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BIOLOGICAL CONTROL OF CUCUMBER DOWNY MILDEW CAUSED BY *Pesudopronospora cubensis* (BERK.& CURT.) ROSTOW UNDER GREENHOUSE CONDITIONS.

El-Kafrawy, A. A. and A. A. Ismail
Plant Pathology Research Institute, Agric. Research Center, Giza , Egypt

ABSTRACT

In green house trial, all tested cucumber cultivars were differed in their response to downy mildew disease Beto Star cultivar exhibited the highest percentage of infection (74.84%) and considered the highest susceptible genotype tested, while Shourk cv. was the least susceptible one (33.71%). All commercial biocides applicator as protected treatment reduced downy mildew disease severity in both Tokh and Sakha locations. Rhizo-N resulted in the highest efficacy in controlling the downy mildew disease (62.65 and 61.17%), while Plant Guard was the lowest in this respect, in comparison with Shampion fungicide . In addition, all bioagent treatments, which reduced downy mildew infection significantly improved the plant growth and increased the plant height, the number of flowers and fruit yield, when they were sprayed as protected treatments. Rizo-N exhibited the best effective one , where it resulted increasing in plant height, number of flowers and fruit yield(286cm/ plant , 91.90/plant and 49.94 kg/polt), respectively followed by Shampion fungicide and Blight Stop ,while Plant Guard was the least effective one . The curated commercial biocides treatment showed the lowest efficacy in controlling the downy mildew disease and plant growth parameters compared with Equagen Pro fungicide resulted in the highest efficacy in controlling the disease and increased plant height, number of flowers and yield at Tokh and Sakha, respectively. Infected cucumber leaves exhibited more activity of oxidative enzymes than healthy in both treatments of biocides as protected or curetted. On the other hand ,activity of the determined enzymes were decreased when Equagen Pro fungicide was used curetted treatment compared to the other biocides .

INTRODUCTION

Downy mildew of cucumber is widely distributed all over the world and its host range includes great number of plants in family Cucurbitaceae (Spencer,1981) The causal organism *Pseudpronospora cubensis* (Berk.&Curt.) Rostow is considered one of the most important diseases of cucumber and was found to attack the plants in open fields and protected cultivation (Shama *et al.* 1998 and Sharma *et al.* 2003) . Downy mildew causes severe damage to cucumber plants and often is a limiting factor in the production cucurbits crops (Lebeda and Vrban 2004). The reaction of different cucumber varieties to *P.cubensis* differed in their reaction to the disease . The highest disease severity was recorded on Beto Star and Pasandra , while the lowest disease severity was on Marmar and Primo. (Vaskuti and Feher,1999 and Abdel-Karem,Eman,2002). The discovery of new products of biological control agents that are commercially available in the world and the demonstration of their effects in reducing incidence and disease severity have opened a new promising avenues for practical application in agriculture and for promoting environmental . *Trichoderma harzianum* strain T.39, developed at the Volcani Center in Israel and marketed as Trichodex 20 P. The reported mode of action of T.39 is competition for nutrients and interference with

production of lytic enzymes by the pathogen and induced resistance. *Bacillus.subtilis* strain QST 713 produced by Agra Quest Inc. and marketed as Serenade. This product is advertised to have a spectrum of activity including over 40 plant diseases including common greenhouse disease such as gray mold, downy and powdery mildew . The bacterium is presumed to work through a number of mode of action such as competition, parasitism, antibiosis and induction of systemic acquired resistance (SAR) (Timothy and Richard,2001). Georgieva(2003) found that the protective treatments with bioagents were more effective than curative treatments because they prevent the pathogen from invading and colonizing on tissues plants. Haggag-Wafaa(1997) suggested that *T.harzianum* can increase plant growth of radish, since it produces plant hormone such as Indole-3 acetic acid (IAA) and GA3 . Also *B. subtilis* produce large concentration of IAA and GA3 which, increased shoot: root elongation and yield of sugar beet. *Spherothica fuliginea* was parasitized by common phylloplane yeast and *Tilleiopsis* sp. The first compound was preparation of cellulose produced by a compound had an eliciting effect, triggering, peroxidase, polyphenoloxidase and chitinase activity to produce systemic acquired resistance(SAR) with the production of ethylene and salicylic acid . (Martinez *et al.* 1999).

