



Professor Duraisamy Saravanakumar
Director, School for Graduate Studies and Research

THE UNIVERSITY OF THE WEST INDIES
ST AUGUSTINE

Pesticide Risk Reduction

Focus on Health Impacts & Alternatives

Pests & Diseases



Use of chemical Pesticides



HHPs

Trade impacts

Development of resistance



Impact of pesticides

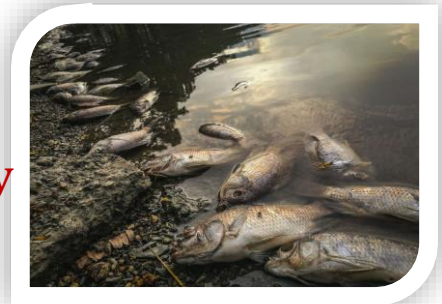
Human Health



Food Safety



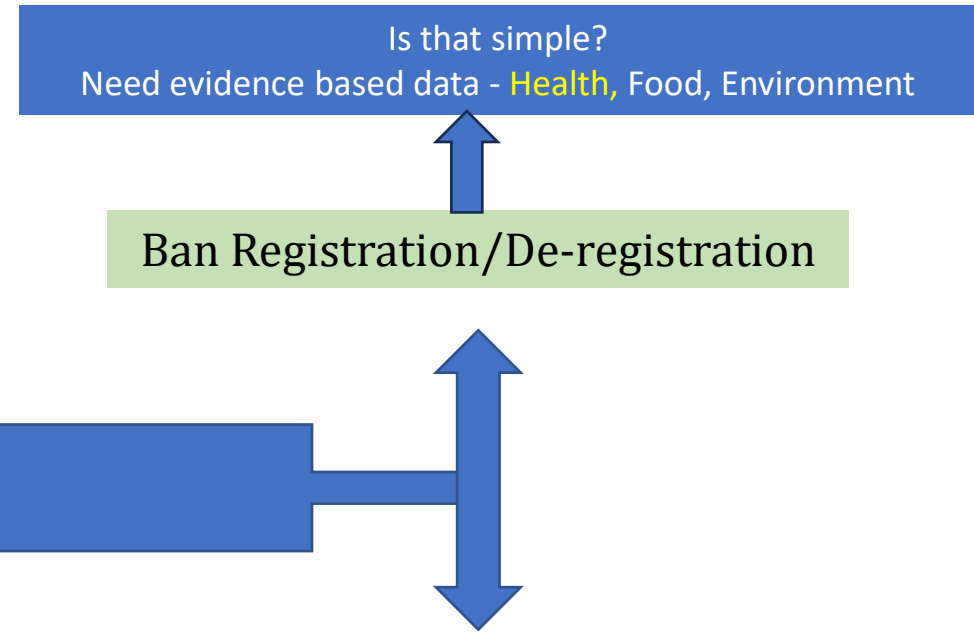
Environmental toxicity



How to reduce the risk of pesticides?

- Replace the hazardous pesticides
- Reduce the use of pesticides

How to replace or reduce the risk of pesticides?

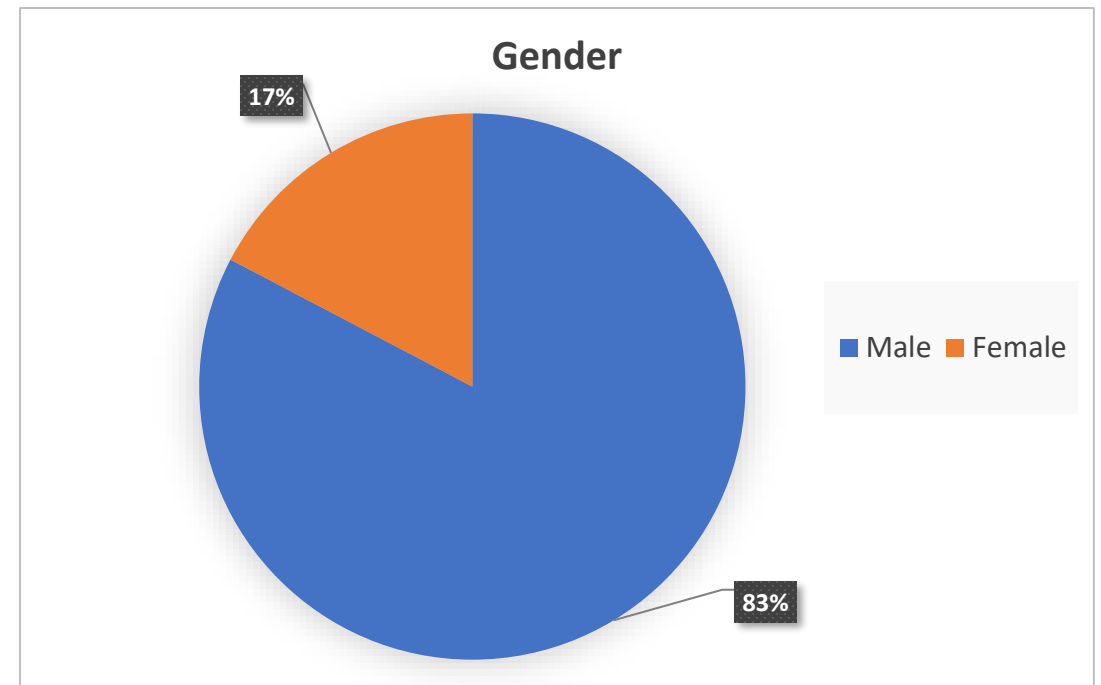
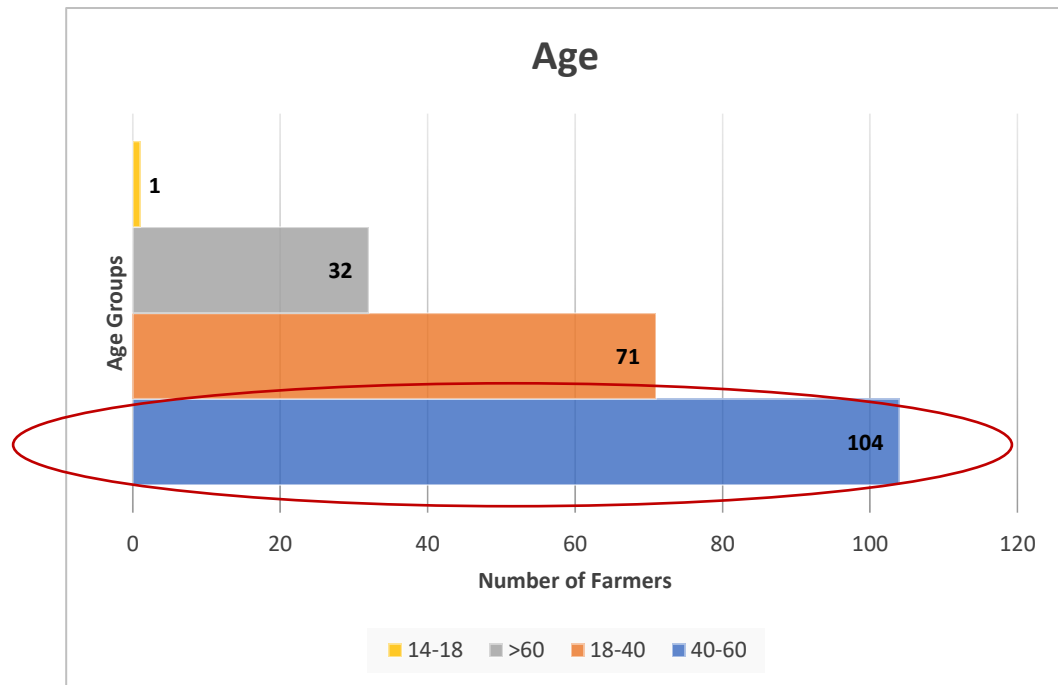


91% of the farmers had less than 5 ha of land



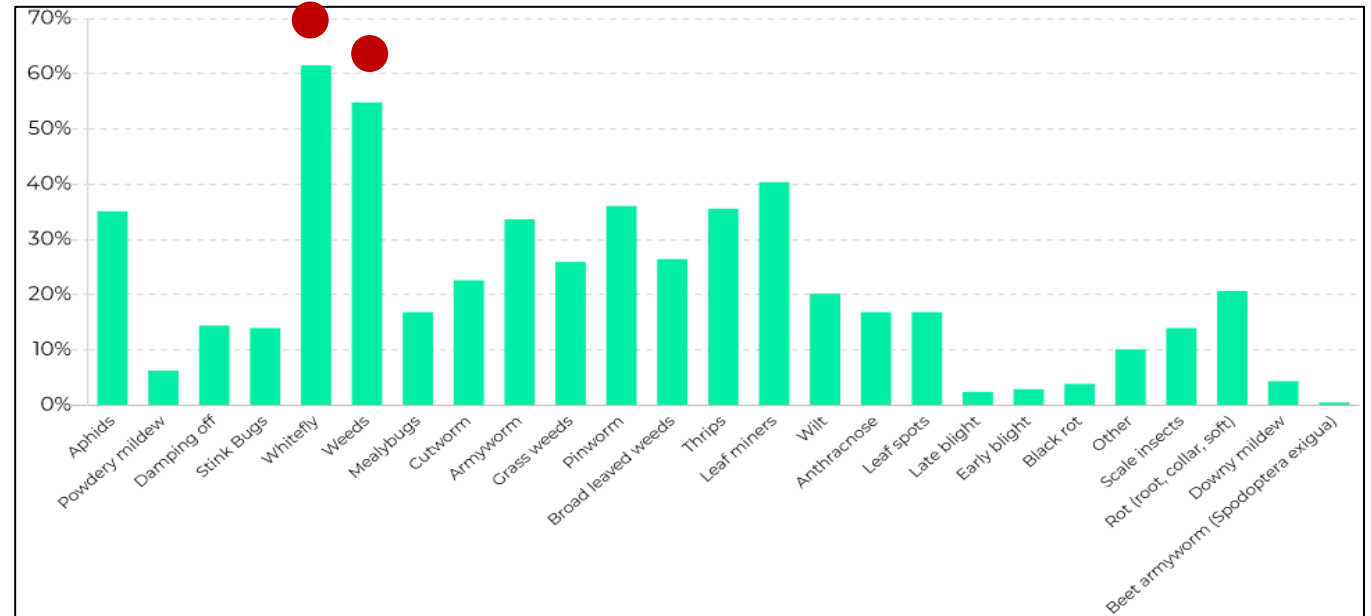
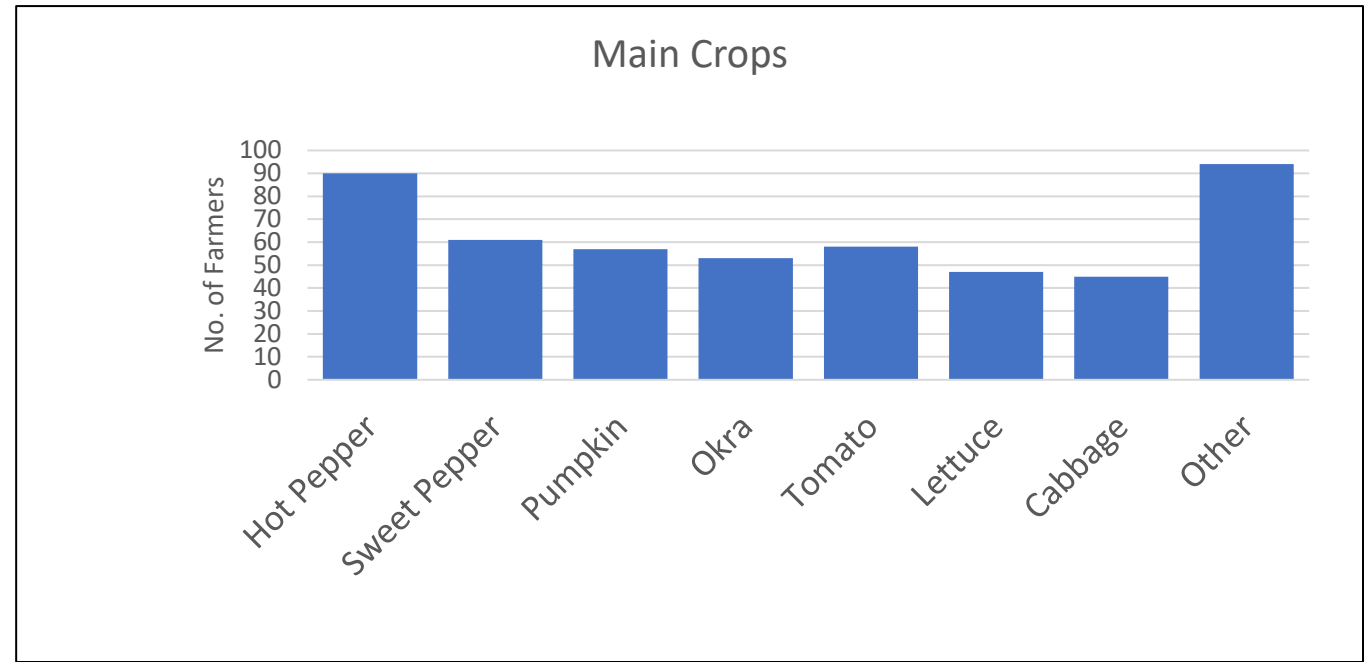
Adopted from: Jodhan, D.; Minter, D.W. (2006).

