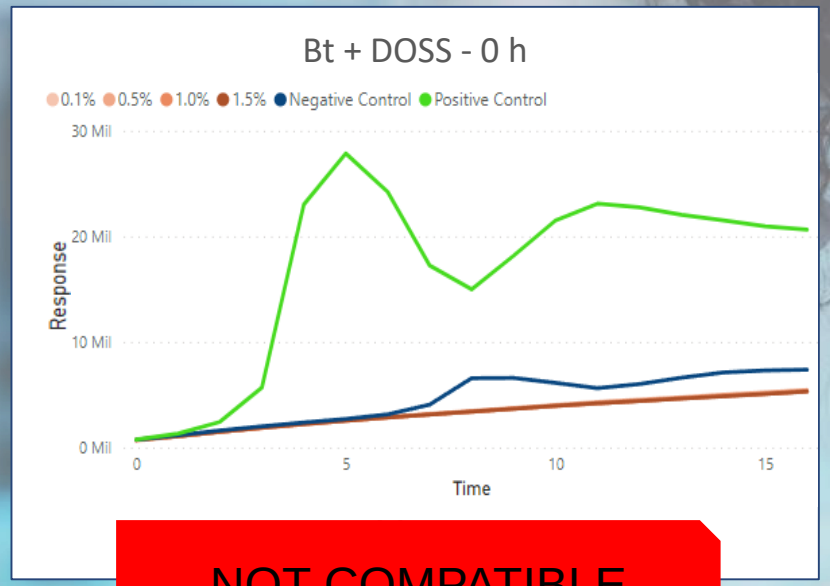
Microorganism	Formulation type	Use rate
Bacillus thuringiensis (G+)	WG	0.1%
Bacillus amyloliquefaciens (G+)	SC	2%
Pseudomonas chlororaphis (G-)	FS	2%
Beauveria bassiana (F)	OD	0.13%
Trichoderma atroviride (F)	WG	0.20%

Biocompatibility with microorganisms In-can

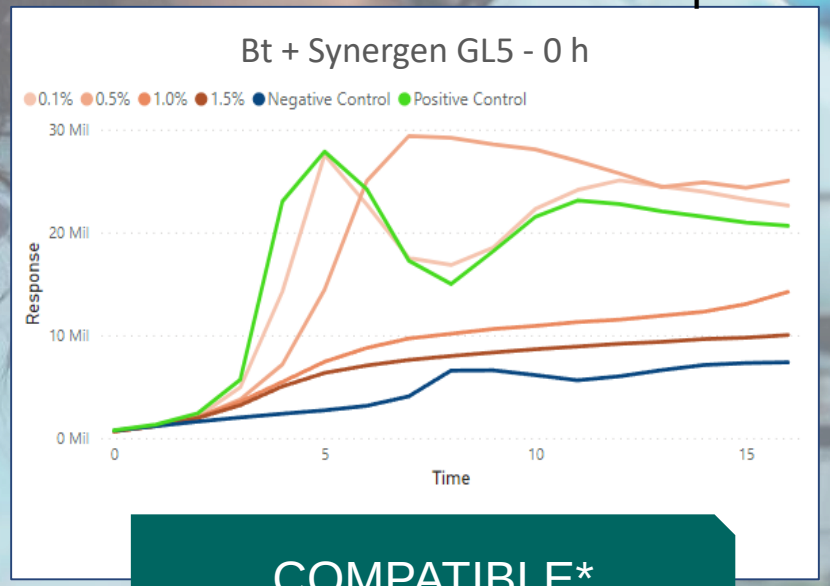


Results of high-throughput fluorescence and viability curves
Biocompatibility requires data interpretation

