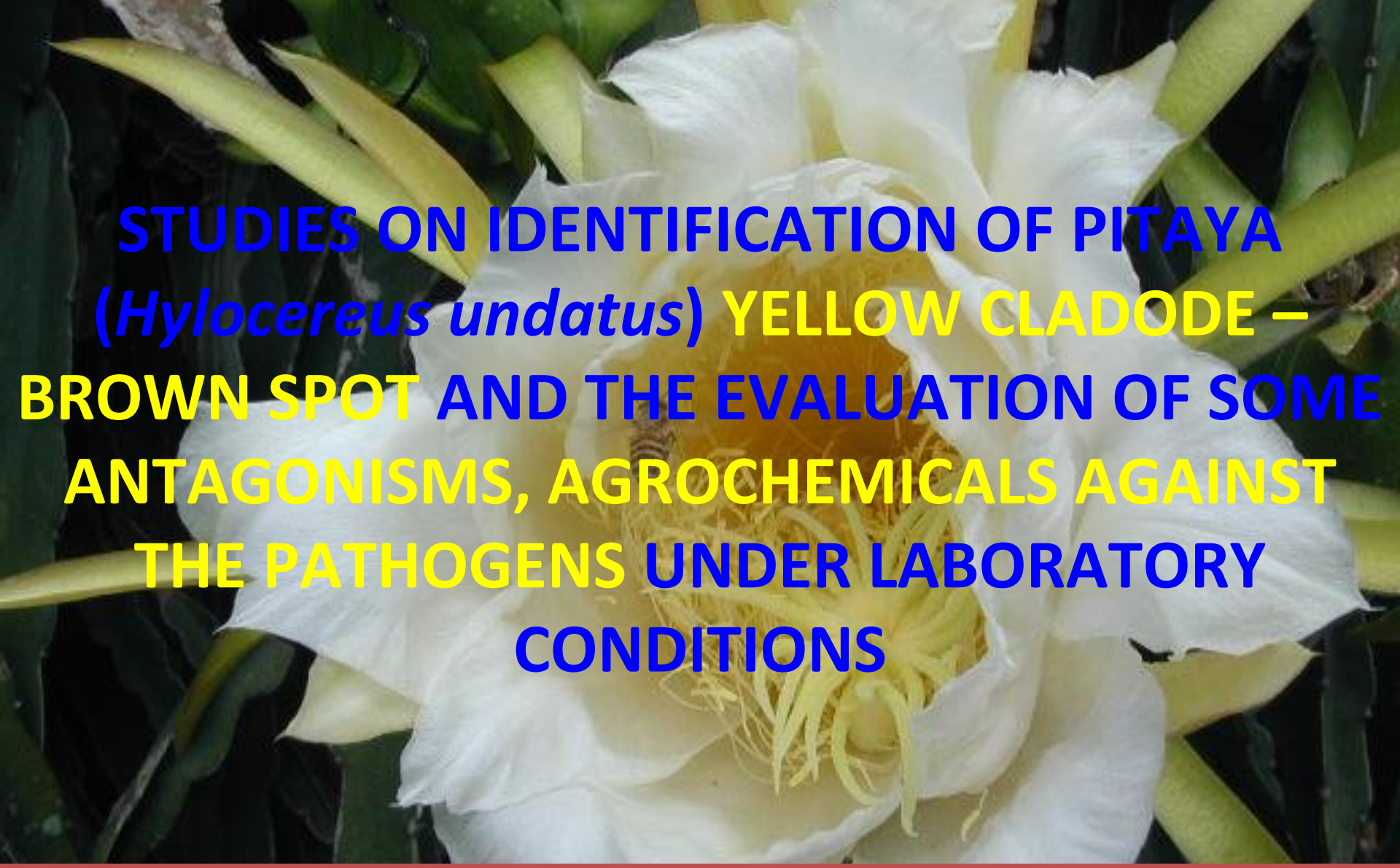

STUDIES ON IDENTIFICATION OF PITAYA (*Hylocereus undatus*) YELLOW CLADODE – BROWN SPOT AND THE EVALUATION OF SOME ANTAGONISMS, AGROCHEMICALS AGAINST THE PATHOGENS UNDER LABORATORY CONDITIONS