Age & Gender – 208 Farmers

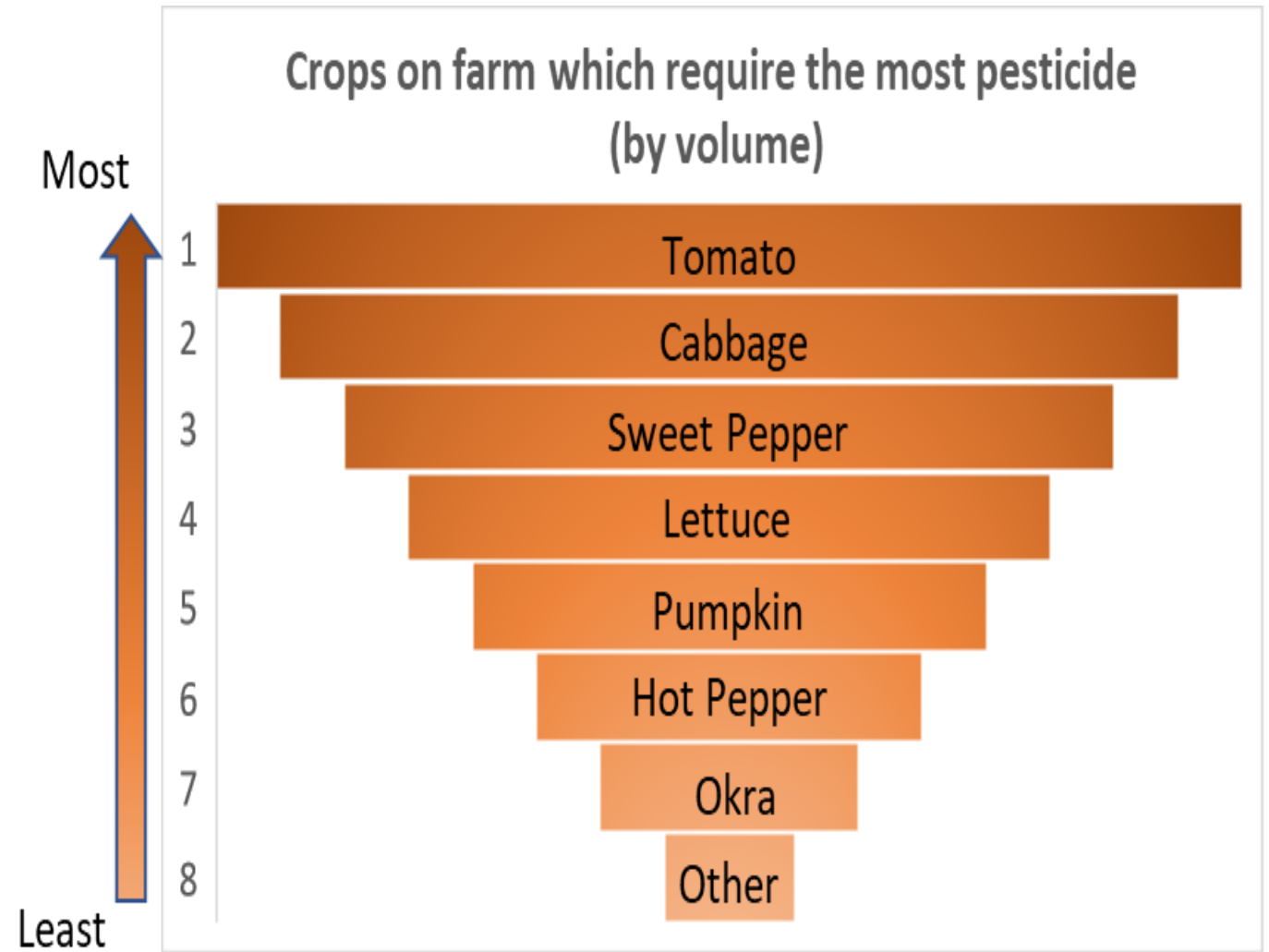


36 Female, 172 Male - 208 Farmers

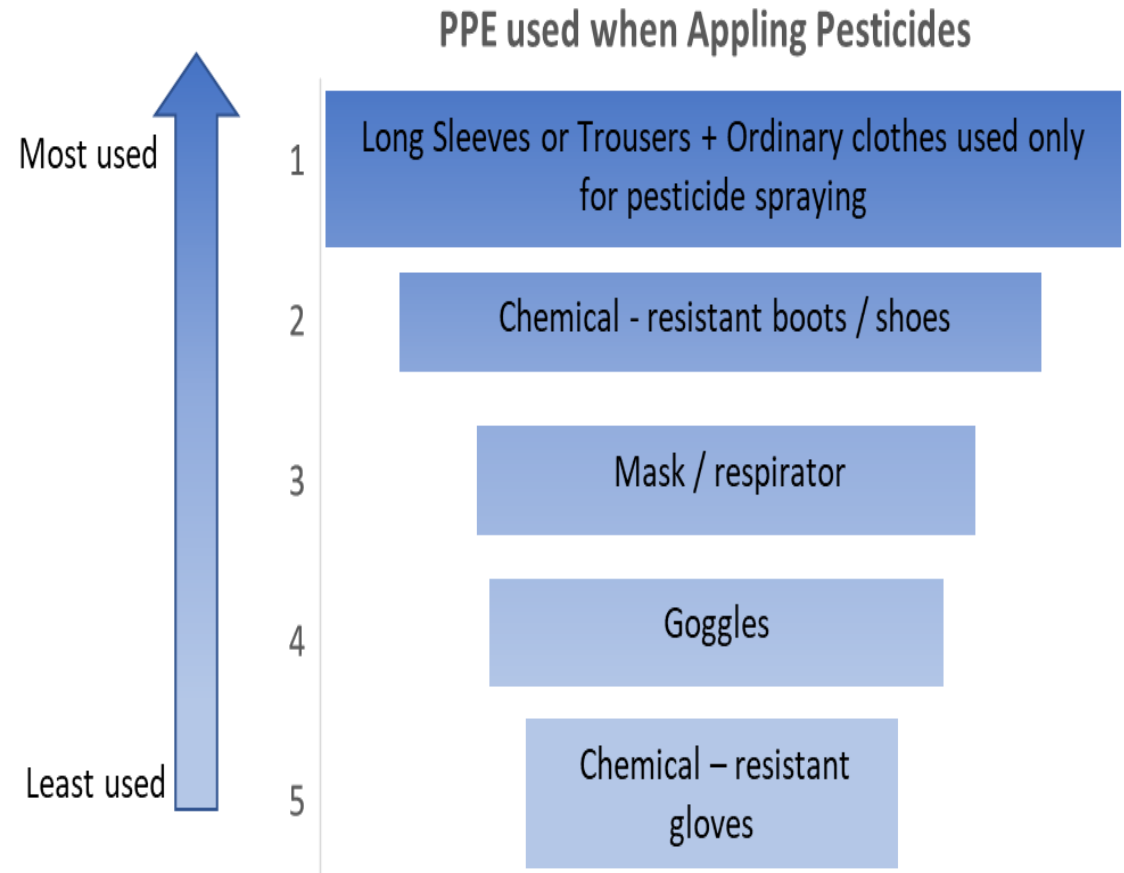
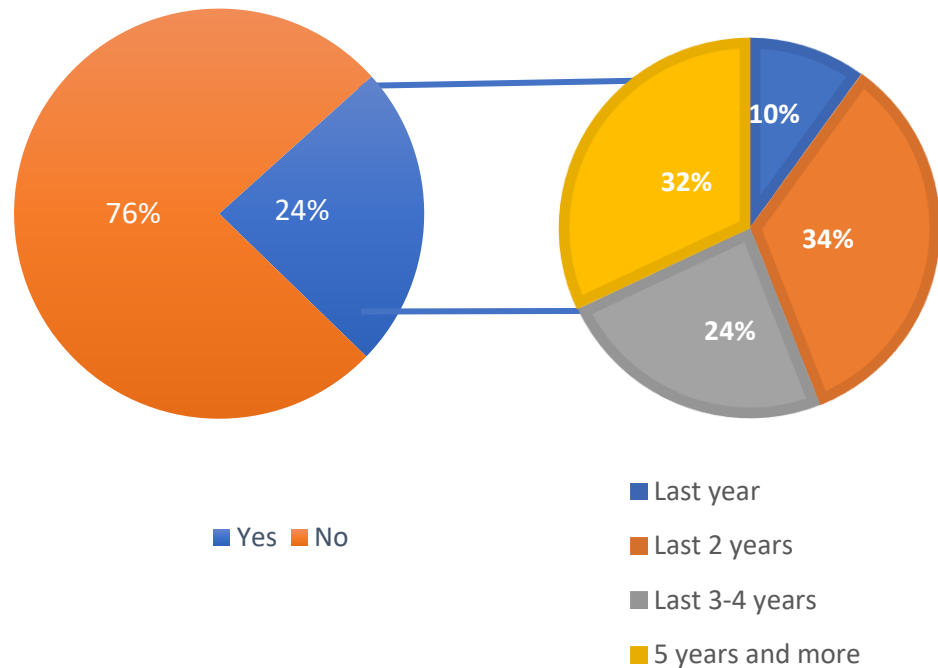
Main Crops Cultivated & Pests require more pesticides



