CICR
ICAR - Central Institute for Cotton Research
(An ISO 9001:2008 Certified Organisation)

Studies to identify most virulent strains of entomopathogenic fungi for microbial control of whitefly (*Bemisia tabaci*) infesting cotton in North India

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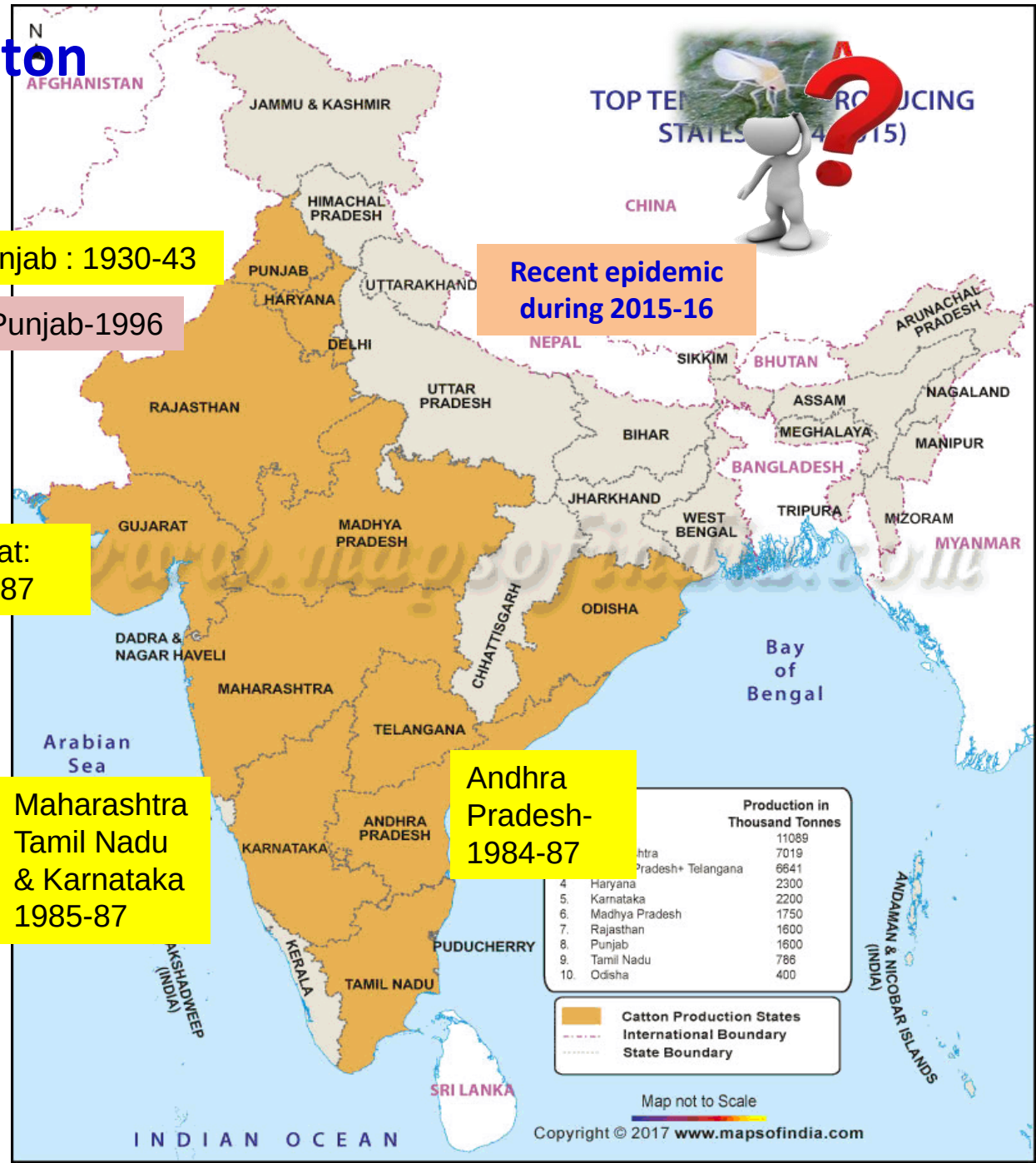
Whitefly a serious threat to cotton

- One of the 600 most destructive pest species
- Recorded on more than 900 plant species and transmitting more than 60 plant viruses worldwide
- **Direct loss to the cotton crop yield upto 28.1%**
- **Indirect loss-CLCuD causes yield losses upto 88.4 %**