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I. INTRODUCTION

- In Vietnam, pitaya (*Hylocereus undatus*) is considering one of the most important tropical fruits and promoting income for producers at Binh Thuan, Tien Giang and Long An.
- At present, the growing area of pitaya is approximately 25,000 ha with the production about 468,300 tons and average yield 24 to 30 tons/ha.
- It has exporting to thirty countries of whole the world.

- However, due to the expansion of planting area and production, it is also facing many problems: anthracnose, bacteria fruit rot, yellow cladode-brown spot, fruit flies, thrips, etc.
- Yellow cladode- brown spot has widespreadly attacking most of fruit growing regional in the South.
- It is usually occuring in the dry season, on the top of plants, initial symptoms appear on upper stem surfaces as small, pinpoint with reddish-brown in colour. Scabs are usually surrouned by yellow halo and spots expand will cause large yellow areas and finally secondary infesion also attack (special stem rot) if in rainy season (Hieu *et al*., 2012).





- In the world, there are very few research on this issue and some of them proved that light factor is relative to yellow cladoe phenomenon (Mizrahi và Nerd, 1999; Sven, 2002; Thomson, 2002; Crane và Balerdi, 2005



II. MATERIALS AND METHODS

The experiments were carried out in the Plant Pathology Laboratory, Plant Protection Division, Southern Horticultural Research Institute, during March to November 2011. Fungal isolates used in the experiments were sub-cultured on Potato Dextrose Agar (PDA) for further examinations.

II. MATERIALS AND METHODS

- **Isolation and identification of pathogen:**

  + The symptomatic parts of plants were collected at Chau Thanh dist., Long An province and Cho Gao dist., Tien Giang province, brought to laboratory and then isolations were made following by Agrios (2005).

  + Pieces of plant samples taken from the margin of healthy and diseased tissues were briefly disinfected with 70% alcohol and thoroughly washed with three changes of sterile distilled water. These pieces were transferred aseptically into PDA plates.

  + Plates were incubated in incubator at 25 ± 10C and the single colonies of fungus were then transferred onto fresh petri dishes to obtain pure culture.

II. MATERIALS AND METHODS

- Identification of pathogen associated with the disease was done by comparing its different morphological characters like growth behaviour, colour, and shape, etc. following keys in literature of Barnett and Hunter (1998), Nelson *et al*. (1983).
- *Sequence of 28S rRNA of Bipolaris* sp. and *Fusarium* sp.: Pure cultures of fungus were sent to NK-BIOTEK Laboratory at HCMC for sequencing using 28S rRNA gene. Using BLAST SEARCH software for comparison of isolated fungus DNA sequence with other relative sequences from gene bank for identification of species.

II. MATERIALS AND METHODS

- **Pathogencity tests**: pathogencity test was conducted in nethouse following Koch's postulate which described by Agrios (2005).

+ In nethouse, thirty cuttings of one year old stem were cultured in plastic pots containing sterilized soil which prepared 8 months before. Each seedling was incubated with 3 bits (5mm in diameter) of fresh mycelium of fungus at separate position on stem surface which was injured by the help of sterilized needle, however control pots were not incubated.

+ To maintained moisture for fungus developing by provide frequently sterilized water.

II. MATERIALS AND METHODS

- ***In vitro* evaluation of bioagents**: Different of antagonistic bacteria viz., *Bacillus subtillis, B. megaterium, Pseudomonas fluorescens* and *Trichoderma* which were isolated from rhizosphere and fruit surface of different crops were obtained from Plant Protection Division.

  These antagonistic fungi were screened in *in vitro* condition for their efficacy in suppressing the growth of fungus by Dual Culture Technique (Dennis and Webster, 1971).