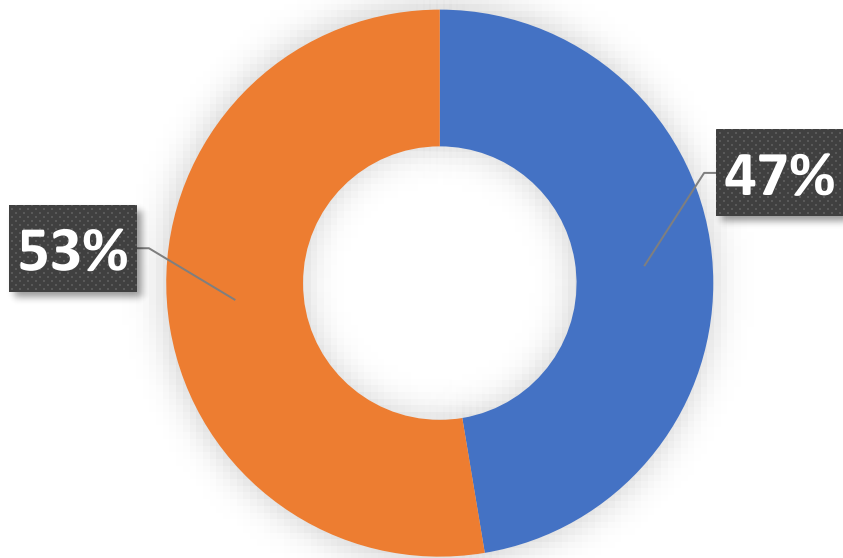
Crops require
most pesticides



Farmers PPE training and type of PPE used

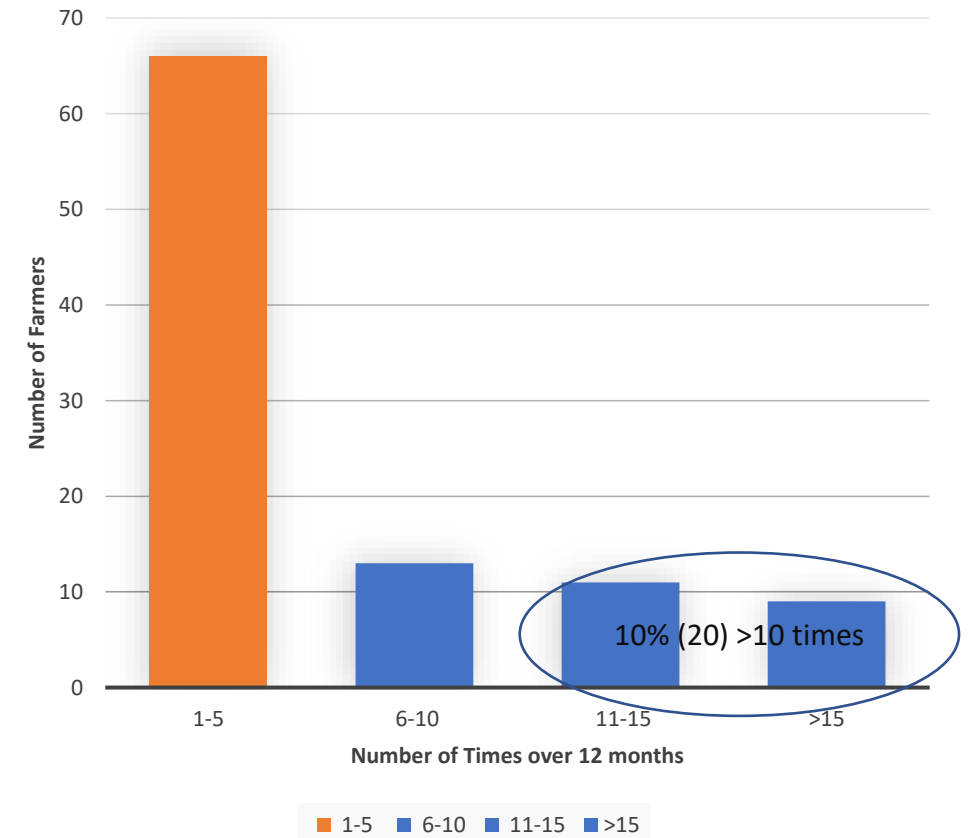


Farmers felt unwell over past 12 months within 24 hrs after application



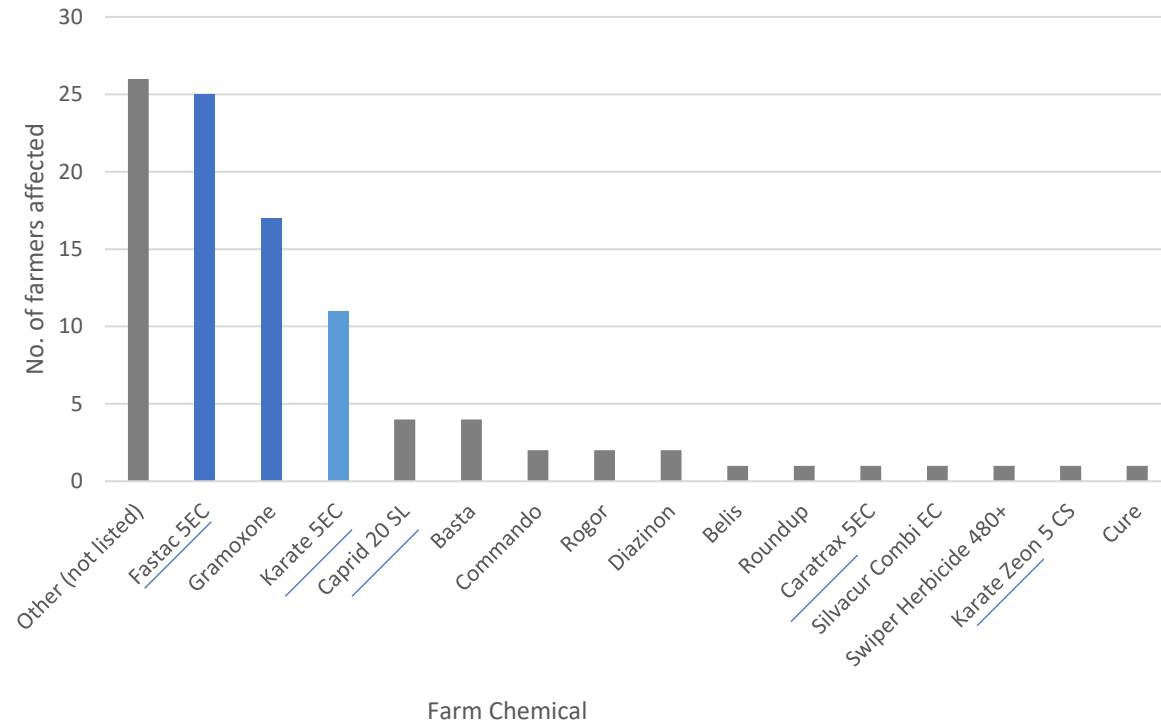
■ Yes ■ No

Times have Farmer felt unwell within 24 hours of using pesticides over 12 months (N=99)

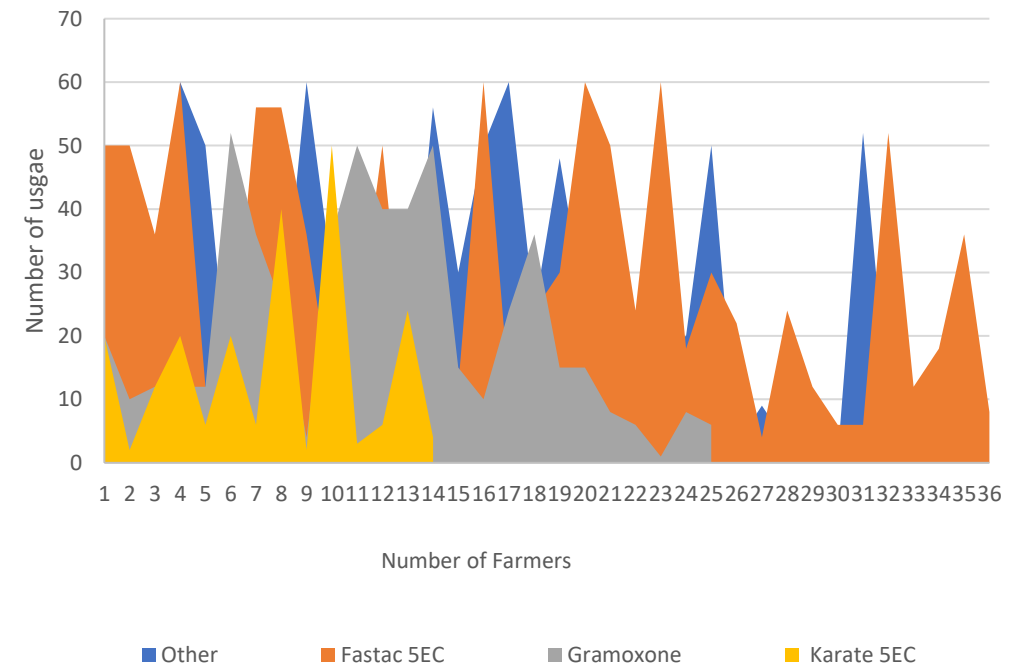


Pesticides reported with health impact

Pesticides used in the last 12 months which affected health

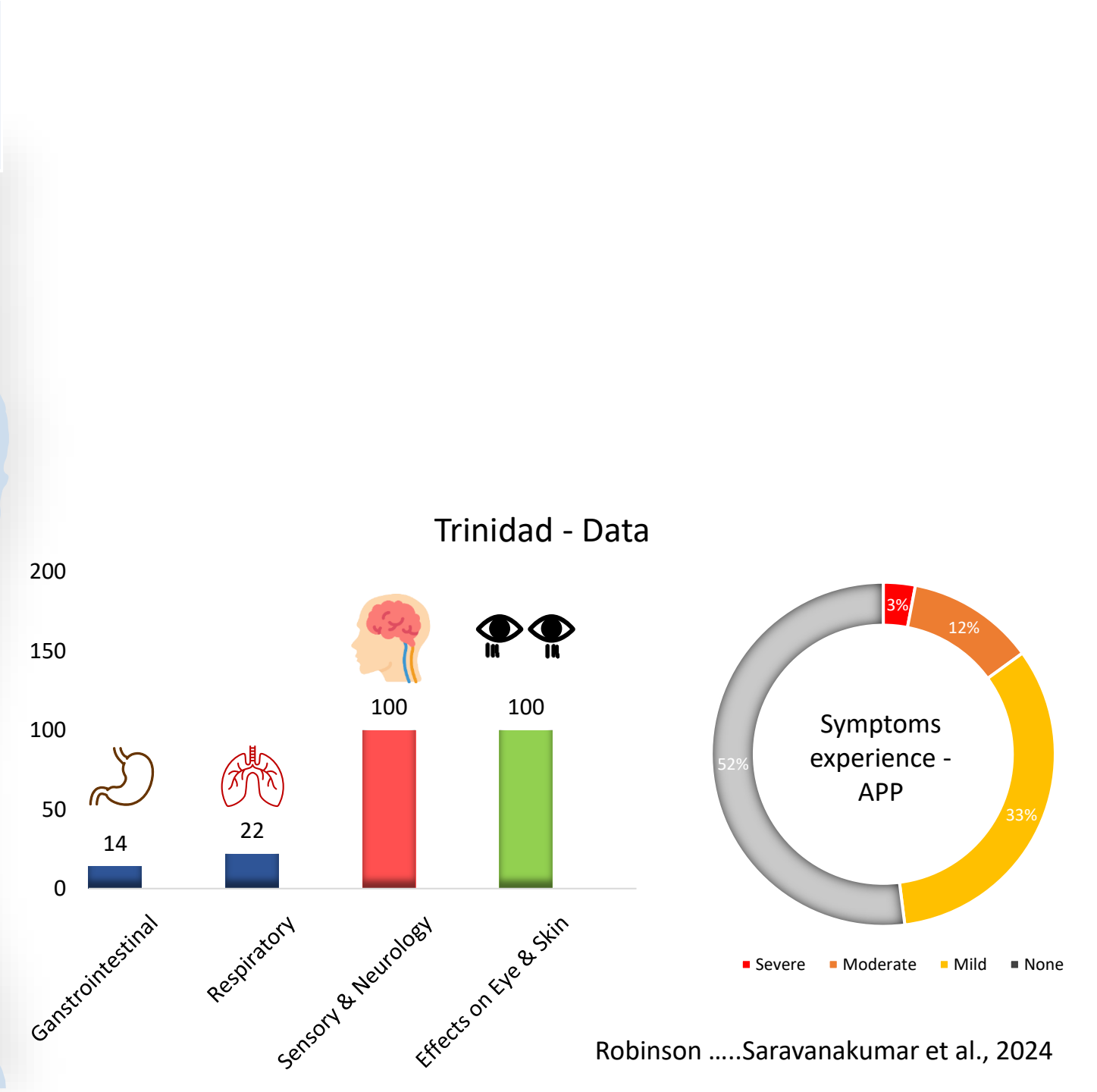
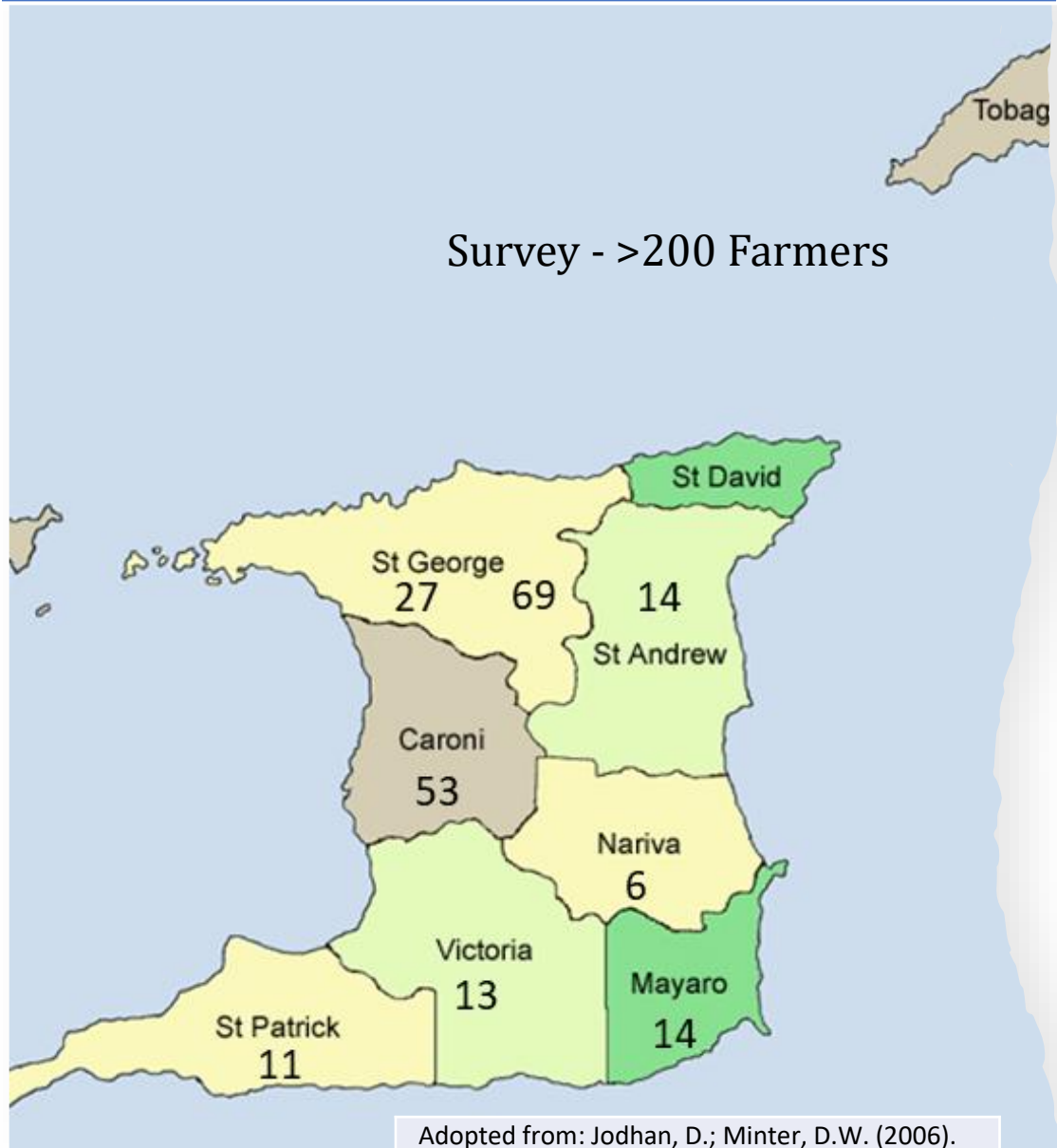


Times of application over 12 months time

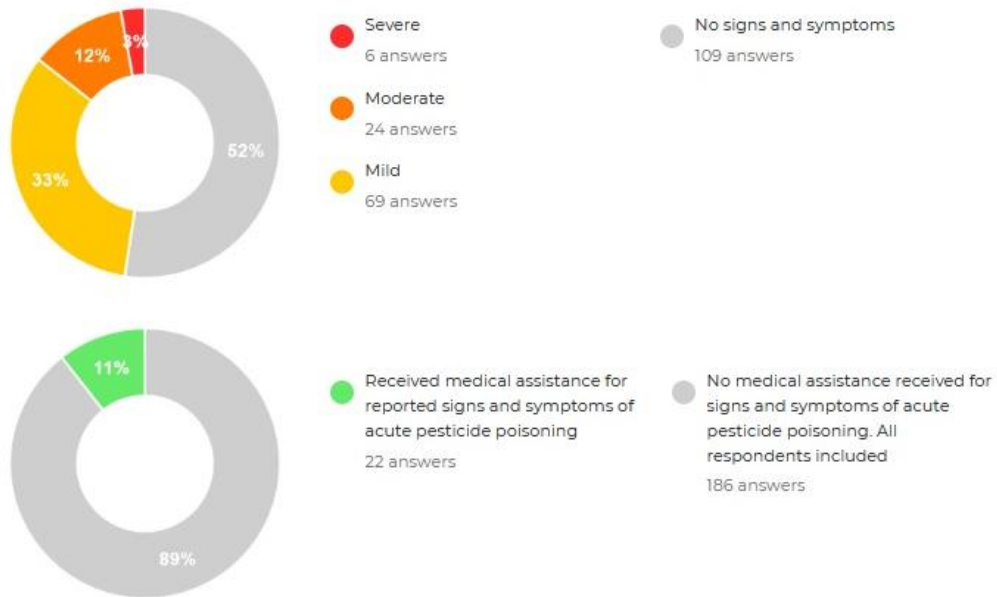


*Supertak (Alpha Cypermethrin), Agrinet (Methomyl), Ethrine-Plus (Ethion & Cypermethrin)