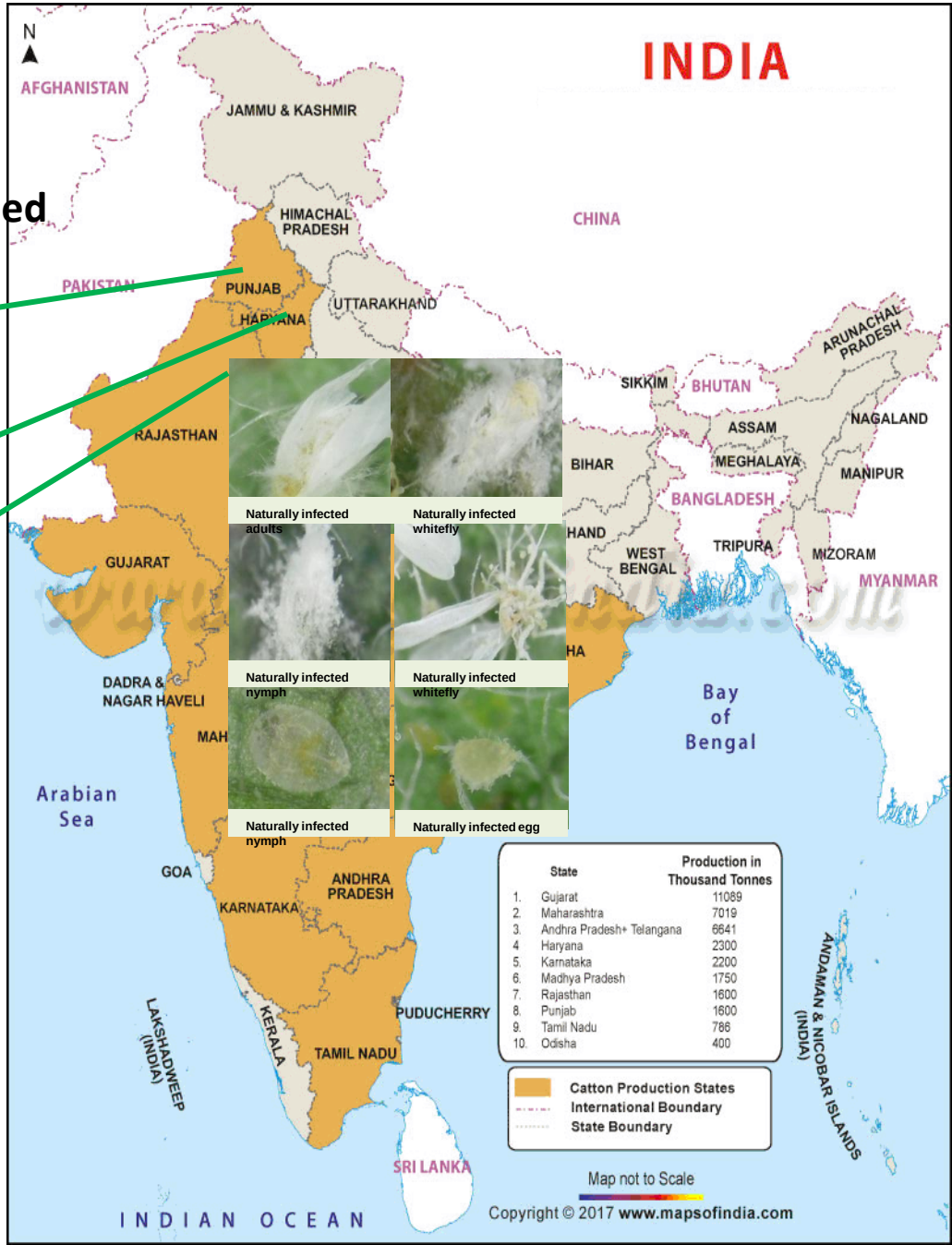
- Among 109 pesticides, 35 insecticides including 6 mixtures
- Indiscriminate use of chemical pesticides causing pesticide resistance
- Among 14, only 2 biopesticide products are available for whitefly management in India



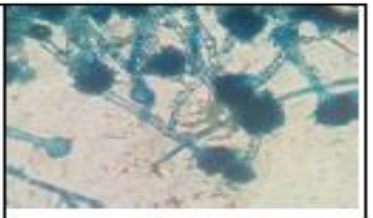
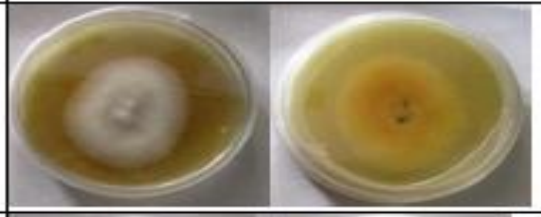
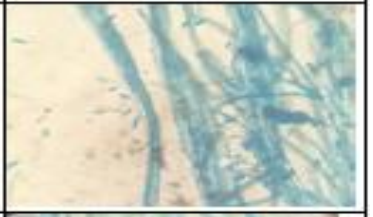
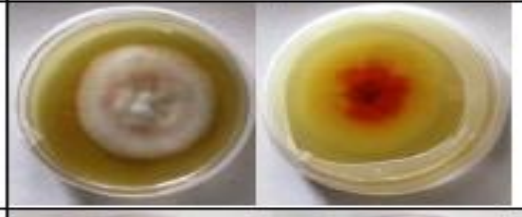
Survey & collections of EPFs