II. MATERIALS AND METHODS

- **Screening of fungicides *in vitro***: Poinsoned Food Technique (Grover and Moore, 1962) was employed to screen the fungicides against fungus.

+ For *Bipolaris crutacea*, an experiment was conducted in Plant Pathology Laboratory to study the effect of various agro-chemicals on fungi under *in vitro* conditions. The experiment was laid out in Completely Random Design (CRD) and replicated thrice. Six fungicides (treatments) were tested at producer recommend concentrations viz. Man 80WP (Mancozeb), Coc 85WP (Copper oxychloride), Norshield 58WP (Cuprous oxide), Viroval 50WP (Iprodione), Daconil 75WP (Chlorothalonil), Biogreen (Oligo-chitosan) along with control treatment

II. MATERIALS AND METHODS

- Similarly, for *Fusarium equiseti*, eight fungicides viz. Nativo 750WG (Tebuconazole + Trifloxystrobin), Phytocide 50WP (Dimethomorph), Amistar Top (Azoxystrobin + Difenoconazole), Aliette 80WP (Fosetyl Aluminium), Amistar 250SC (Azoxystrobin), Ridomil 68WP (Mancozeb + Metalaxyl), Score 250EC (Difenoconazole), Funomyl 50WP (Benomyl) and control (no treated) were tested for disease control.

- Statistical analysis of data: Data were subjected to statistical analysis after proper transformation wherever required as described by Gomez and Gomez (1984).

III. RESULTS AND DISCUSSION:

- **Isolation and identification of pathogen:** Among 40 samples collected and isolated from infested cladode, there were three different fungi species associated with disease: *Bipolaris* sp., *Fusarium* sp. and *Alternaria* sp. However, out of them, the two of *Bipolaris* sp. and *Fusarium* sp. appeared with high proportion in PDA culture, whereas *Alternaria* sp. was only negligible appearance (Table 1).

Table 1. Fungi isolated from diseased samples

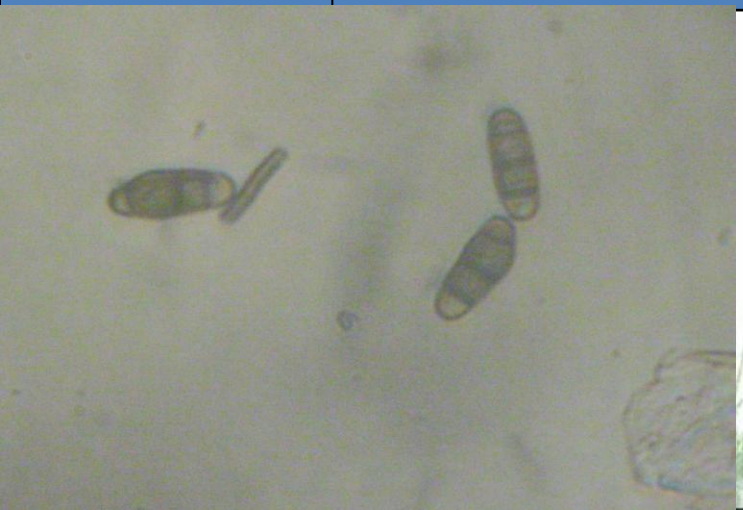
Collection areas	Fungus appearance frequencies (%)		
	Bipolaris sp.	*Fusarium* sp.	*Alternaria* sp.
Tiengiang	62.0	68.0	5.0
Longan	45.0	50.0	10.0