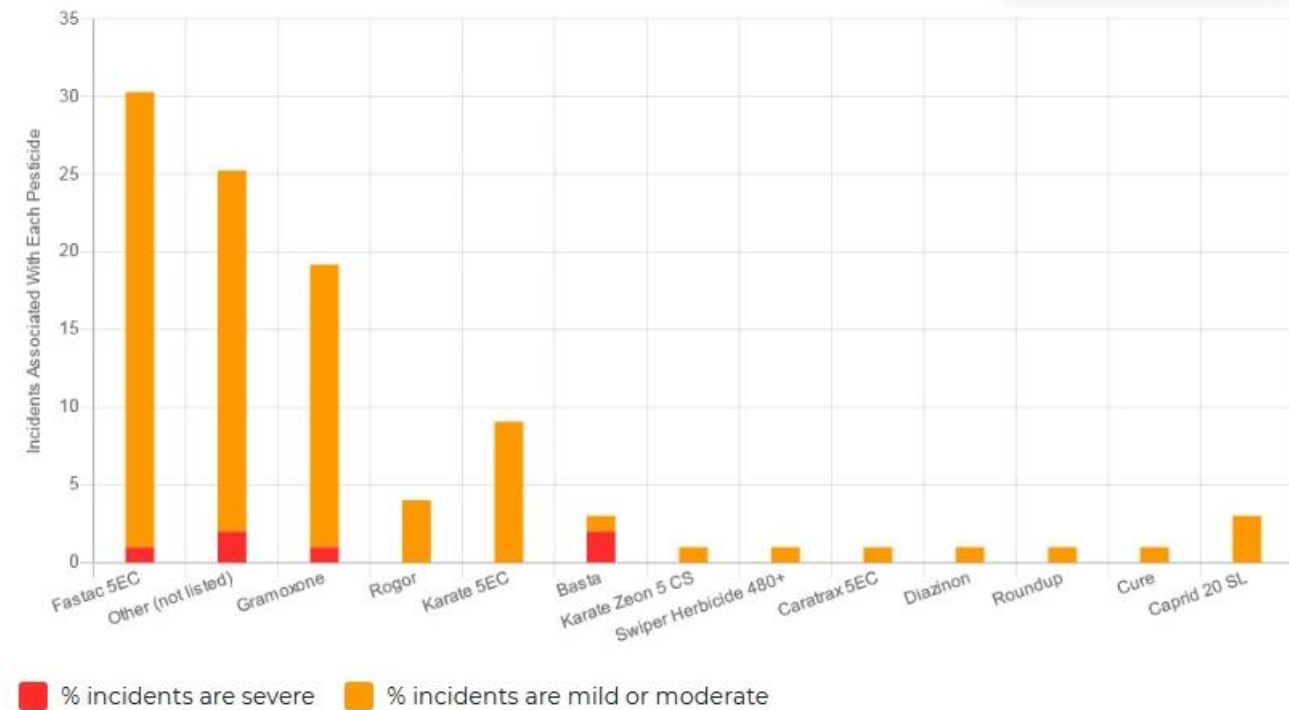
Symptoms associated with HHPs



Percentage of people reporting signs & symptoms consistent with acute pesticide poisoning



Percent incidents associated with pesticides



Reports of pesticide incidents in Trinidad from the surveying of 208 participants



This report assisted the regulatory authorities to make decision to **DE-REGISTER** some of the HHPs!!!

How to reduce the risk of pesticides?

- Replace the hazardous pesticides
- Reduce the use of pesticides

How to replace or reduce the risk of pesticides?

Is that simple?
Need evidence based data - **Health**, Food, Environment

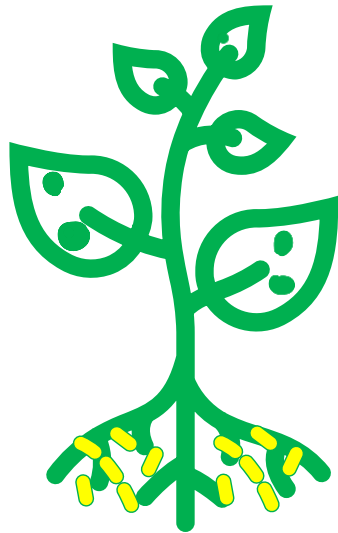
Ban Registration/De-registration

Alternatives

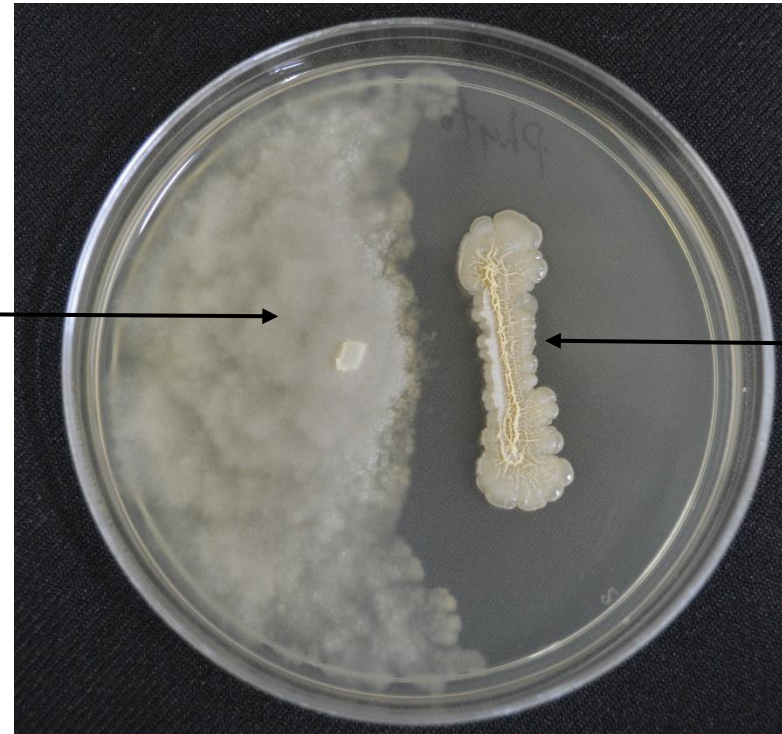
- (i) Integrated management practices
 - Cultural
 - **Biologicals**
 - **Crop Resistance (Genetic)**
 - Low risk pesticides/mitigate risk

Biocontrol

- Use of microorganisms, genes or gene products in the management of plant diseases



**Plant disease
causing fungi**

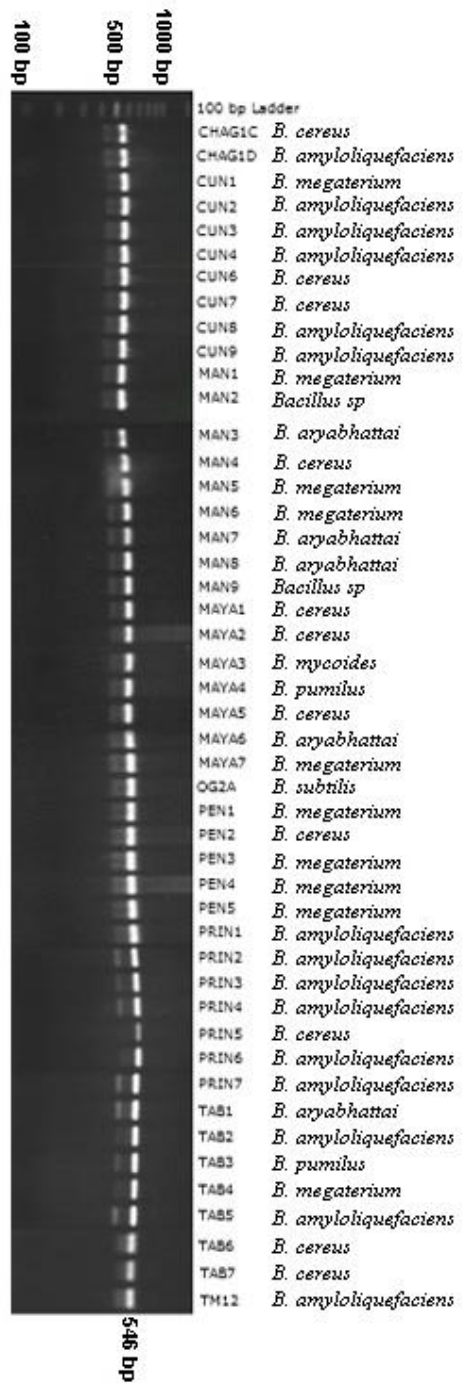


**Plant Beneficial
bacteria**

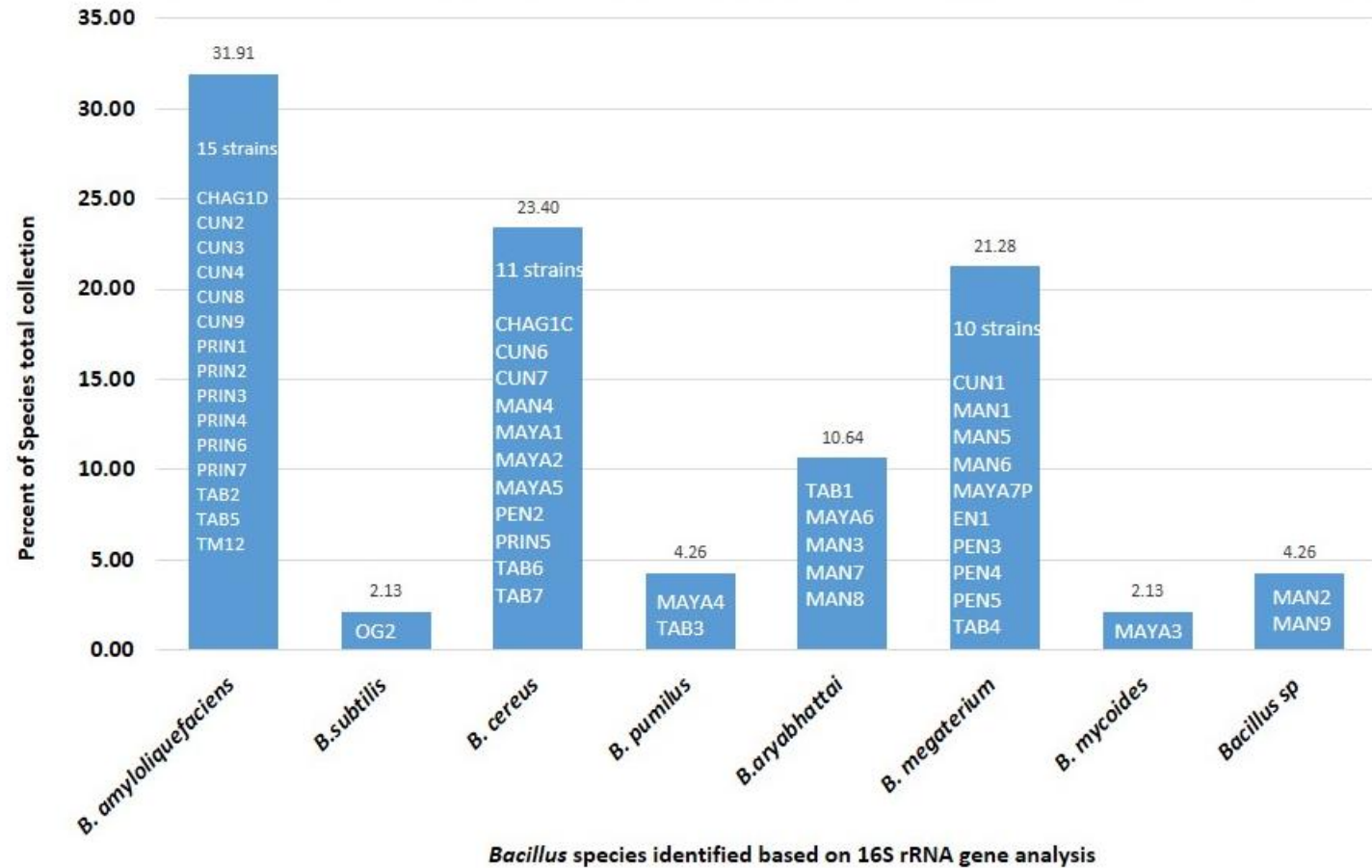
Vegetable disease control under tropical conditions

- Intensive collection of rhizosphere soil samples from various ecosystems of T&T with an intend of identifying beneficial microbes
- Isolation of fungal and bacterial cultures resulted in 200 isolates
- Based on morphology & biochemical characterization, a major group of organisms appeared *Bacillus* species