- ~ 500 samples collected from 19 location in 11 districts
- 105 entomopathogenic fungal strains isolated and purified

State	District	Village	Samples	Purified isolates
Punjab	Mansa	Sardulgarh	50	10
		Mansa	40	5
	Abohar	Abohar	38	12
		Panchkoshi	15	2
	Bathida	Talwandisaabo	25	2
	Muktsar	Muktsar	15	2
	Sangrur	Sangrur	15	1
	Faridkot	Faridkot	25	5
	Districts-6	**Locations- 8**	**223**	**39**
Haryana	Sirsa	Sirsa	75	21
		Begu	20	6
		Rangdikhera	35	10
		Musaibwala	35	7
		Kalanwali	15	3
		Chormar	20	3
		Handi Khera	12	3
	Hisar	Hisar	15	2
	Fathehabad	Fathehabad	12	1
	Districts-3	**Locations- 9**	**239**	**56**
Rajasthan	Hanumangarh	Sangaria	15	1
	Sriganganagar	Sriganganagar	25	9
	Districts-2	**Locations - 2**	**40**	**10**
Total	**Districts-11**	**Locations - 19**	502	105



Characterization of new EPF collections

Local 0085	Initial white later turn to Brownish-golden suppressed, scattered, powdery growth, Dark reddish brown pigment **Diameter: 50x62 mm**	W F CICR RS		*Aspergillus* spp. rough spores, round tip	
Local 0086	Dull white to light Brownish raised undulated growth with regular margin, yellowish pigment. **Diameter: 50x57 mm**	W F CICR RS		*Acremonium*, Like 54/55 culture Like 76 culture	
Local 0087	Pinkish white raised uniform colony, yellowish pigment **Diameter: 55x55 mm**	CICR RS Nymphs		?? Septet sticky mycelium, no sporulation Like 70 culture	
Local 0088	Dull white to light pink colour with raised colony, lower surface dark red to brown pigmentation, more sporulating **Diameter: 80x75 mm**	CICR, Nymph,		*Fusarium* 	
Local 0089	Dull white to ashy colour, raised fluffy growth, dull white to yellowish pigment **Diameter: 90x90 mm**	Shri Ganganagar		***Pecilomyces javanicus***	
Local 0090	Initially colonies are in scattered, Purple white with yellowish growth slightly raised, Lower surface no pigmentation **Diameter: 90x88 mm**	Shri Ganganagar		***Pecilomyces javanicus***	

EPF strains at ICAR-CICR

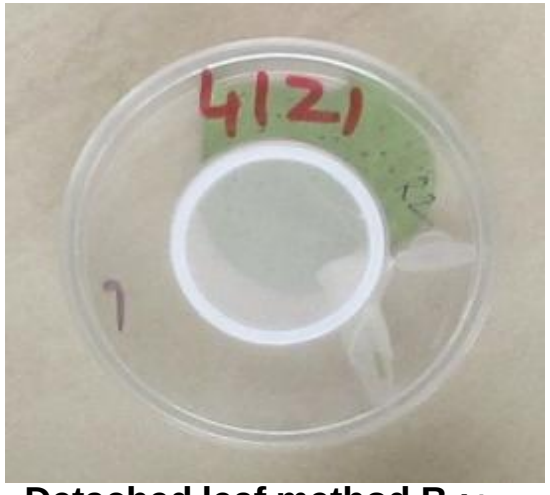
S.no	Genus	New isolate	MTCC	NAIMCC	Total
1.	*Isaria*		49	6	55
2.	*Paecilomyces*	20			20
3.	*Beauveria*	08	122	31	161
4.	*Acremonium*	18			18
5.	*Aspergillus*	13			13
6.	*Fusarium*	23			23
7	*Lecanicillium*	02		13	15
8.	*Hirsutella*	03	08		11
9	*Tolypocladium*	01			01
10	*Aschersonia*	01			01
11	*Metarrhizium*			20	20
12	*Nomureae*		11		11
13	*Pochonia*			3	03
14	*Phaeoisaria*			2	02
	Unidentified	16+3			19
	Total=373	108	190	75	373

Standardization of bioassay protocol for large scale evaluation against whitefly :