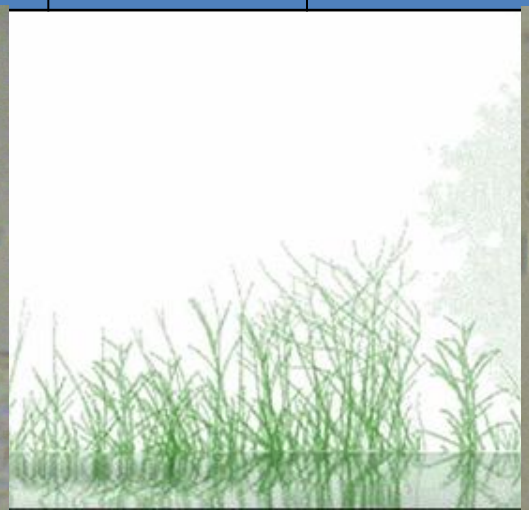
III. RESULTS AND DISCUSSION

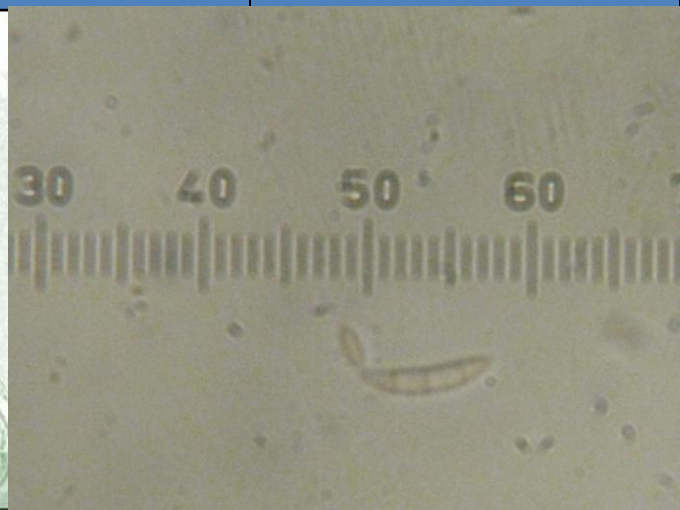
Morphology identification:

Table 2. Morphology characteristics of isolated fungus

Isolates				
			Dimension (µm)	Septation
Bipolaris sp.	Elliptical, straight or curved,	Yellow brown, yellow	19.56±4.97 x 9.3±1.46	1-4 septates (commonly 2-3 septate)
Fusarium sp.	Oval to comma-shaped	White/PDA, tan to brown	17.5±6.06 x 4.58±2.68	1-4 septates (commonly 2-4 septate)







III. RESULTS AND DISCUSSION

- **Pathogencity test:** All of the two isolates tested were virulent on dragon fruit (*Hylocereus undatus*) both nethouse and in the field. These spots were appeared at 5 days after inoculating with fungus mycelium. Scabs were brown spot symptom with yellow halo which were induced on cladode surface of the plants after inoculation with the isolates were similar to those observed in the field (Table 3).

Table 3. Proportion of scab formation after artificial incubated

Isolated fungus	Scab formation (%)	
	Nethouse	Field
Bipolaris sp.	56.25	62.50
Fusarium sp.	52.08	60.42

Fig.1. Inoculated with *Bipolaris crustacea*

Fig.2. Inoculated with *Fusatium equiseta*

III. RESULTS AND DISCUSSION:

* **Sequence of 28S rRNA gene of *Bipolaris* sp. and *Fusarium* sp.**

- **The sequences of 28S rRNA gene of *Bipolaris* sp. and *Fusarium* sp. revealed 188bp and 179bp rDNA sequence, respectively and when we made BLAST SEARCH from the gen bank on NCBI, the results revealed that *Bipolaris* sp. and *Fusarium* sp. rDNA was identity as *Bipolaris crustacea* and *Fusarium equiseti* with up to 99% and 100% respectively.**

III. RESULTS AND DISCUSSION:

Evaluation of effective of antagonisms again pathogens

Table 4. Effective of antagonist against *B. crustacea*

Treatments	Percent inhibition (%)					
	2D.A.I	3 D.A.I	4 D.A.I	5 D.A.I	6 D.A.I	7 D.A.I
B. subtillis	6.19c	**24.50b**	**37.66b**	**36.60b**	**35.41b**	**45.63b**
B. megaterium	3.09d	8.17d	17.49d	26.04c	29.17c	32.67d
P. fluorescent 8	**15.47b**	13.60c	29.57c	**38.11b**	**33.76b**	40.83c
SOFRI-Trichoderma	**24.75a**	**35.37a**	**68.58a**	**73.55a**	**77.01a**	**80.27a**
CV (%)	**1.13**	**2.08**	**2.84**	**2.89**	**1.95**	**1.87**
LSD (0,01)	**0.45**	**1.13**	**2.23**	**2.04**	**1.67**	**1.70**

D.A.I: Day after innoculated. Values in the same column followed by the same letter were not statistically significant. Oginal data were converted to arcsin before analysis.