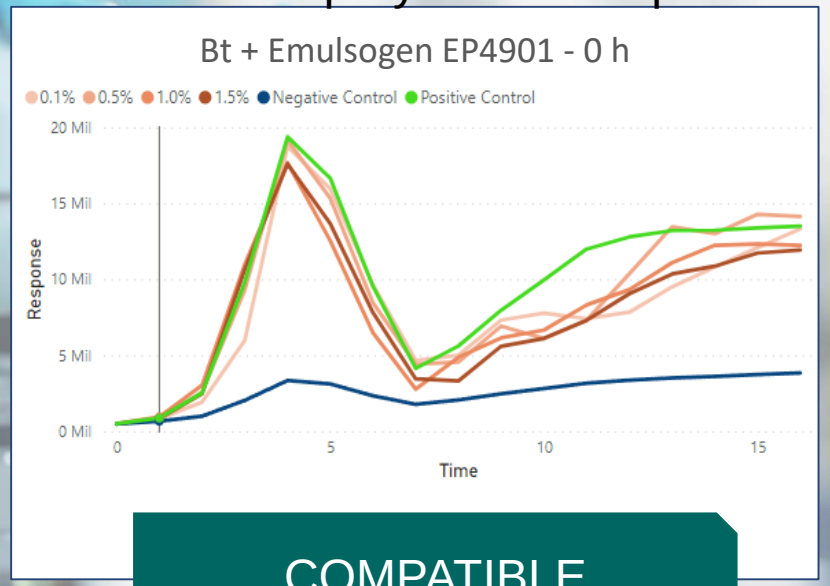
Experimental results – Spray tank 0h exposure



NOT COMPATIBLE



COMPATIBLE*



COMPATIBLE

* Risk of inhibition depending on concentration and exposure

Data management and result processing, many datapoints and growing…

Selection of tank mix or in-can

Biological Data

in-can Data

Microorganism selection

Select Organism Type & Name.

- Bacteria
  - Bacillus amyloliquefaciens (G+); SC; 2%
  - Bacillus thuringiensis (G+); WG; 0,10%
  - Pseudomonas chlororaphis (G-); FS; 2%
- Fungi
  - Beauveria bassiana; OD; 0,13%
  - Trichoderma atroviride; WG; 0,20%

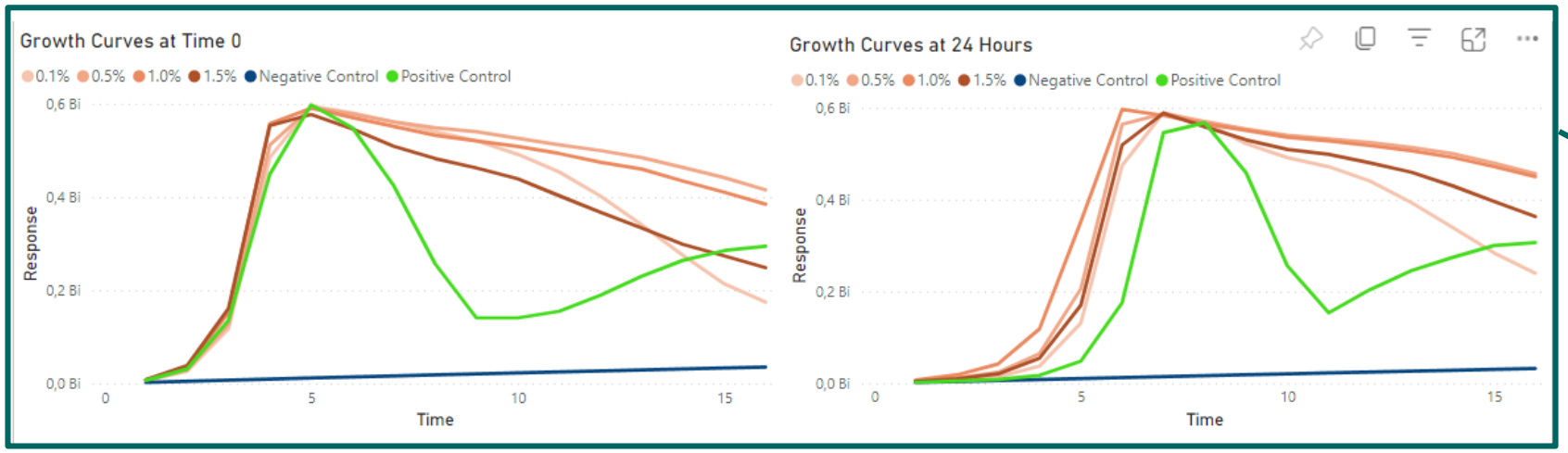
Select Sample by Function.