5 *in vitro* + *modified polyhouse* methods

Leaf disc method: Petridisc -0.2% water agar- Leaf disc 20 mm dia

Detached leaf method A: Insect Breeding disc -0.2% water agar



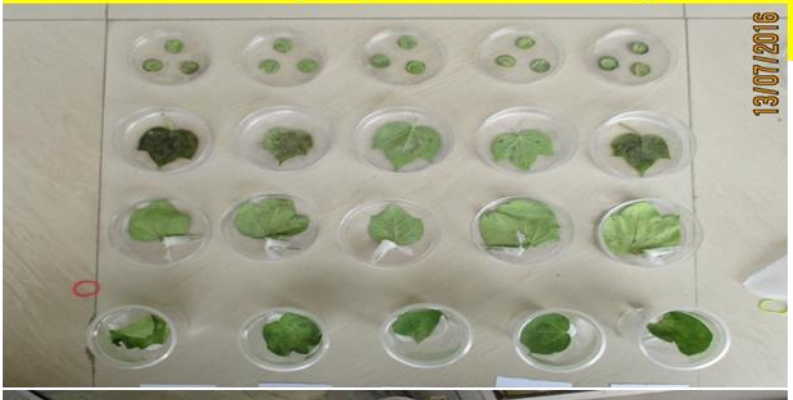
Detached leaf method B : Insect Breeding disc - Leaf with 2 ml -2% Sucrose solution

Detached leaf method C: Plastic cups 15 cm height - Leaf with 10 ml -2% Sucrose solution

Detached leaf method D: Big Plastic trays- Leaf on aluminum mesh support with 2 ml -2% Sucrose solution