III. RESULTS AND DISCUSSION:

Table 5. Effective of antagonist against *F. equiseti*

Treatments	Percent inhibition (%)					
	1 D.A.I	2 D.A.I	3 D.A.I	4 D.A.I	5 D.A.I	6 D.A.I
B. subtillis	**15.16a**	**15.25a**	**19.77b**	**30.79b**	**37.44b**	**53.00b**
B. megaterium	18.24a	12.20b	16.20c	38.15d	30.95c	48.50c
P. flourescent 8	15.16a	12.20b	15.82c	25.33e	26.23d	44.67d
SOFRI-Trichoderma	7.58b	12.20b	**42.29a**	**59.67a**	**66.82a**	**75.33a**
CV (%)	8.42	1.47	1.5	1.04	1.23	0.82
LSD (0,01)	2.66	0.44	0.61	0.57	0.69	0.57

D.A.I: Day after innoculated. Values in the same column followed by the same letter were not statistically significant. Ogrinal data were converted to arcsin before analysis.

III. RESULTS AND DISCUSSION:

Evaluation of effective of fungicides against pathogens

Table 6. Effect of some fungicides against *B. crustacea*

Sr.no	Treatments	Percent inhibition (%)						
		1D.A.I	2D.A.I	3D.A.I	4D.A.I	5D.A.I	6D.A.I	7D.A.I
1	Man	**100a**	**100a**	**100a**	**100a**	**100a**	**100a**	**100a**
2	Super Cook	89.74b	84.90c	84.62c	80.25c	81.88c	81.34c	82.17b
3	Norshield	89.74b	93.04b	93.68b	94.08b	94.70b	94.77b	**95.03a**
4	Viroval	**100a**	**100a**	**100a**	**100a**	**100a**	**100a**	**100a**
5	Daconil	**100a**	87.44c	84.82c	77.20c	76.25d	73.80d	68.12b
6	Biogreen	0.0c	0.0d	0.0d	0.0d	1.33e	1.89e	9.35c
	CV (%)	**3.68**	**3.89**	**2.67**	**4.20**	**2.81**	**3.11**	**7.21**
	$LSD_{0,01}$	**5.24**	**5.29**	**3.60**	**5.55**	**3.74**	**4.13**	**9.68**

D.A.I: Day after innoculated. Values in the same column followed by the same letter were not statistically significant. Orginal data were converted to arcsin before analysis.