The objective of the present investigation was to study the effect of some commercial biocides such as Plant Guard, Blight Stop and Rhizo-N used as foliar spray on Beto Star cucumber cv. as protected and curated treatment in controlling downy mildew caused by *P.cubensis* .

MATERIALS AND METHODS

I – Varietal reaction:-

Four hybrid cultivars i.e Beto Star, Sherouk, Mena and Nile were used in this study . The experiments were carried out at ToKh and Sakha locations under greenhouse conditions during 2005 and 2006 seasons . Sedling of cucumber cultivars ,30 days after sowing were transplanted in rows of 7m length and 1m width were prepared for each treatment .The treatments were arranged in complete blocks design with four replicates, each plot had 28 plants. Culture practices were followed as usual . The disease severity was determined from starting symptoms appearance till the end of the growing period .

2- Effect of different biocides on downy mildew disease severity:

The three selected biocide namely, Plant Guard, Rhizo-N and Blight stop were evaluated under greenhouse conditions for their control efficiency .

Table(1): The commercial formula and the rate of application of the tested biocides:

Commerial formulation	Bioagents	Rate of application	Source
Plant Guard	*Trichoderma harzianum* $30x10^6$ spores/ml.	2.5 ml/l	El-Nasr company Sadat city.
Rhizo-N	*Bacillus subtilis* $32x10^6$ Cells/g. *Trichoderma harzianum*	2.5 g /l	El-Nasr company Sadat city.
Blight Stop	$3x10^6$spores/ml .	10 ml/l .	Plant pathology Inst. ARC.

Table (2): **The protected and systemic fungicide, active ingredient, active material and rate of application:**

Fungicids	Active ingredient %	Rate of application	Active material
Shampion	77	2.5 g/l	Copper hyderoxide
Equagen Pro	52.5	0.45 g/l .	5-methyl-5-(4-phenoxyphenyl-3-phenylamino) -2,4- oxozolidinedi one.

Cucumber seedling of Beto Star cv. were used in this experiment to study the effect of biocides on downy mildew disease development . The biocides were used as protected and curated treatments.

1- Protected treatment :-

In this case the biocides were sprayed before symptoms appearance of the downy mildew disease and used Shambion at 2.5g/L. as protected fungicides after transplanting in greenhouse and repeated 15 days intervals.

2- Curated treatment :-

The biocides were sprayed after symptoms appearance and used Equagen Pro 0.45g/L . as systemic fungicide. The disease severity was assessed weekly starting from symptoms appearance till the end of the growing period. Data were recorded as a mean percentage of the two season 2005 and 2006. Also, plant height, number of flowers and the fruit yield were also calculated

III Determination of oxidative enzymes :-

Leaves of healthy and diseased plants of Beto Star cultivar were collected before and after inoculation and treatment of bioagents . Fresh leaves were cut at the base for rough determination of oxidative enzymes . Enzymes extraction were prepared as described by Maxwell and Bateman (1967).

Peroxidase , polyphenoloxidase and Catalase activity were determined according to the methods described by Allam and Hollis(1972), Broesch (1954) and Colowick and Kaplan(1955).

Disease assessment :-

The disease was determined according to the next scale and was calculated using the following equation developed by Kremer and Unterstahofer, (1967).

$$R = \frac{\sum (a \times b)}{N \times K} \times 100$$

where : -

R = Disease index.
a = Number of leaves within infection grade.
b = Number of value of each grade.
N = Total number of leaves.
K = The highest degree of infection in category.

Table (3): The scale used to estimate the infection degree of the different downy mildew sample:

Numerical value or infection category	Infection	Mildew response
0	Mildew free	R
1	10% of the leaf surface was infected	LS
2	11-25% of the leaf surface was infected	MS
3	26-50% of the leaf surface was infected	S
4	51-100% of the leaf surface was infected	HS

R = Resistance. **S = Susceptible .**
LS = Least Susceptible . **HS = Highly Susceptible .**
MS = Moderately Susceptible .

RESULTS AND DISCUSSION

I - Varietal reaction :

The reaction of four cucumber cultivars i.e Beto Star, Sherouk,Mena and Nile to *P. cubensis* was studied under greenhouse condition.