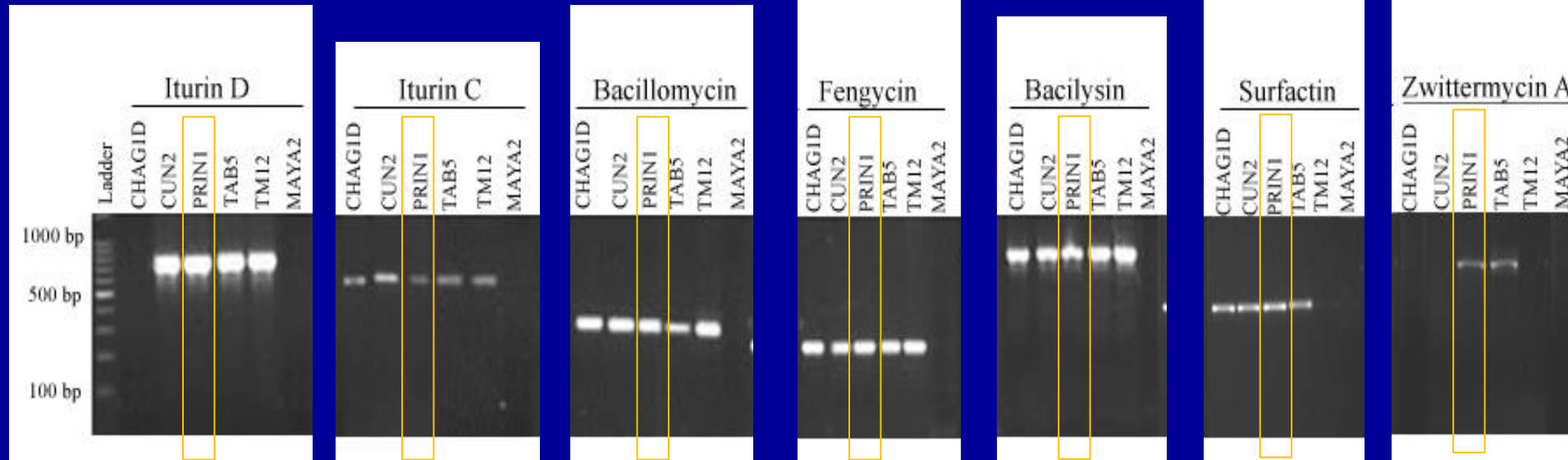




Identification of rhizosphere strains from rhizosphere soils of Trinidad based on 16S rRNA gene analysis



Detection of lipopeptide genes in *B. amyloliquefaciens*



Bacilysin (815 bp)
Iturin D (800 bp)
Zwittermycin (779 bp)
Iturin C (594 bp)
Surfactin (441 bp)
Bacillomycin D
Fengycin (220 bp)

Iturin A synthetase D (ituD)	5'-CCCCTGTTCTAGATGATCGGAGGAATCTC-3' 5'-TGCATCGATTCTGTCCATCTAACCGGCATC-3'	D: 95°C for 1 min A: 55°C for 1 min E: 72°C for 90S
---------------------------------	---	---

Iturin A synthetase C (ituC)	5'-CCCCCTCGGTCAAGTGAATA-3' 5'-TTGGTTAAGCCCTGATGCTC-3'	D: 94°C for 30 S A: 65°C for 45 S E: 72°C for 90 S
---------------------------------	--	--

Bacillomycin D Synthetase (BmyB)	5'-TGAAACAAAGGCATATGCT-3' 5'-AAAAATGCATCTGCCGTTCC-3'	D: 94°C for 1 min A: 58°C for 1 min E: 72°C for 1 min
-------------------------------------	---	---

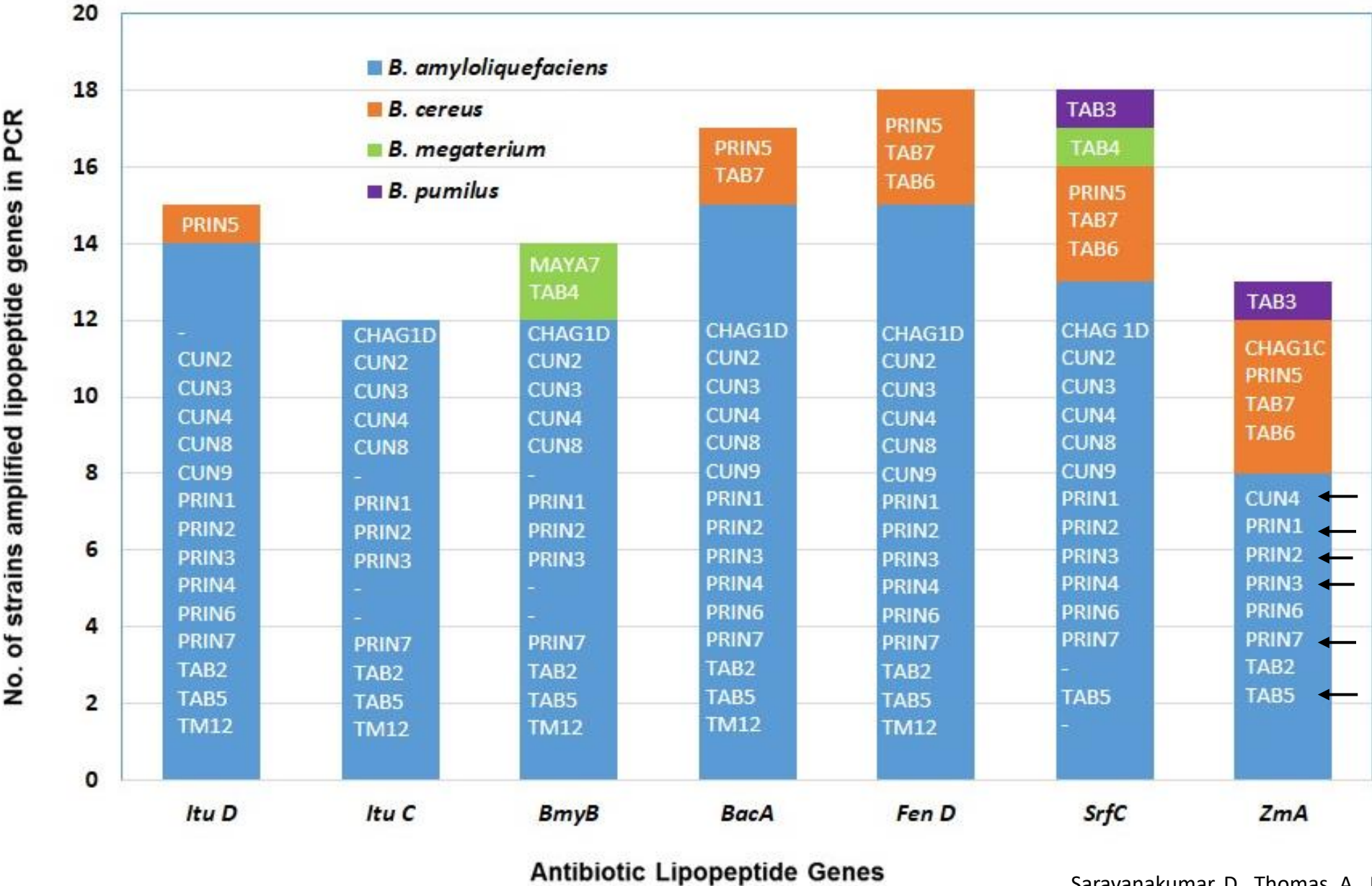
Fengycin synthetase (fenD)	5'-CCTGCAGAAGGAGGAGAAGTGAAG-3' 5'-TGCTCATCGTCTTCCGTTTC-3'	D: 94°C for 1 min A: 56°C for 1 min E: 72°C for 1 min
-------------------------------	--	---

Bacilysin synthetase A (bacA)	5'-CTTCTCCAAGGGGTGAACAG-3' 5'-TGTAGGTTTCACCGGCTTTC-3'	D: 94°C for 1 min A: 56°C for 1 min E: 72°C for 1 min
----------------------------------	--	---

Surfactin (SurC)	5'-ACAGTATGGAGGCATGGTC-3' 5'-TTCCGCCACTTTTTCAGTTT-3'	D: 95°C for 1 min A: 55°C for 1 min E: 72°C for 1 min
---------------------	---	---

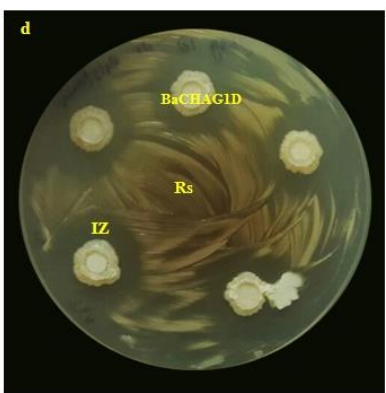
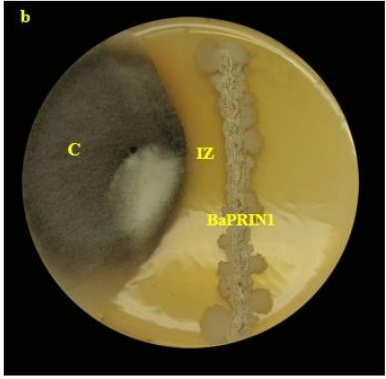
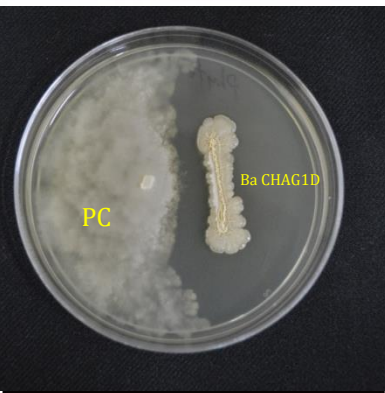
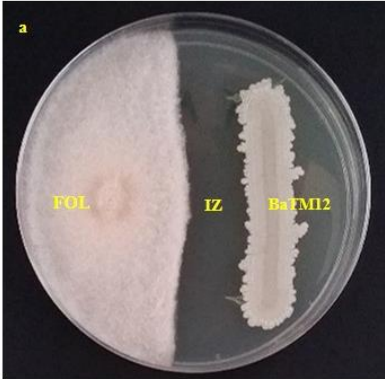
Zwittermicin A (ZmA)	5'-TTGGGAGAATATACAGCTCT-3' 5'-GACCTTTTGAAATGGGCGTA-3'	D: 94°C for 1 min A: 57°C for 30S E: 72°C for 1 min
-------------------------	--	---

Bacillus strains showed positive to PCR amplification of antibiotic lipopeptide genes



Strains	Percent mycelium inhibition of fungal pathogens				Inhibition against bacterial pathogen
	<i>F. oxysporum</i> <i>f.sp. lycopersici</i>	<i>C. gloeosporioides</i>	<i>C. lactuca-</i> <i>sativae</i>	<i>A.Solani</i>	<i>R. solanacearum</i>
CHAG 1C	27.86 ^d	52.98 ^{e-l}	39.12 ^{l-p}	35.29 ^{ef}	++
CHAG 1D	52.86 ^{ef}	67.26 ^{j-l}	53.36 ^{pqr}	47.06 ^{gh}	++++
CUN 1	13.57 ^{abcd}	29.76 ^{a-h}	6.16 ^{ab}	17.65 ^{bc}	+
CUN 2	49.29 ^{ef}	59.52 ^{f-l}	52.23 ^{pqr}	52.94 ^{hi}	++++
CUN 3	52.86 ^{ef}	59.52 ^{f-l}	51.29 ^{q-r}	58.82 ^{ij}	++++
CUN 4	50.25 ^{ef}	63.69 ^{8-l}	54.26 ^{ppq}	58.82 ^{hi}	++++
CUN 6	20.71 ^{bcd}	25.60 ^{a-f}	30.33 ^{e-m}	11.76 ^{ab}	++++
CUN 7	45.71 ^e	10.71 ^{ab}	53.16 ^{pqr}	35.29 ^{ef}	-
CUN 8	52.86 ^{ef}	54.17 ^{e-l}	54.26 ^{pqr}	64.71 ^j	+++
CUN 9	52.86 ^{ef}	58.33 ^{f-l}	62.33 ^s	47.06 ^{gh}	+++
MAN 1	6.43 ^{ab}	66.67 ^{j-l}	3.33 ^a	3.33 ^a	-
MAN 2	20.71 ^{bcd}	14.88 ^{abcd}	28.16 ^{d-k}	11.76 ^{ab}	-
MAN 3	13.57 ^{abcd}	12.50 ^{abc}	20.24 ^{b-h}	23.53 ^{cd}	++
MAN 4	45.71 ^e	51.79 ^{e-l}	16.16 ^{a-f}	23.53 ^{cd}	-
MAN 5	17.14 ^{abcd}	35.12 ^{a-k}	8.54 ^{abc}	11.76 ^{ab}	+
MAN 6	17.14 ^{abcd}	30.95 ^{a-i}	16.26 ^{a-f}	11.76 ^{ab}	+
MAN 7	10.00 ^{abc}	65.56 ^{j-l}	3.36 ^a	3.33 ^a	+
MAN 8	24.29 ^{cd}	12.50 ^{abc}	14.26 ^{a-e}	17.65 ^{bc}	+
MAN 9	17.14 ^{abcd}	1.19 ^a	12.44 ^{abcd}	23.53 ^{cd}	+
MAYA 1	6.43 ^{ab}	28.57 ^{a-g}	38.36 ^{t-p}	17.65 ^{bc}	+
MAYA 2	20.71 ^{bcd}	47.02 ^{c-l}	12.36 ^{abcd}	17.65 ^{bc}	-
MAYA 3	2.86 ^a	13.69 ^{abc}	23.14 ^{c-j}	5.88 ^a	+
MAYA 4	45.71 ^e	32.74 ^{a-j}	43.12 ^{k-q}	23.53 ^{cd}	++++
MAYA 5	20.71 ^{bcd}	44.05 ^{b-l}	20.24 ^{b-h}	17.65 ^{bc}	-
MAYA 6	24.29 ^{cd}	35.71 ^{a-k}	29.36 ^{e-l}	11.76 ^{ab}	++
MAYA 7	10.00 ^{abc}	33.33 ^{a-j}	38.33 ^{t-p}	23.53 ^{cd}	++
OG2A	52.86 ^{ef}	49.40 ^{d-l}	53.24 ^{pqr}	52.94 ^{hi}	++++
PEN 1	10.00 ^{abc}	45.24 ^{b-l}	18.33 ^{a-g}	5.88 ^a	-
PEN 2	17.55 ^{abcd}	19.64 ^{a-e}	45.16 ^t	25.36 ^{cde}	++
PEN 3	10.00 ^{abc}	66.07 ^{j-l}	22.24 ^{b-i}	17.65 ^{bc}	+
PEN 4	6.43 ^{ab}	45.83 ^{b-l}	31.44 ^{f-n}	17.65 ^{bc}	++
PEN 5	5.59 ^{ab}	42.86 ^{b-l}	12.23 ^{abcd}	11.76 ^{ab}	+
PRIN 1	52.86 ^{ef}	66.07 ^{j-l}	74.26 st	47.06 ^{gh}	+
PRIN 2	52.86 ^{ef}	58.93 ^{f-l}	51.22 ^{m-q}	47.06 ^{gh}	++++
PRIN 3	52.86 ^{ef}	55.95 ^{f-l}	53.44 ^{pqr}	52.94 ^{hi}	++
PRIN 4	52.86 ^{ef}	62.50 ^{8-l}	74.26 ^r	41.18 ^{fg}	++++
PRIN 5	24.29 ^{cd}	54.76 ^{e-l}	34.21 ⁸⁻ⁿ	41.18 ^{fg}	++
PRIN 6	50.68 ^{ef}	69.64 ^{k-l}	53.22 ^{pqr}	67.12 ^j	+++
PRIN 7	51.68 ^{ef}	64.88 ^{h-l}	51.22 ^{m-q}	52.94 ^{hi}	++++
TAB 1	13.57 ^{abcd}	13.69 ^{abc}	42.16 ^{k-q}	17.65 ^{bc}	-
TAB 2	45.71 ^e	35.71 ^{a-k}	46.16 ^{m-r}	58.82 ^{ij}	++++
TAB 3	49.29 ^{ef}	49.41 ^{d-l}	51.22 ^{q-r}	66.47 ^j	++++
TAB 4	13.57 ^{abcd}	42.26 ^{b-l}	42.36 ^{k-q}	17.65 ^{bc}	-
TAB 5	51.48 ^{ef}	61.91 ^{8-l}	58.16 ^{qrs}	41.18 ^{fg}	++++
TAB 6	27.86 ^d	66.67 ^{j-l}	52.26 ^{pqr}	29.41 ^{de}	++
TAB 7	17.14 ^{abcd}	50.60 ^{e-l}	47.33 ^{q-r}	29.41 ^{de}	++
TM 12	52.96 ^{ef}	57.14 ^{f-l}	52.23 ^{pqr}	58.82 ^{ij}	++++