III. RESULTS AND DISCUSSION

Evaluation of effective of fungicides against pathogens

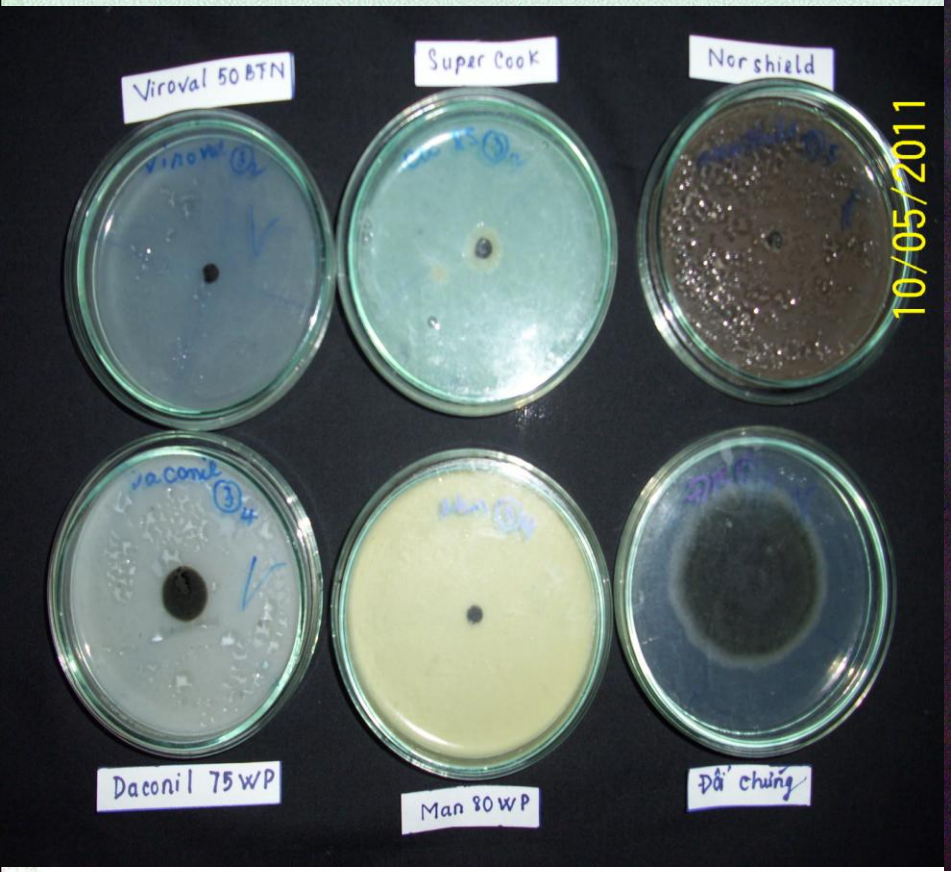
Table 7. Effect of some fungicides against *F. equiseti*

Sr.no	Treatments	Percent inhibition (%)					
		1D.A.I	2D.A.I	3D.A.I	4D.A.I	5 D.A.I	6D.A.I
1	Nativo	**100a**	**100a**	**100a**	**100a**	**100a**	**100a**
2	Phytocide	45.80c	51.99e	61.90e	73.82d	73.83e	70.44d
3	Amistar Top	95.59a	84.87c	89.25c	90.49c	87.76c	87.63c
4	Aliette	**100a**	**100a**	100a	**100a**	**100a**	**100a**
5	Amistar	71.08b	47.20e	62.54e	61.62e	63.04f	64.96e
6	Ridomil	**100a**	**100a**	**100a**	**100a**	**100a**	**100a**
7	Score	**100a**	87.55b	92.10b	92.05b	93.37b	94.74b
8	Funomyl	94.61a	62.40d	71.25d	76.12d	77.19d	70.30d
	CV (%)	**10.67**	**5.21**	**2.46**	**2.10**	**1.63**	**1.28**
	$LSD_{0,01}$	**9.14**	**3.92**	**1.93**	**1.67**	**1.29**	**1.20**

IV. CONCLUSIONS

- Isolations of yellow cladode – brown spot were identified that *B. crustacea* and *F. equiseti* which were the main organisms related with the disease.
- All tests of biological agents were inhibited mycelium growth of the fungus *B. crustacea* and *F. equiseti* in the laboratory, specialy the SOFRI-Trichoderma gave highest ability of inhibition against both of *B. crustacea* and *F. equiseti* under *in vitro* conditions.
- Treatments of Man 80WP and Viroval 50WP were completely inhibited *B. crustacea*, whereas Ridomil 68WP, Aliette 80WP and Nativo 750WG proved highly effective (100%) to *F. equiseti*, followed by Score 250EC.
- For further research should be confirm these results to fields as well as make the problems clear with the studies on abiotic factors (light, temperature, radiation) which are effectively stimulating develop of pathogen or not.