Pesquisar

- Selecionar tudo
- Adjuvants
- Antifoaming agents
- Candidates for rainfastness agents
- Candidates for UV protectors
- Carrier/ cosolvents
- Dispersing agents
- Emulsifiers
- OMRI
- Other
- Preservatives
- Rheology modifiers
- Wetting agents

Period	0				24			
Sample	1.5%	1.0%	0.5%	0.1%	1.5%	1.0%	0.5%	0.1%
	130	128	127	126	188	175	175	139
	16	10	7	16	18	18	16	15
	171	187	167	154	226	218	216	203
	186	183	196	161	247	214	188	158
	128	154	148	141	164	171	178	162
	153	155	157	151	217	171	179	165
	129	128	127	127	156	142	195	139
	199	183	186	144	205	197	239	234
	16	16	16	16	20	20	20	20
	149	161	154	167	242	240	241	225
	153	155	152	140	211	211	220	199
	238	236	208	167	243	244	242	226
	172	148	131	120	191	208	190	189
	121	122	118	114	211	180	209	158
	141	138	131	124	176	188	196	197
	170	157	139	125	235	225	230	204
	198	195	172	129	238	238	230	173

Coformulant overview for quick identification



Growth curves at tank mix exposure times
0 h (left)
24 h (right)

Clariant High-throughput Microbial Solutions

Sustainable

Data based decision making

Selection of the best option for further developments

Dr. Katharina Grundler
Care Chemicals
AD Crop Solutions
25.10.2023

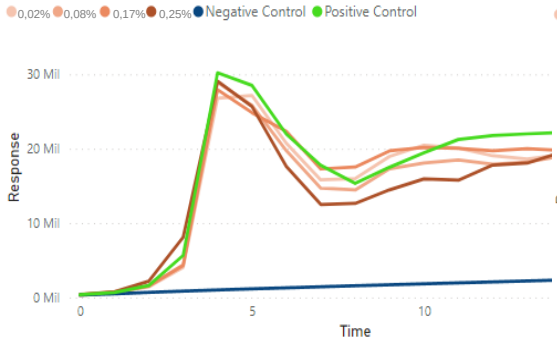
Greater chemistry

Novel Solution: Optimize your microbe-based formulation

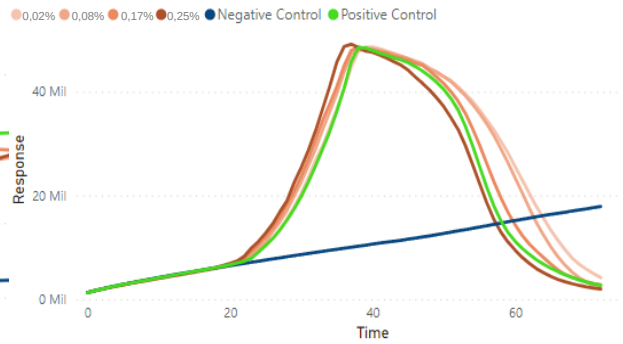
Very good biocompatibility ✓

In-can (at 0,1%)	Viability assay Spray Tank (0,02 to 0,25%)				
B. amyloliquefaciens	*B. thuringiensis*	*B. amyloliquefaciens*	*P. chlororaphis*	*Trichoderma atroviride*	*Beauveria bassiana*
✓	✓	✓	✓	✓	✓