Testing pathogen suppressing activity of LP producing strains against vegetable pathogens



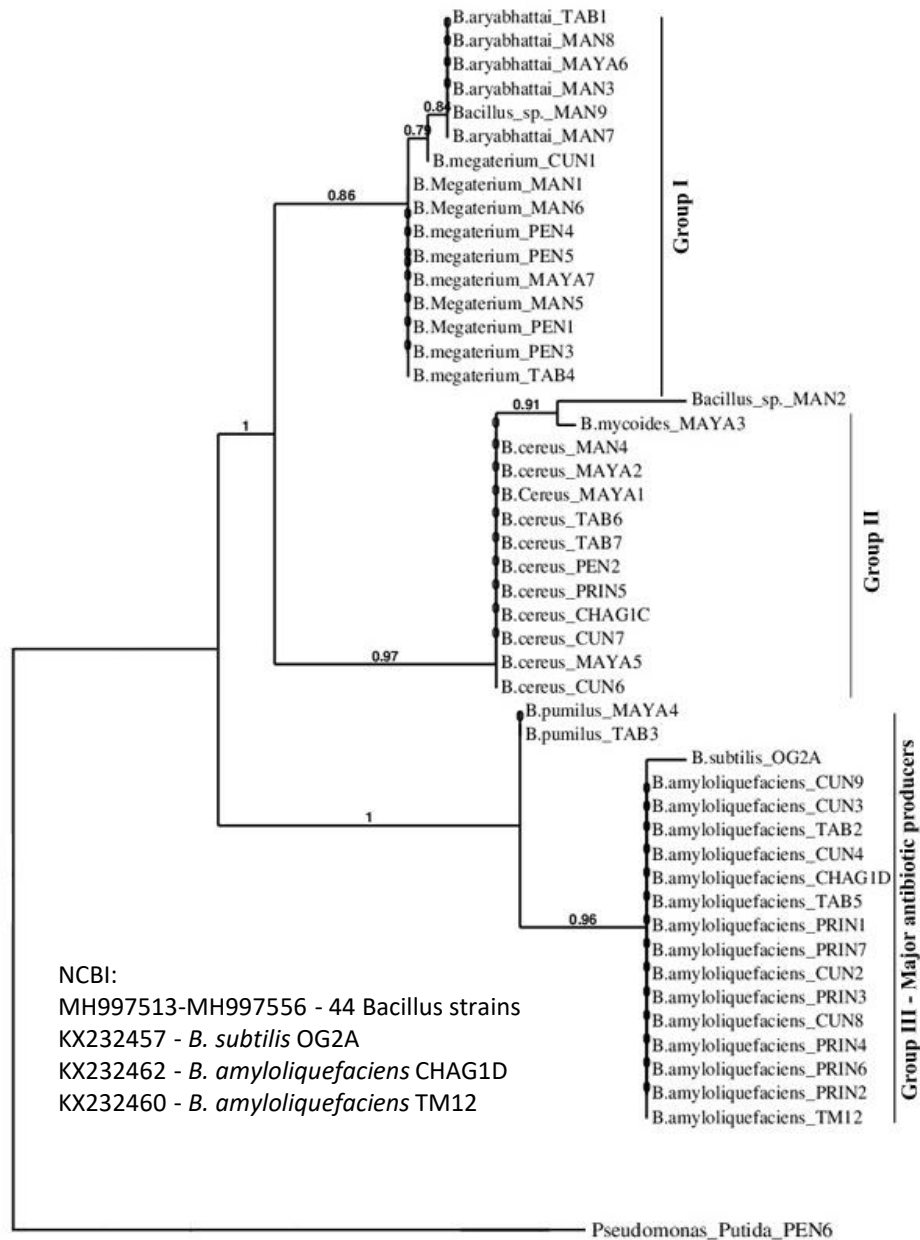
a TM12 against *Fusarium oxysporum* f.sp. *lycopersici* (Fol).

b PRIN1 against *Cercospora lactuca-sativae* (C).

c CHAG1D (Ba CHAG1D) against *Phytophthora capsici* (Pc).

d CHAG1D (Ba CHAG1D) against *Ralstonia solanacearum* (Rs).

IZ: Inhibition Zone



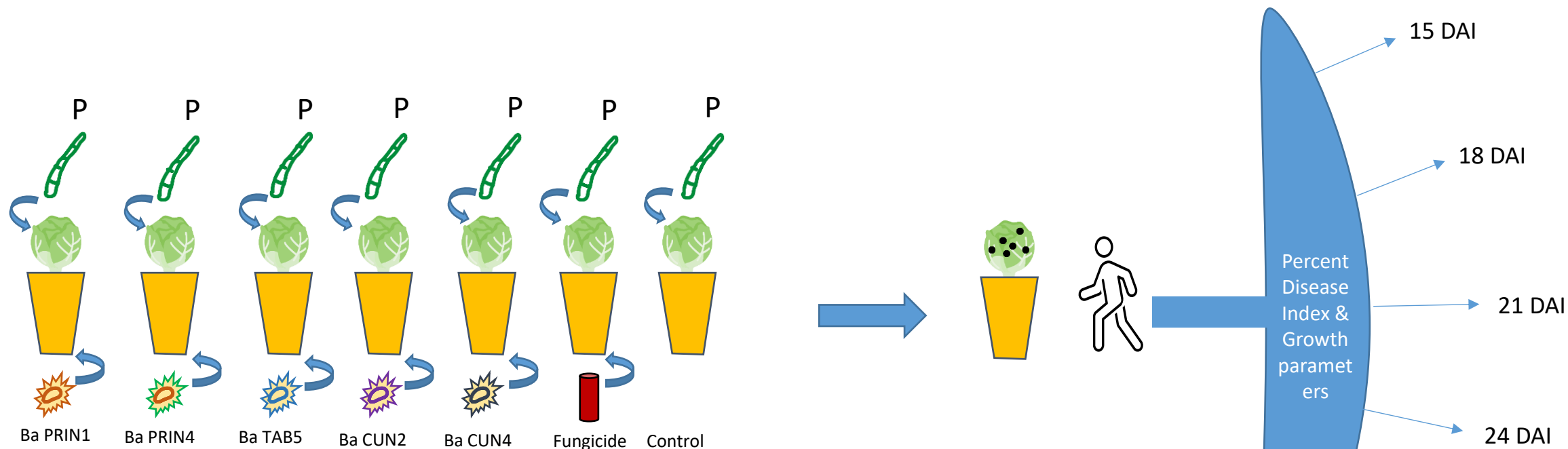
Phylogeny of *Bacillus* strains based on sequence analysis of 16S rRNA gene

Clustering of antibiotic producers

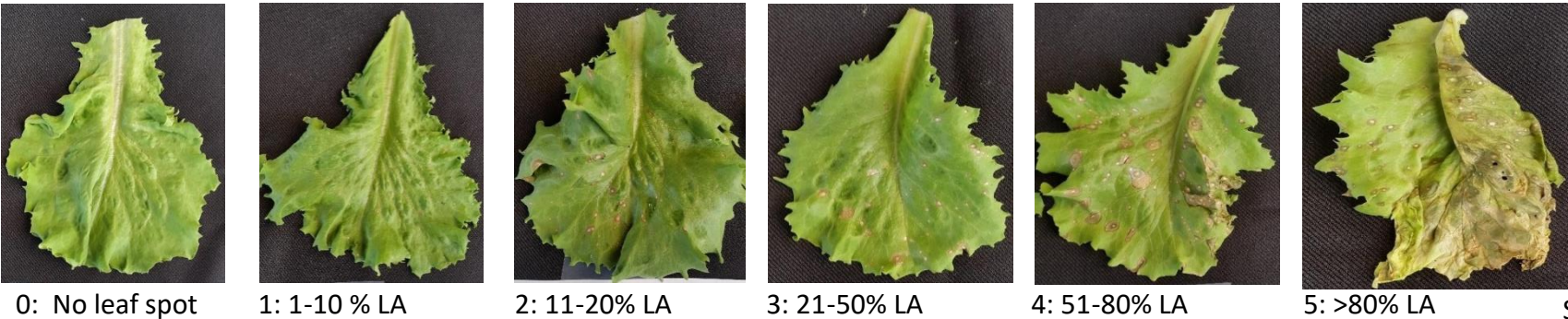
Tested growth promoting activity of all *Bacillus* strains



Screening of *B. amyloliquefaciens* strains against *Cercospora* LS against artificial inoculation



Disease assessment for *Cercospora* leaf spot in lettuce



Treatment	No. of lesions /plant				Percent Disease Index			
	15 DAI	18 DAI	21 DAI	24 DAI	15 DAI	18 DAI	21 DAI	24 DAI
<i>B. amyloliquefaciens</i> PRIN 1	10.46 ^a	13.05 ^a	19.81 ^a	30.56 ^a	3.90 ^a	7.22 ^{ab}	11.67 ^{ab}	21.67 ^b
<i>B. amyloliquefaciens</i> PRIN 4	38.17 ^a	38.69 ^a	48.20 ^a	62.98 ^a	9.44 ^a	13.33 ^b	18.89 ^b	31.67 ^b
<i>B. amyloliquefaciens</i> TAB 5	33.26 ^a	51.59 ^a	60.93 ^a	93.10 ^a	7.22 ^a	11.67 ^b	22.22 ^b	42.22 ^b
<i>B. amyloliquefaciens</i> CUN 2	17.76 ^a	26.69 ^a	45.09 ^a	461.26 ^b	4.44 ^a	6.67 ^{ab}	14.44 ^{ab}	31.67 ^b
<i>B. amyloliquefaciens</i> CUN 4	29.02 ^a	50.21 ^a	79.91 ^a	106.60 ^a	10.56 ^a	12.22 ^b	22.22 ^b	38.89 ^b
Fungicide	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
Control	1075.14 ^b	978.81 ^b	1113.68 ^b	1060.74 ^c	47.78 ^b	66.11 ^c	77.78 ^c	91.11 ^c

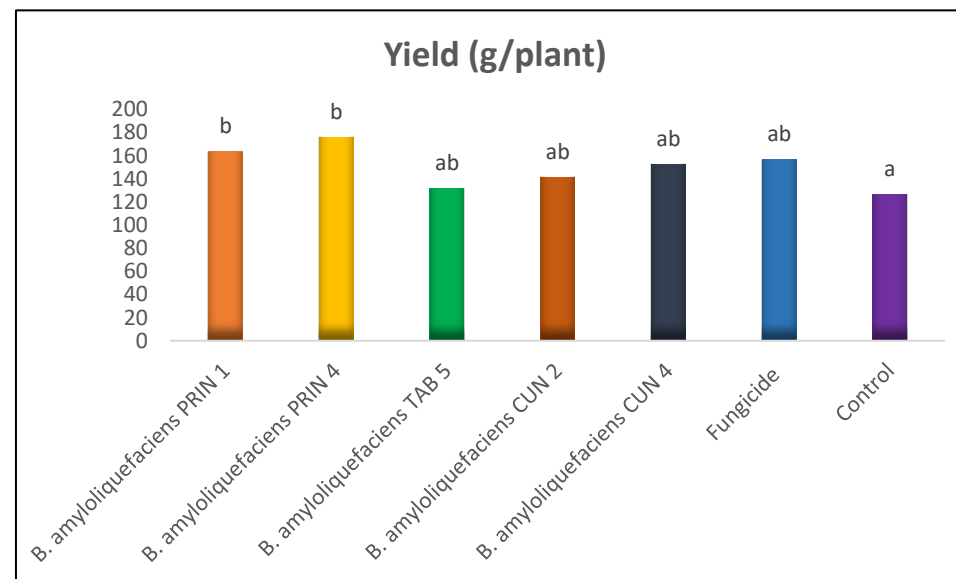
Bacillus amyloliquefaciens strains against Cercospora leaf spot of lettuce under artificial inoculation settings