Pathogencity testing in nethouse



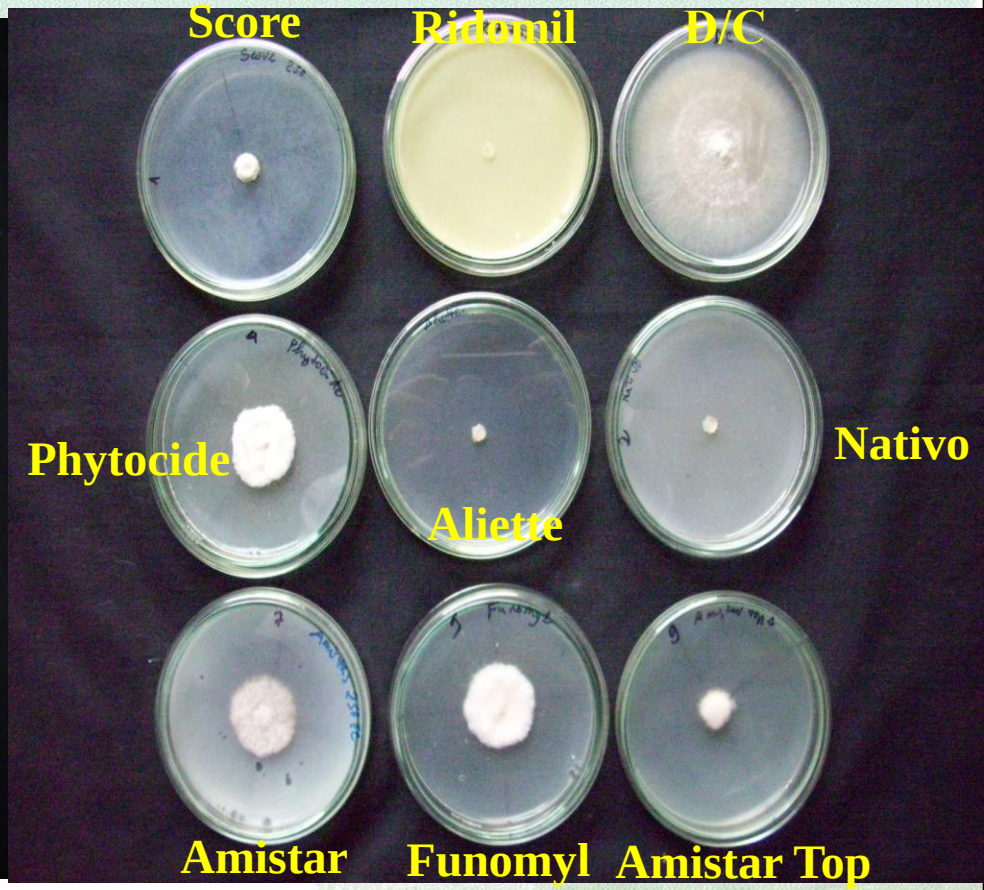


Fig 3. Chemicals testing against to *B. crustacea* at 7 D.A.I

Fig 4. Chemicals testing against to *F. equiseti* at 7 D.A.I

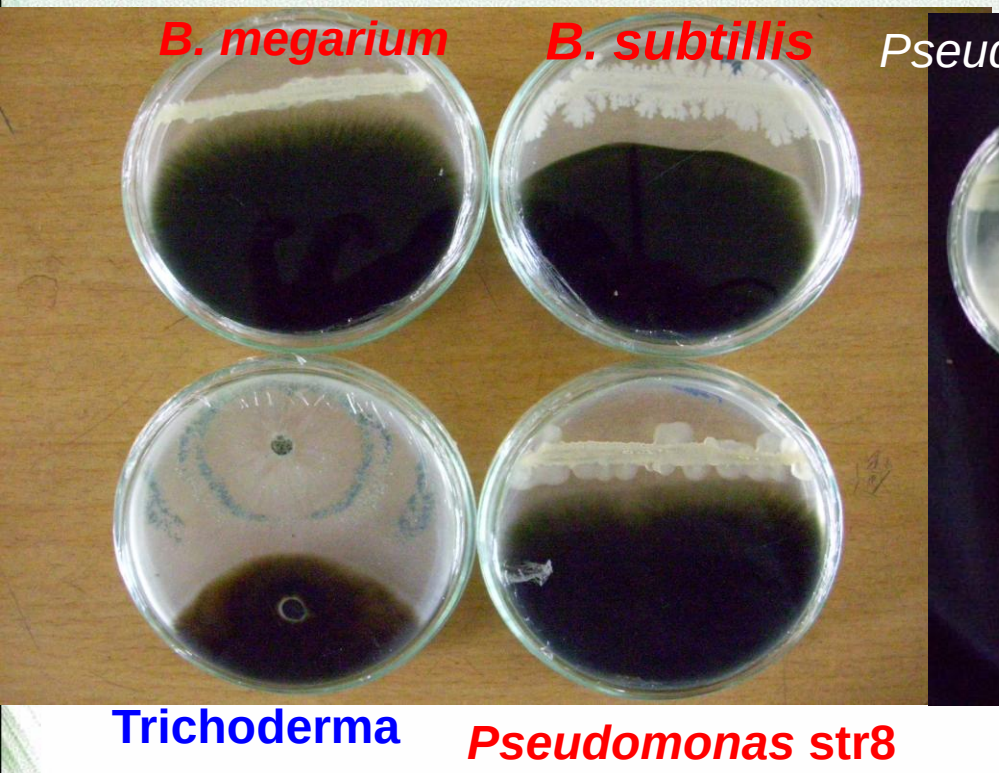