New modified bioassay method for large scale evaluation of EPFs against whitefly

5 *in vitro* + 1 *polyhouse* testing methods

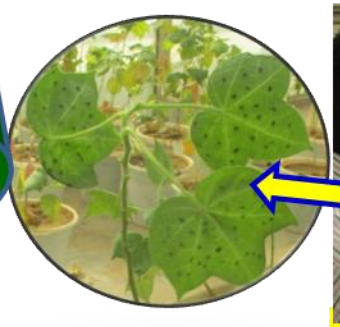


One month old potted plants

Plants exposure for egg laying

Plants incubation for 10 days

Modified method



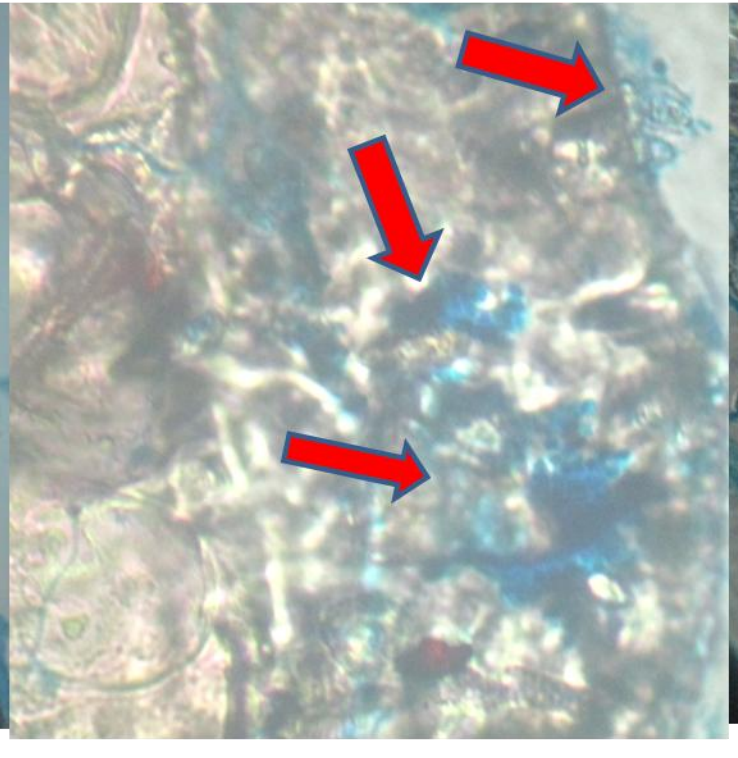
Marking of nymphs

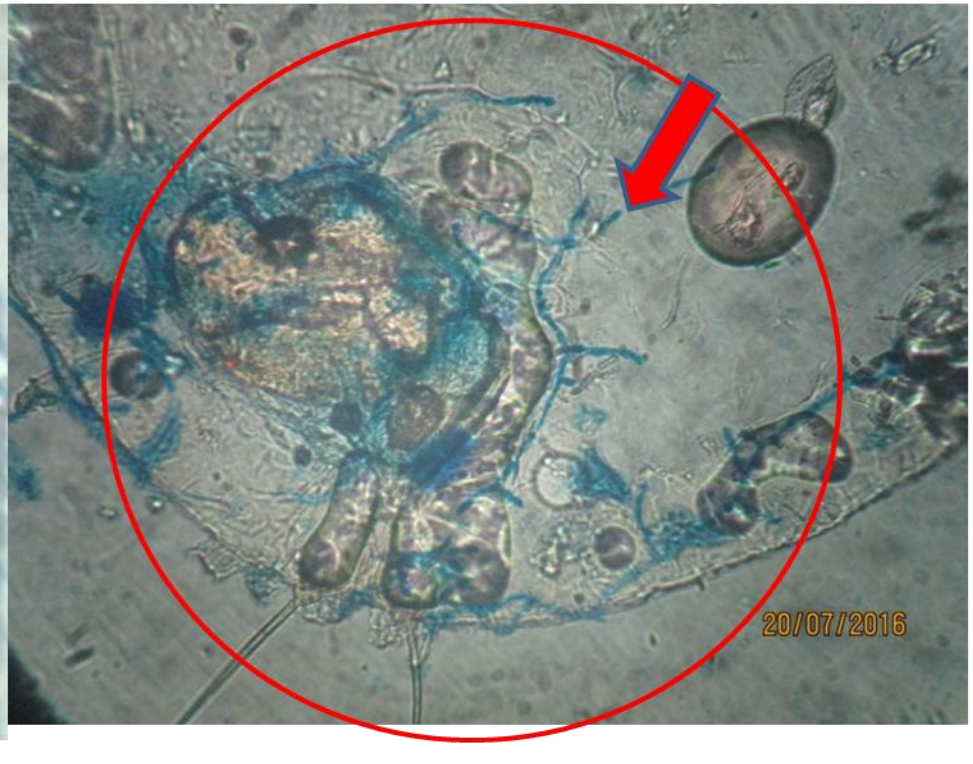
Confirmation

Observation at 3, 5, 7 DAI

Spray with EPF spore suspensions

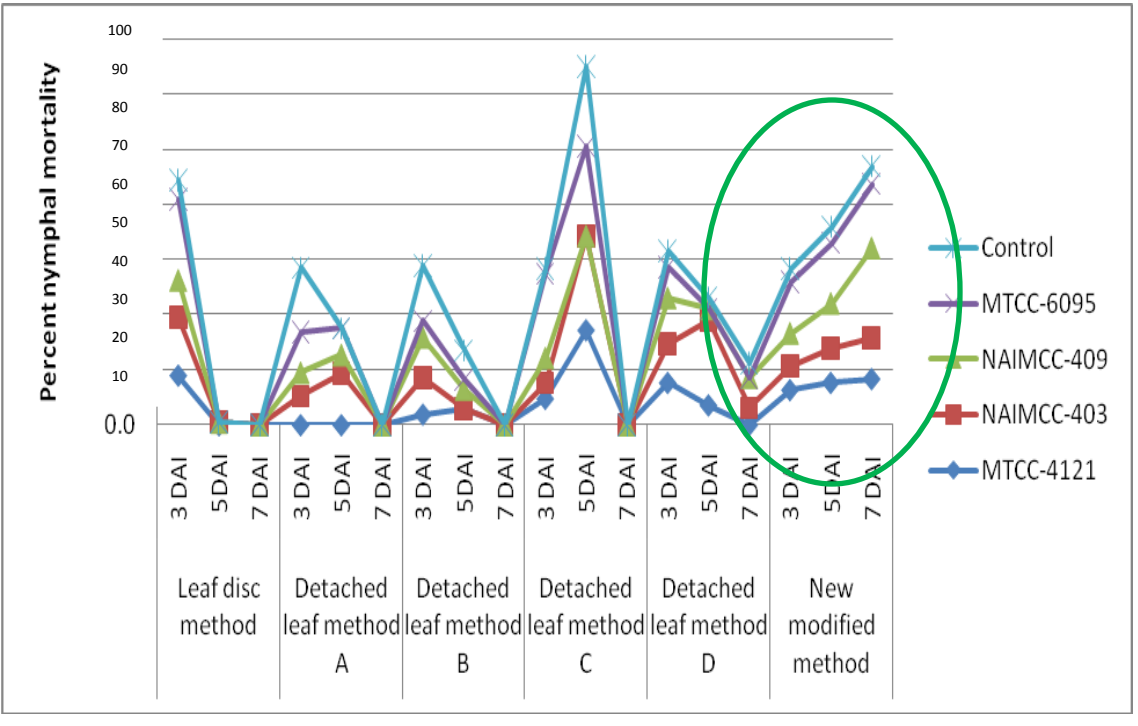
Confirmation - Laboratory & microscopic study