Spray Tank, *Bt,* Viability curve at 0h



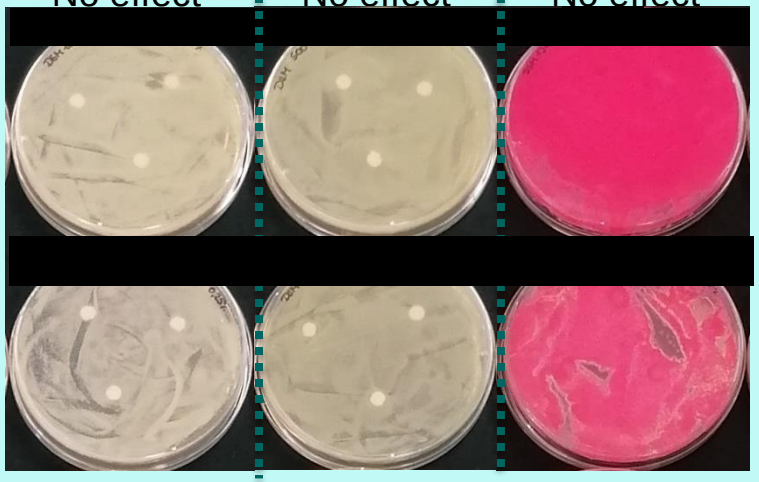
Spray Tank, *T. atroviride,* Viability curve at 0h



✓ Compatible at all tested concentrations under a tank-mix condition/ 24 h incubation or in-can for 12months

Disk diffusion assay (0,17% and 0,25%)

Bacillus subtilis DSM10	*Pseudomonas chlororaphis* DSM50083	*Beauveria bassiana* DSM1344
No effect	No effect	No effect