Figure 5. Antagonists testing against to ***B. crustacea*** at 7 D.A.I

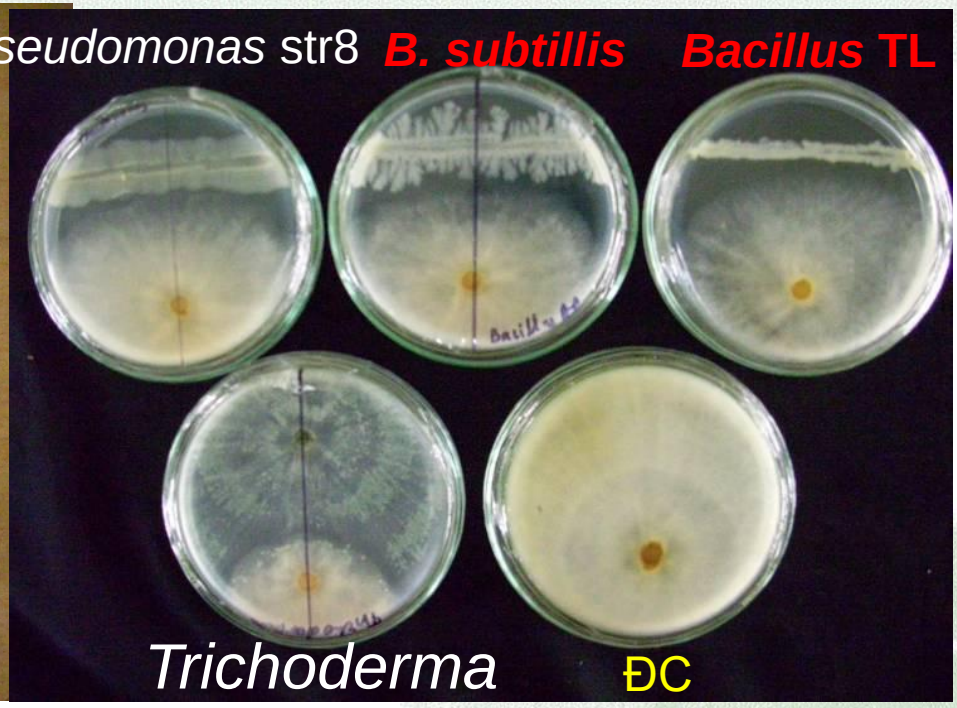


Figure 6. Antagonists testing against to *F. equiseta* at 7 D.A.I

THANK YOU FOR YOUR ATTENTION