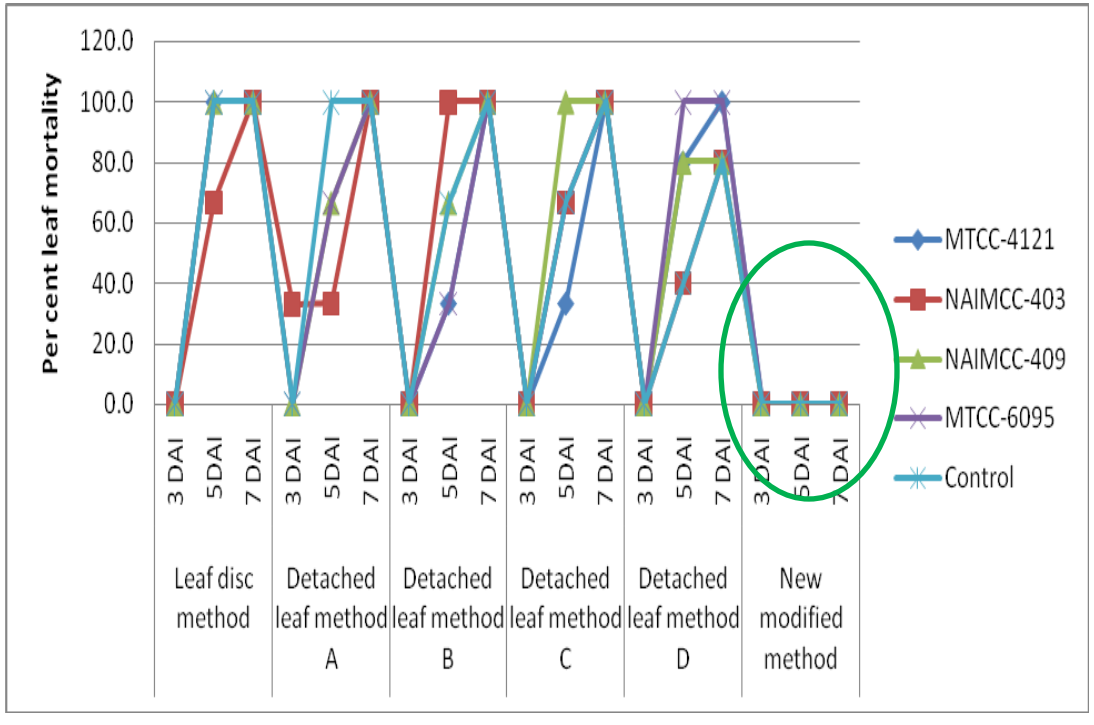




Comparative trend of the whitefly nymph mortality & survival of cotton leaf under 6 different methods

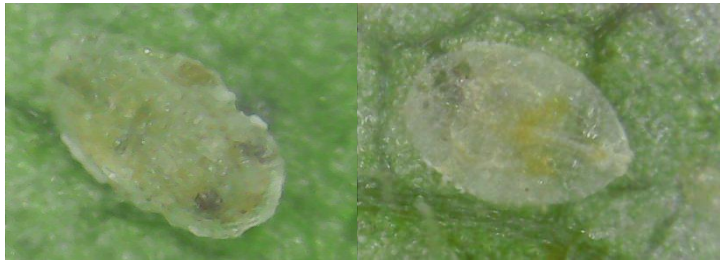
Observation on mortality of nymphs: at 3, 5 & 7 DAI