Control
Disease index - 91.11%
Lesions/plant - 1060.74



Ba PRIN1
Disease index - 21.67%
Lesions/plant - 30.56



Saravanakumar, D.,
Thomas, A.,
Banwarie, N. 2019
EJPP

Field evaluation of *Ba* PRIN1A in lettuce

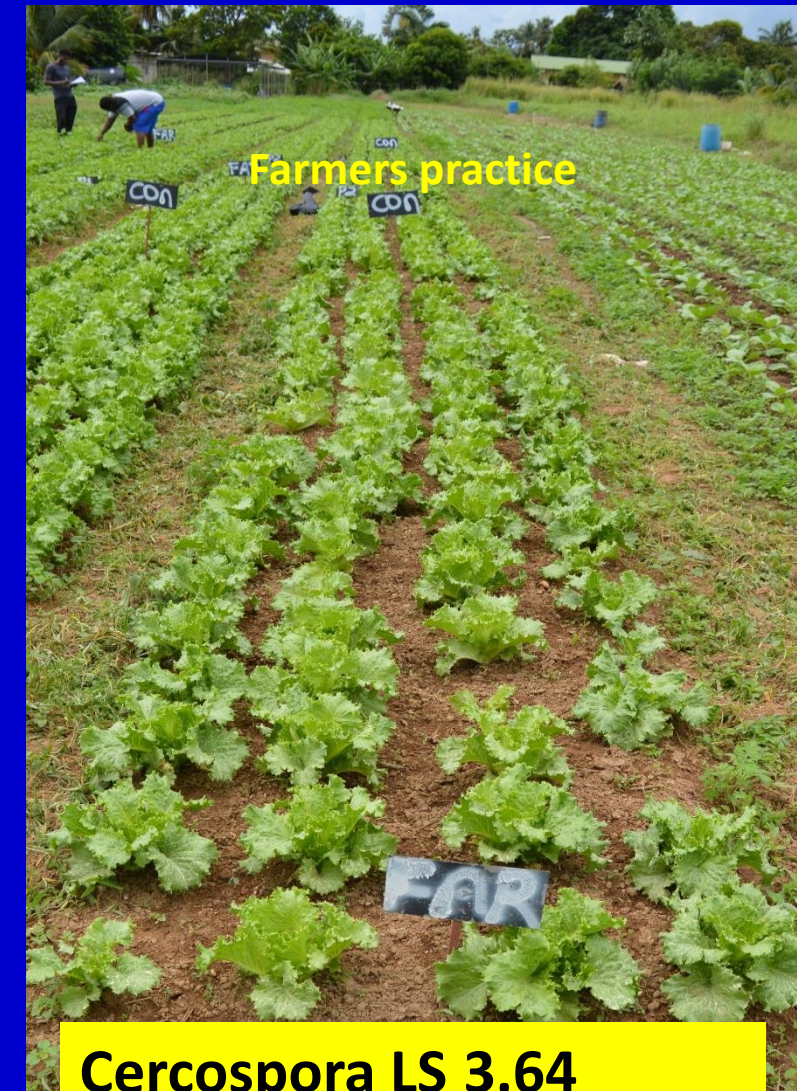


Cercospora LS	17.66
Bacteria LS	15.09
Yield (g)	201.49



B. amyloliquefaciens PRIN1A
(i) control of Leaf spots of lettuce
(ii) growth promotion of lettuce

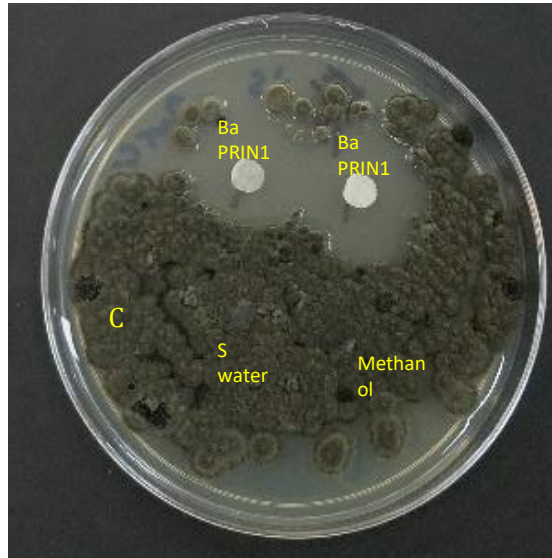
Cercospora LS	2.22
Bacteria LS	8.70
Yield (g)	281.87



Cercospora LS	3.64
Bacteria LS	4.53
Yield (g)	247.64

How does it suppress the pathogen?

Antifungal activity of lipopeptide extract of *Ba* PRIN1 against *C. lactucae-sativae* & *Bacterial pathogen*



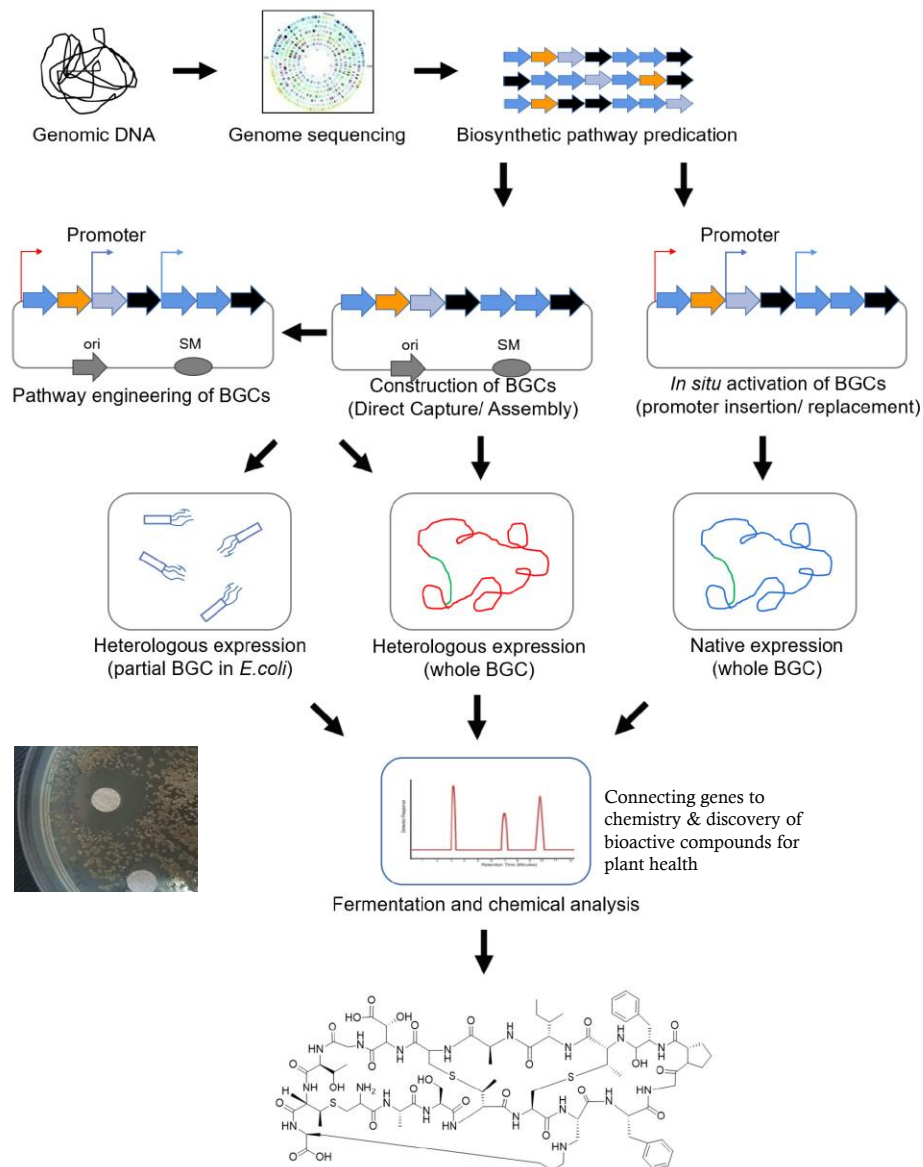
Degeneration of conidial (a) and mycelial (b) structure due to the activity of lipopeptide extract of *Ba* PRIN1



- a. Degeneration and lysis of conidia
- b. Lysis of mycelium
- c. Normal conidia and mycelium (inset) in control

Novel Anti-fungal, anti-bacterial and anti-oomycete compounds discovered using column chromatography, HPLC & ^1H NMR

Genome mining for discovery of novel compounds

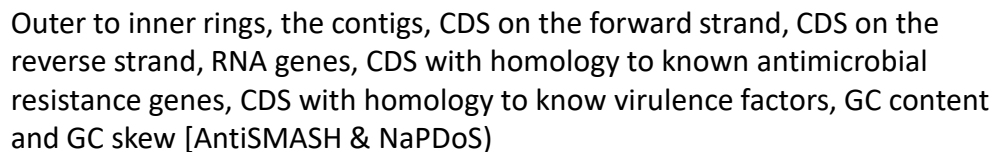


DNA sequencing & analysis of 4 *Bacillus* strains

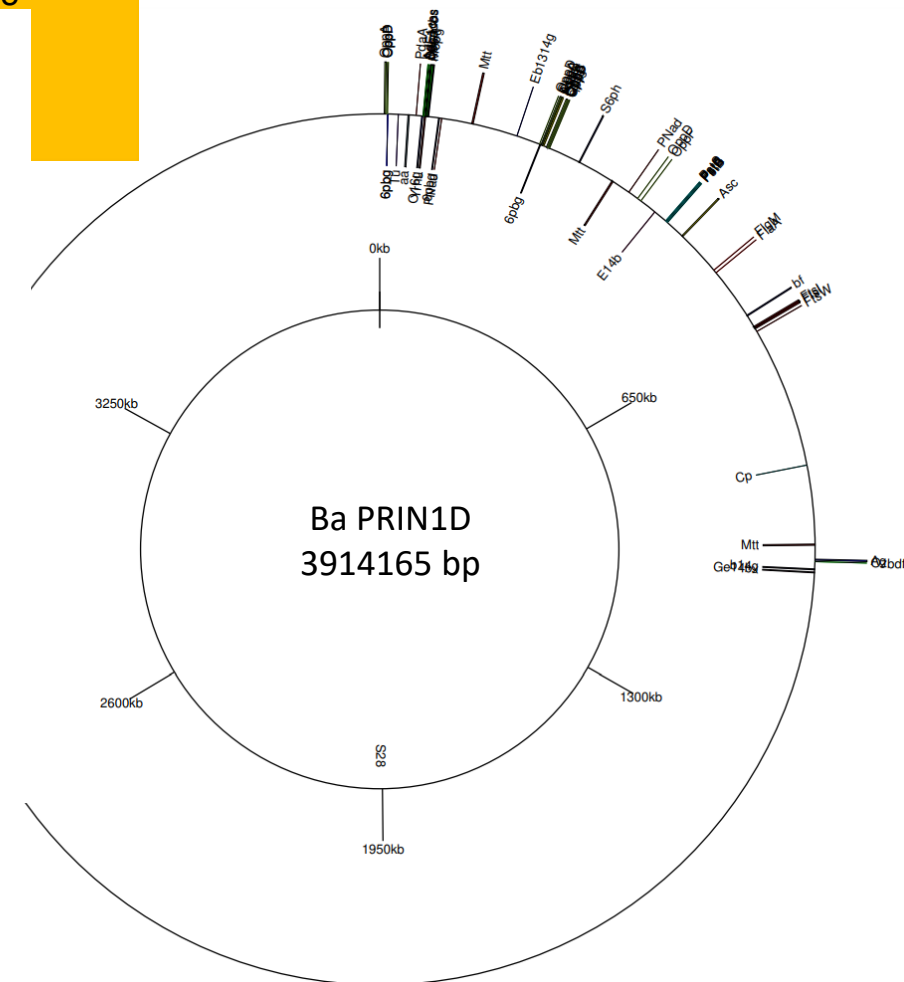
Overview	1.1	1.2	1.3	1.4	1.5	1.6	2.1	2.2	3.1	4.1	4.2	5.1	5.2	5.3	5.4	10.1	11.1	12.1	13.1	16.1	
Identified secondary metabolite regions using strictness 'relaxed'																					
Region	Type	From	To	Most similar known cluster		Similarity															
Region 1.1	NRPS  , betalactone  , transAT-PKS 	1	86,616	fengycin 	NRP	80%	Antifungal Antibacterial Antifungal & Antibacterial														
Region 1.2	transAT-PKS-like  , transAT-PKS  , NRPS  , T3PKS 	142,819	251,989	bacillaene 	Polyketide + NRP	100%															
Region 1.3	transAT-PKS 	473,771	561,977	macrolactin H 	Polyketide	100%															
Region 1.4	lanthipeptide-class-ii 	725,813	754,702																		
Region 1.5	terpene 	875,632	896,372																		
Region 1.6	PKS-like 	978,386	1,019,630	butirosin A / butirosin B 	Saccharide	7%															
Region 2.1	NRPS  , RiPP-like 	73,698	125,490	bacillibactin 	NRP	100%															
Region 2.2	other 	674,148	715,566	bacilysin 	Other	100%															
Region 3.1	transAT-PKS 	1	33,921	difficidin 	Polyketide + NRP	46%															
Region 4.1	transAT-PKS  , NRPS 	1	46,583	rhizoctin A 	Other	6%															
Region 4.2	NRPS 	116,656	182,063	surfactin 	NRP:Lipopeptide	82%															
Region 5.1	transAT-PKS-like 	1	45,657	difficidin 	Polyketide + NRP	53%															
Region 5.2	T3PKS 	161,201	202,301																		
Region 5.3	terpene 	270,928	292,811																		
Region 5.4	NRPS 	315,381	336,976	plipastatin 	NRP	30%															
Region 10.1	NRPS 	1	22,379	locillomycin 	NRP + Polyketide	35%	Antibacterial and antiviral														
Region 11.1	transAT-PKS-like 	1	22,767	difficidin 	Polyketide + NRP	26%															
Region 12.1	NRPS 	1	12,916	fengycin 	NRP	20%															
Region 13.1	NRPS 	1	9,227	fengycin 	NRP	13%															
Region 16.1	NRPS 	1	6,493																		