The results obtained Table (4) indicated that, the tested cultivars were differed in their response to *P.cubensis*. The cultivars Beto Star and Mena were highly susceptible showing percentage of infection 74.84% and 50.70%, respectively . The two cultivars Sherouk and Nile were susceptible .However, the lowest percentage of infection was observed on Sherouk cv. (32.69 and 33.71%) at Tokh and Sakha, respectively.The differences between the two cultivars Beto Star and Sherouk were highly significant, while the other tested cultivars fall in between the results obtained at Tokh location were more or less similar to those at Sakha location .These results are in accordance with those obtained by Abd El-Karem, Eman(2002) indicated that the highest disease severity was recorded on Beto Star, Pasandra and Rawa, while the lowest disease severity was on Marmar and Premo. As regard to, resistance or suscepitibillity may be attributed to a number of internal or external factors which may decrease or increase the change and degree of infection. Several kinds of resistance may physiological process or chemical compound and may depend upon the lock of the nutrient or substance that is either performed or formed only response to infection these results were reported by many investigators, Neykov and Dobrev (1988), El-Zayat *et al.* (1993) and Ibrahim (2007).

Table (4): Response of some cucumber cultivars to downy mildew caused by *P.cubensis* under greenhouse conditions at Tokh and Sakha location during 2005-2006 seasons.

Cultivars	Disease severity (%)							
	Tokh			Sakha			Combined data	Downy mildew response
	2005	2006	Mean	2005	2006	Mean		
Beto Star	71.18	73.95	72.57	76.35	77.87	77.11	74.84	HS
Sherouk	30.79	34.58	32.69	33.46	35.98	34.72	33.71	S
Mena	42.20	47.15	44.68	53.04	56.72	54.88	50.70	Hs
Nile	38.62	41.34	39.98	42.96	46.30	44.63	42.31	S
L. S. D at 5%	6.41	5.75	-----	8.45	9.76	-----	-----	-----

HS = Highly susceptible **S = Susceptible**

2- Effect of spraying different biocides on downy mildew disease of cucumber :

Plant Gurad, Blight Stop and Rhizo-N were sprayed for either protected or curated treatments for controlling downy mildew disease on highly susceptible Beto Star cucumber cv. as foliar spray.Results obtained in Table (5) showed that Plant Gurad, Rhizo-N and Blight Stop were significant in controlling downy mildew infection when used as protected treatment Rhizo-N proved to be the most effective resulting, in controlling the disease (62.65%)followed by Blight Stop (59.90%) . Plant Guard exhibited the lowest efficacy (40.97%).In this respect. Shampion fungicide had the best efficacy in reducing disease severity(69.44%) when used as protected treatment in both season 2005 and 2006 at Tohk location. The results obtained at Sakha were more or less similar to those at Tokh locality . Similar results in(Table 6) were obtained ,since plant Guard, Rhizo-N and Blight Stop when used as curetted treatment were less effective in controlling downy mildew disease . Rhizo-N was higher than Blight Stop or Plant Guard in this respect, Which recorded 41.18, 32.73 and 20.81%, respectively. Equagen Pro fungicide had the best efficacy in controlling the disease severity (88.72%) when, used as curetted treatment in both season 2005 and 2006 at Tokh location. The results obtained at Tokh location were more or less similar to those at Sakha location. These results are in line with those reported by Bedlan (1997), Elad *et al.*(1999) Vmesha *et al.*(1999) and Georgieva (2003), they found that the protective treatments were more effective than curative treatments because they prevent the pathogen from invading and colonizing the plants. Regarding Rhizo-N *(Bacillus Subtilis*) which showed considerable effect in controlling downy mildew disease. This might be due to that bacteria produce more antibiotics (bacteriocin and subtilisin) which act as inhibitors to pathogenic fungi (Asaka & Shado,1996 and Farahat,1998). The biocide Serenade produced by Agra Quest Inc. (Davis, CA) is the latest product based on strain QST 713 of *B.subtilis*. The products is advertised to have a spectrum of activity including over 40 plant disease including common greenhouse disease such as gray mold, powdery and downy mildew . The bacterium is presumed to work through a number of mode of action such as competition, parasitism, antibiosis and induction of systemic aquired resistance (SAR). The products marketed primarily as a tool to prevent resistance to chemicals and as an alternative to chemicals in areas where fungicide- resistance pathogens have developed. *Trichoderma harzianum* strain T.39, developed at the Volcani Center in Israel and marketed as Trichodex 20P. The reported mode of action of *T.harzianum* T39 is competition for nutrients and interference with production of lytic enzymes by the pathogen, thus in addition to slowing the germination of the pathogen،s conidia and T39 also prevents the penetration of the host tissue and the maceration process (Timothy and Richard,2001). Haggag-Wafaa(1997) Suggested that, *T.harzianum* can increase plant growth of radish, since it produces plant hormone such as Indole-3 acetic acid (IAA). Also *B.subtilis* produce large concentration of IAA which, increased shoot; root elongation of sugar beet. *Bacillus* spp that elicit induced systemic resistance (ISR) also elicit plant growth promotion. Studies on mechanisms indicate that elicitation of (ISR) by *Bacillus* spp is associated