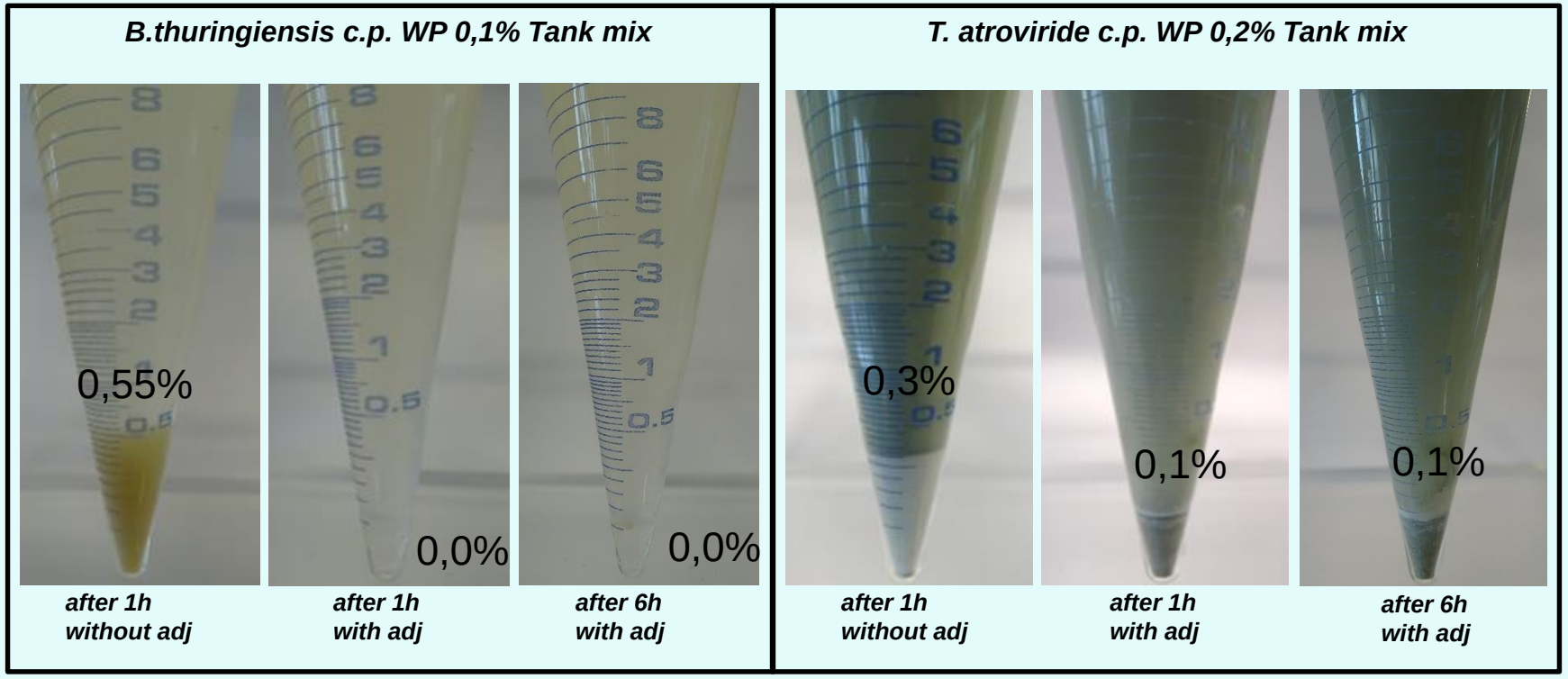
Negative control - DOSS

Novel Solution for Biologicals: Adjuvant

Function:
New dispersant/stabilizer agent for biologicals in low use concentration (pH stable, high electrolyte load)

In-can dose ~ 0,8%

Tank-mix dose 0,1-0,2%



IMPROVED TANK-MIX STABILITY

Product solutions for biologicals

	Clariant product	Formulation type	In-Can (1-10%)	Spray Tank (0.1 to 1.5%)					Regulatory		
			Bacillus amyl.	*Bacillus turigensis*	*Bacillus amyl.*	*Pseudomonas chlororaphis*	*Trichoderma atroviride*	*Beauveria bassiana*	REACH	EPA	OMRI eligible
EMULSIFIERS	Emulsogen EL types	SC, OD, SE	✓	✓	✓	✓	✓	✓	■	■	■
	Emulsogen TS types	SC, OD, SE	✓	✓	✓	✗	✓	✓	■	■	■
	Emulsogen M	SC, OD, SE	✓	✓	✓	✓	✓	✓	■	■	■
	Emulsogen MTP types	OD, SE	✓	✓	✓	✓	✓	✓	■	■	
	Emulsogen ELO 200	OD	✓	✓	✓	✓	✓	✓	■	■	

✓ Compatible at all tested concentrations under a tank-mix condition/ 24 h incubation or in-can for 6months
✓ Compatible at some of the tested concentrations under a tank-mix condition/ 24 h incubation or in-can for 6months
✓ Compatible with delayed growth at some or all tested concentrations under a tank-mix condition/ 24 h incubation or in-can for 6months

Product solutions for biologicals

	Clariant product	Chemistry	In-can (1- 10%)	Spray Tank (0.1 to 1.5%)					Regulatory		
			Bacillus amyl.	*Bacillus turigensis*	*Bacillus amyl.*	*Pseudomonas chlororaphis*	*Trichoderma atroviride*	*Beauveria bassiana*	REACH	EPA	OMRI eligible
WETTING & DISPERSANTS	Dispersogen LFH	SC, SE	✓	✓	✓	✓	✓	✓	▪	▪	
	Genapol PF 80	SC, SE, WP, WG	✓	✓	✓	✓	✓	✓	▪	▪	▪
	Dispersogen PSL 100	SC, OD	✓	✓	✓	✓	✓	✓	▪	▪	
	Genapol 10500	SC, OD, SE, WG	✓	✓	✓	✓	✓	✓	▪	▪	▪

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Product solutions for biologicals

	Clariant product	Chemistry	In-can (1- 10%)	Spray Tank (0.1 to 1.5%)					Regulatory		
			Bacillus amyl.	*Bacillus turigensis*	*Bacillus amyl.*	*Pseudomonas chlororaphis*	*Trichoderma atroviride*	*Beauveria bassiana*	REACH	EPA	OMRI eligible
ADJUVANTS	Synergen SOC	OD, SE	✓	✓	✓	✓	✓	✓	▪	▪	
SOLVENTS	Genagen NBP	SC, SE	✓	✓	✓	✓	✓	✓	▪	▪	

✓ Compatible at all tested concentrations under a tank-mix condition/ 24 h incubation or in-can for 6 months
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✓ Compatible with delayed growth at some or all tested concentrations under a tank-mix condition/ 24 h incubation or in-can for 6 months

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Thanks to the whole team:

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