Microbes	Size (bp)	Proteins with functional assignments	Secondary metabolites
Ba CHAG 1D	3,920,902	3,281	14 types of antimicrobial compounds present in <i>B. amyloliquefaciens</i> strains , NRPs, RPs, PKs, NRPs-PKS, RiPPs
Ba PRIN1	3,920,153	3,282	Locillomycin (+), Lanthipeptide Class ii
Ba CUN9	3,914,165	3,289	Rhizoctin (+),
<i>B. subtilis</i> OG2	4,087,451	3,604	Subtilosin A (+), subtilomycin (+) Difficidin (-), Macrolactin (-), locillomycin (-), Lanthipeptide Class i

Whole Genome Sequencing of Ba PRIN1D



Plant colonization & growth-related genes



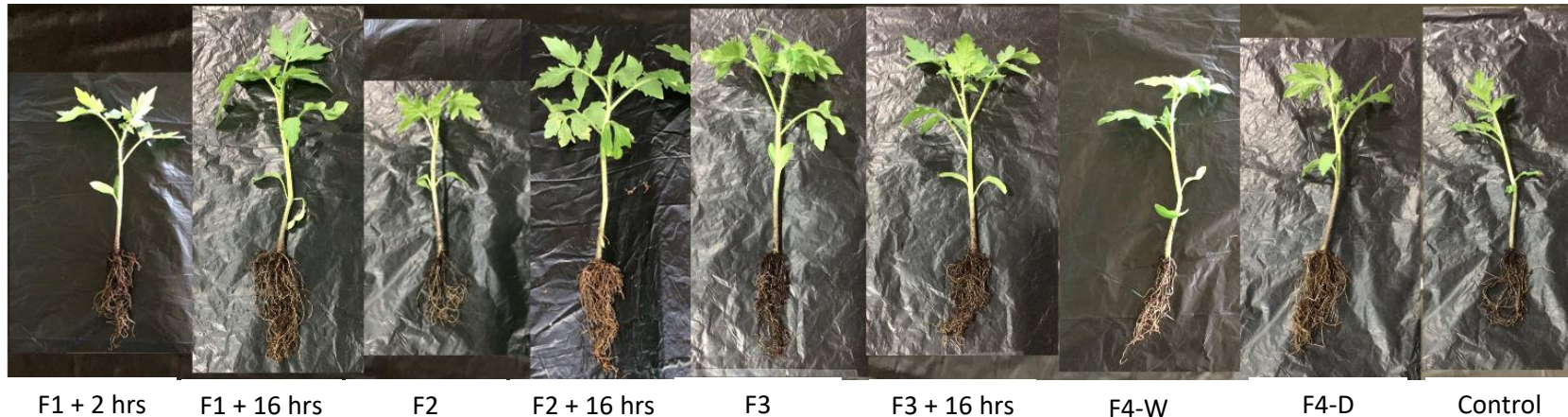
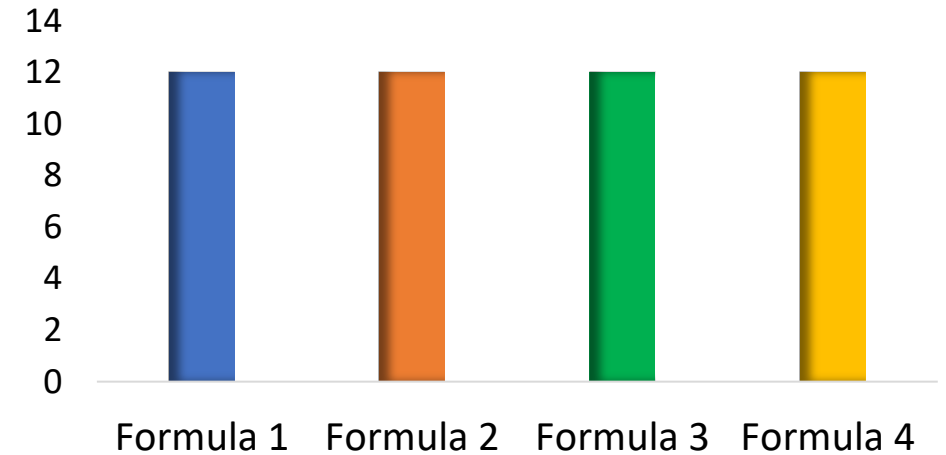
Development of microbial formulation for plant health management

Shelf life

Formulations for different delivery systems

Efficacy enhancement

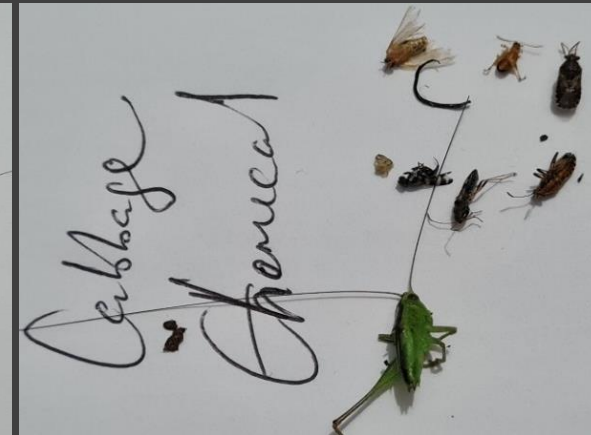
Shelf Life (Months)





Developed the bio-product after series of lab, greenhouse & field experiments - **Biophyt 1.0**

Could be a potential component of integrated disease management practices in the region!

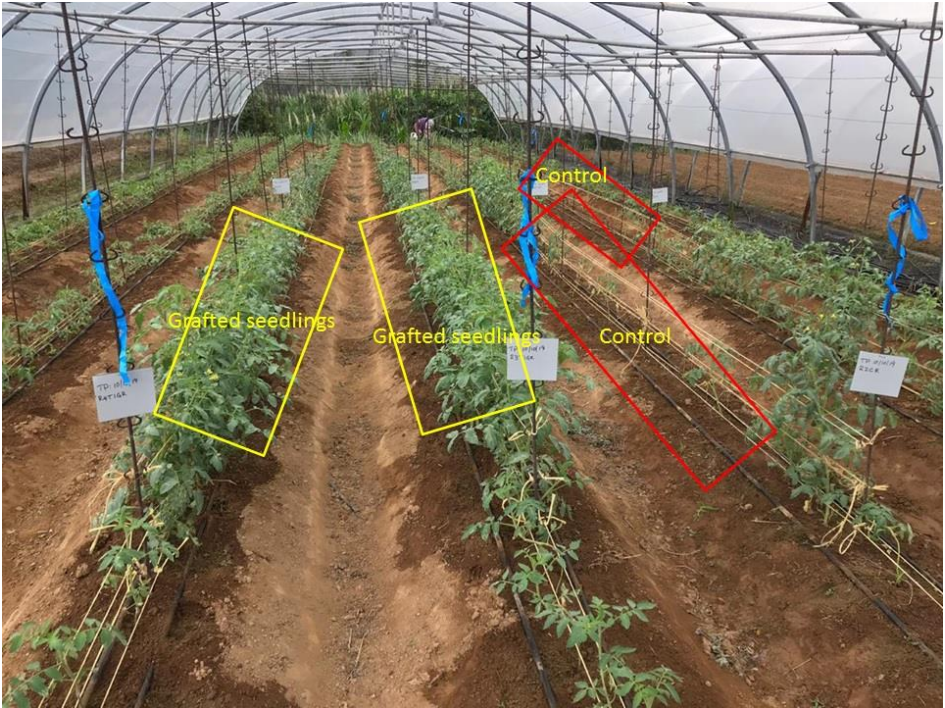
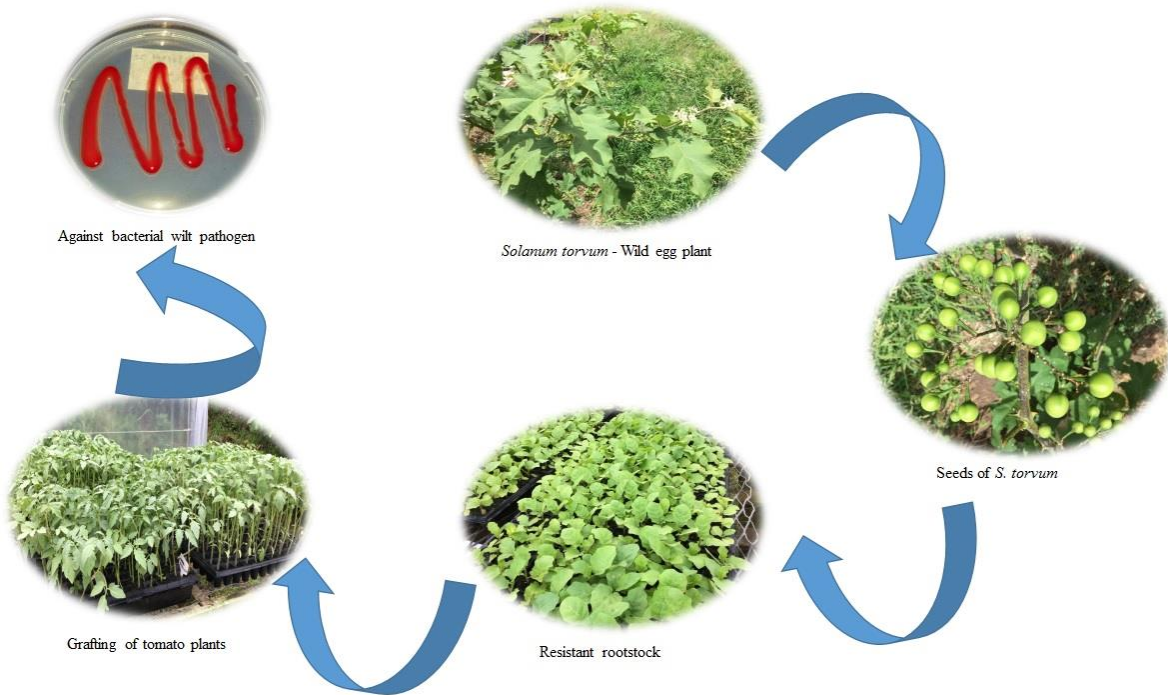


Crop Resistance against Bacterial wilt in tomato - St Lucia

- Tropical condition – high disease severity
- No crop rotation – limited land
- No effective chemical
- No resistant variety
- Semi-protected cultivation



Grafting as technique for enhancing disease resistance



Grafting procedure utilized to prepare planting material for compatibility study

A. Preparation of rootstock (*S. torvum*) by cutting the stem horizontally, B. V-shaped incision with 5 mm depth, C. Preparation of scion (tomato seedling with similar stem diameter) with V shaped wedge, D. Placement of scion wedge into V shaped incision of the rootstock, E. Union of scion and rootstock

DAP	Disease incidence (%)	
	Grafted on ST	Non-Grafted
7	0.00 ^f	35.00 ^{de}
14	0.00 ^f	51.67 ^{cd}
21	0.00 ^f	51.67 ^{cd}
28	0.00 ^f	61.67 ^{bcd}
35	0.00 ^f	83.34 ^{abc}
56	6.67 ^{ef}	98.33 ^a
63	6.67 ^{ef}	100.00 ^a
77	10.00 ^{ef}	100.00 ^a
Fruit yield / plant (kg)		
	4.5	0.0*

Performance of grafted and non-grafted tomato plants in semi-protected trials against naturally infected bacterial wilt pathogen

[Smith, J & Saravanakumar, D, 2022 Journal of Plant Disease Protection]

How to reduce the risk of pesticides?

- Replace the hazardous pesticides
- Reduce the use of pesticides

How to replace or reduce the risk of pesticides?

Regional Perspectives:

Food Safety: Lack of pesticide residue data
Environment: Lack of environmental assessment
Registration: No expedited or provision for registration of biopesticides
Trainings: Extension officers / ICTs
Farmers: Knowledge transfer and information

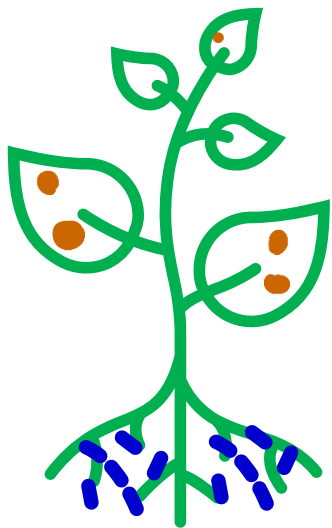
Is that simple?
Need evidence based data - **Health**, Food, Environment

Ban Registration/De-registration

Alternatives

- (i) Integrated management practices
- Cultural
 - **Biologicals**
 - **Genetic approaches**
 - Low risk pesticides/mitigate risk

Summary



Pesticide Risk Reduction

Ongoing Projects – CPSP & UNEP/FAO

Pesticide Suicide Prevention

Tool to determine Pesticide Risk

Integration of efforts to reduce
pesticide risks in the region