with ultrastructural changes in plant during pathogen attack and with cytochemical alteration Joseph *et al.*(2004).

Table (5) : Effect of different biocides used as protected treatment on cucumber downy mildew disease on Beto Star cv. Under greenhouse conditions at Tokh and Sakha location during 2005 and 2006 seasons.

Bioagents	Tokh		Sakha	
	Disease severity(%)	Efficacy(%)	Disease severity(%)	Efficacy(%)
Plant Guard (2.5ml/l)	40.30	40.9	43.00	40.49
Rhizo-N (2.5g/l)	25.50	62.65	28.06	61.17
Blight Stop (10ml/l)	27.36	59.90	29.85	58.69
Shampion (2.5 g/l)	20.86	69.44	22.25	69.21
Control	68.27	-	72.26	-
L. S . D 5%	4.51	-	5.43	-

Table (6) : Effect of different biocides used as curated treatment on cucumber downy mildew disease on Beto Star cv. under greenhouse conditions at Tokh and Sakha location during 2005 and 2006 seasons.

Bioagents	Tokh		Sakha	
	Disease severity(%)	Efficacy(%)	Disease severity(%)	Efficacy(%)
Plant Guard (2-5ml/l)	56.16	20.81	59.16	20.43
Rhizo-N (2-5g/l)	41.71	41.18	45.08	39.37
Blight Stop (10ml/l)	47.70	32.73	50.15	32.55
Equagen Pro (0.45g/l)	8.00	88.72	9.01	87.88
Control	70.91	-	74.35	-
L. S . D 5%	5.40	------	3.01	------

3-Plant height ,number of flowering and fruit yield :-

The results presented in Table (7) show that, all biocide treatments, which reduced downy mildew infection significantly increased the plant height, the number of flowers and fruit yield, when its used as foliar spray. Protected treatment Rhizo-N exhibited the best effect, where it resulted 286cm plant height, 91.90 no.of flowers/ plant and 49.94 kg/polt fruit yield, followed by Shampion fungicide and Blight Stop while, Plant Guard was the least effect in this regard (228cm/plant) (62.12 no.of flowers/ plant) and (33.47kg/polt), respectively in both the years 2005 and 2006 at Tokh locality. The results obtained at Sakha location were more or less similar to those at Tokh locality.

Results shown in Table (8) illustrate that, all biocides treatment varied in their effect on growth parameters whereas, it were the less effective compared with Equagen Pro fungicide, when the biocides used as foliar spray after symptoms appearance (Curated treatment). Equagen Pro gave the most effective in improving growth plant where it resulted 281cm/plant plant hight, 82.41 no. of flowers and 49.58kg/polt fruit yield. Rhizo-N increased Plant growth parameters, which recorded 243 cm/plant, 64.40 no. of flowers and 28.79kg/polt furit yield, followed by Blight Stop while Plant Guard was the

least effective in this respect, in both years (2005 & 2006) at Tokh locality. The results obtained at Sakha werein the same range of at Tokh. These results are in the line with those obtained by Timothy and Richard(2001)they reported that the biological treatments as Sporodex *(P,flocculosa*), Trichodex, 20p(*T.harzianum*) and Serenade (*B.subtilis*) controlled powdery and downy mildew and improved ratio and quality of the flowering of many crops. *T.harzianum* can increase plant growth of radish, since it produces plant hormone such as Indole Acetic Acid (IAA) and GA3. Also *B.subtilis* produce large concentration of IAA and GA3 which increased shoot ; root elongation and yield of sugar beet.