Healthy

Infected



Infected nymphs with fungal growth

Summary of Bioassay: Percent mortality Range

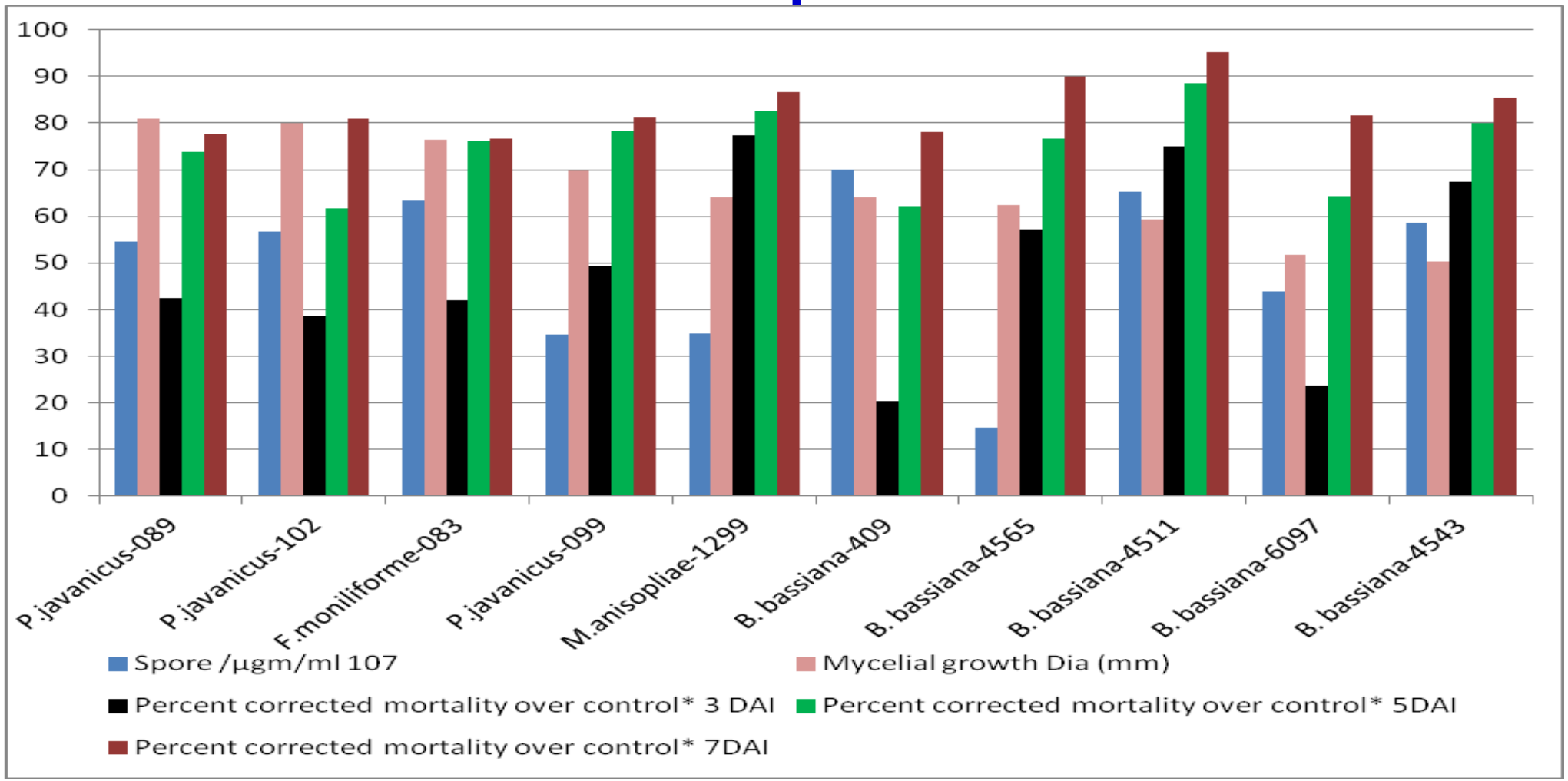
S.No	Spore /µg	Mycelial growth Dia (mm)	Percent corrected mortality over control		
			3 DAI	5 DAI	7 DAI
New EPF - CICR	**1x 10^7**- 3.1 x **10^9**	**24.0 - 90.0**	1.9 - 64.7	3.8 - 76.3	7.8 - 81.1
NAIMCC	5 x 10^7-1.6 10^9	16.8 - 81.0	1.4 - 68.5	7.2 - 80.4	13 - 86.6
MTCC	4. X **10^7**- 6.5 x **10^9**	10.5 - 68.9	0.3 - 83.6	**3.5 - 87.1**	**4.7 - 95.8**

Comparative evaluation of EPF and chemical under polyhouse condition

Treatments	Percent corrected mortality over control		
	3 DAI	5 DAI	7DAI
Beauveria bassiana-6096	21.0(27.3)	50.5(45.3)	75.5(60.3)
Spiromesifen 22.9% w/w SC (1ml/lit)	35.1(36.3)	56.5(48.7)	69.6(56.5)
Beauveria bassiana - 4565	54.9(47.8)	59.3(50.4)	67.8(55.4)
Beauveria bassiana - 4511	18.9(25.8)	39.4(38.9)	64.4(53.4)
Beauveria bassiana - 4537	55.7(48.3)	57.9(49.5)	64.0(53.1)
Beauveria bassiana - 4543	42.7(40.8)	54.5(47.6)	62.9(52.5)
Buprofezin 25%SC (1.6 ml/lit)	15.0(22.8)	18.4(25.4)	59.1(50.2)
Beauveria bassiana - 6097	35.8(36.7)	49.1(44.5)	58.8(50.1)
Beauveria bassiana - 6289	37.4(37.7)	53.6(47.1)	54.7(47.7)
Beauveria bassiana- 6288	22.4(28.2)	38.1(38.1)	54.1(47.4)
Beauveria bassiana - 4542	40.4(39.5)	49.4(44.7)	52.7(46.5)
Pyriproxifen 10% EC (2.5 ml/lit)	41.2(39.9)	42.1(40.5)	52.0(46.1)
Flonicamid 50% WG (0.4 gm /lit)	33.1(35.1)	40.7(39.6)	47.3(43.5)
Beauveria bassiana - 4581	37.4(37.7)	46.3(42.9)	46.8(43.2)
Commercial *L. lecanii* (2 gm /lit)	3.5(10.4)	22.7(28.4)	45.7(42.5)
C.D.	2.895	2.344	2.326
C.V.	4.984	3.302	2.782

(28- 21 Max. Mini Temp & 65-45 % RH)

Performance of Top 10 EPF isolates



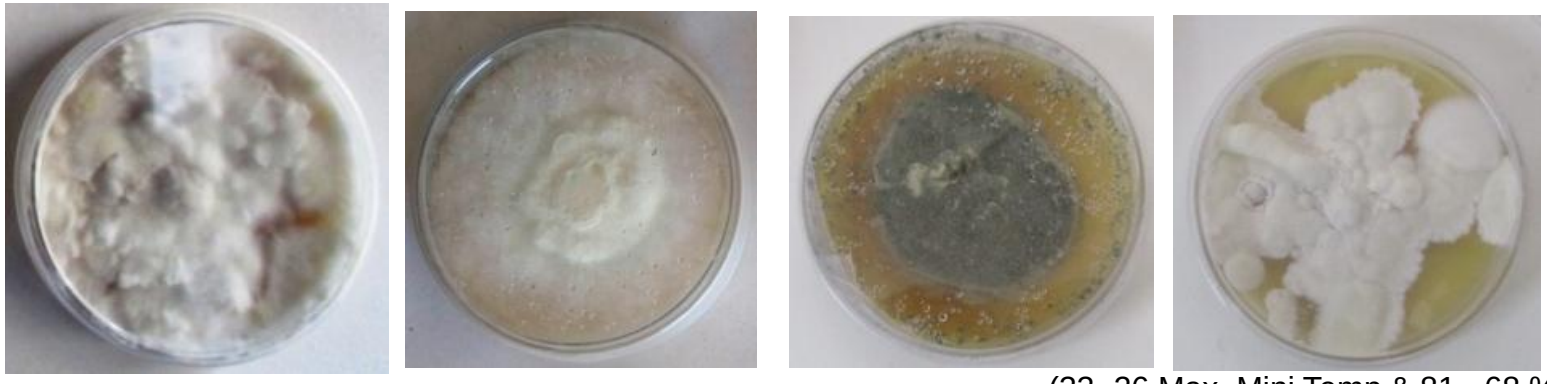
(33- 26 Max. Mini Temp & 81 - 68 % RH)

Scoring: spore production: ≥1x10^{8}spores ; mycelial growth ≥ 50mm dia; mortality > 60% at 5 DAI, 75 % at 7DAI;,

Summary of Probit analysis of selected EPFs

Name	Probit mortality-concentration		Probit mortality-time	
	LC_{50} ($1X10^4$)	Slope ±SE	LT_{50} (days)	Slope ±SE
P. javanicus -0102	**2.977**	3.019 ±0.911	2.854	3.554±0.990
F. miniliformae-0083	3.091	3.197±0.901	2.501	2.789±0.991
M. anisopliae -1299	3.201	3.419 ±0.945	**1.605**	1.902±0.979
B. bassiana -4511	3.143	3.333 ±0.863	2.850	2.779±0.993

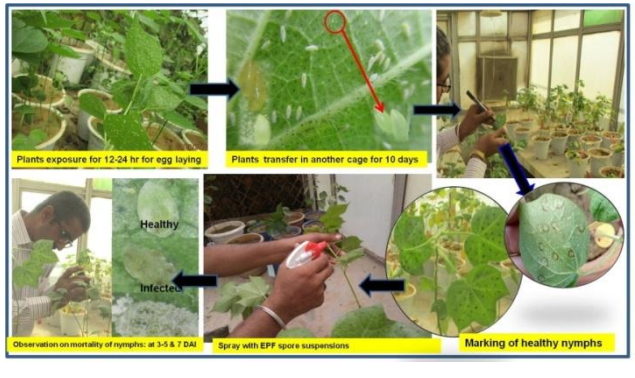
(33- 26 Max. Mini Temp & 81 - 68 % RH)



(33- 26 Max. Mini Temp & 81 - 68 % RH)

Conclusion & Way forward

- New modified polyhouse bioassay method was found to be the most suitable for screening the large number of EPFs
- Short listed *B. bassiana* and *P. javanicus* and *M. anisopliae* were found to be the most virulent against whitefly
- This could provide an alternate option for management of whitefly particularly during early window
- There is a need to study the compatibility of EPFs with IPM/IRM components including chemicals and botanicals; natural enemies and to develop an effective bioformulation with better shelf life.



Thank you !...