Table (7) : Effect of different biocides used as protected treatment on plant hight (cm/plant), number of flowers and yield (kg/polt) of cucumber plant(Beto Starcv.) under greenhouse conditions at Tokh and Sakha location :

Bioagents	Tokh			Sakha		
	Plant hight (cm/ plant)	Number of flowers/ plant	Yield (Kg/ plot)	Plant hight (cm/ plant)	Number of flowes/ plant	Yield (Kg/plot)
Plant Guard (2.5 ml/l)	228	65.12	33.47	223	62.12	32.33
Rhizo-N (2-5 g/l)	286	91.90	49.94	282	90.33	47.99
Blight Stop (10 ml/l)	265	80.11	41.04	261	77.81	39.90
Shampion (2-5 g/l)	274	82.21	42.88	272	80.08	41.56
Control	195	41.00	13.09	187	37.98	12.22
L . S . D at 5%	8.7	9.12	6.98	9.8	10.11	6.15

Table (8) : Effect of different biocides used as curated treatment on plant height (cm/plant), number of flowers and yield (kg/polt) of cucumber plant (Beto Star cv.) under greenhouse conditions at Tokh and Sakha location :

Bioagents	Tokh			Sakha		
	Plant height (cm/ plant)	Number of flowers	Yield (Kg/plot)	Plant height (cm/ plant)	Number of flowers	Yield (Kg /plot)
Plant Guard (2.5ml/l)	202	47.33	20.50	198	45.53	20.22
Rhizo-N (2.5 g/l)	243	64.40	28.79	235	61.50	27.13
Blight Stop (10 ml/l)	234	62.30	27.02	223	60.65	25.50
Equagen Pro (0.45g/l)	281	82.41	49.58	277	80.20	47.95
Control	181	37.42	11.85	175	33.41	10.40
L . S . D at 5%	8.6	12.83	7.98	10.9	11.97	5.08

The oxidative enzymes :

Results shown in Table (9) showed an increase in activity of the oxidative enzymes of the infected cucumber plants in both two treatment i.e. protected and curated with all bioagents Rhizo-N and Blight Stop had the best effect of increasing polyphenoloxidase, peroxidase and catalase of healthy plants.

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Protected treatment of the two mentioned bioacides decreased the activity of oxidative enzymes of the infected plants as compared with Plant Guard and the control. The effect of Shampion fungicide on oxidative enzymes was very close to that of Rizo-N, both in healthy and infected plant. On the other hand, the Equagen Pro followed by Rhizo N used as curated treatment exhibited the best effect of increasing polyphenoloxidase, peroxidase and catalase of healthy plants and decreasing the activity of oxidative enzymes of infected plants as compared with the tested biocides whereas, Plant Guard had reversal effect in this respect. The finding data were in agreement with those reported by Timothy and Richored (2001) they found that, The activity of peroxidase and polytphenoloxidase were higher in capsicum varities resistant to *L.taurica* than in the susceptible one. Zhou & Paulitz (1994) and Chen *et al.* (2000)they found that the biocides (*Pseudomonas corrugata* strain 13 and *P. fluorescens* strain 28 and 63)stimulated higher levels of peroxidase, polyphenoloxidase, catalase and phenylalanine ammonia layse in cucumber without pathogen challenge.

In conclusion, Using antagonistic fungi or bacteria led to highly significant control of the downy mildew disease Rhiz0-N, Blight Stop and Plant Gurad used as protected treatment were effective in controlling the disease and improved the growth parameters with significant difference between them and vice versa in the using antagonistic as curated treatment. However, they could be utilized instead of the used fungicides. In an environmental point of views, utilization of them will decrease air and soil pollution.

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المقاومة الحيوية لمرض البياض الزغبى فى الخيار تحت ظروف الصوبة

أحمد أبوريا الكفراوى وعبد الوهاب عنتر إسماعيل
معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزه

لقد تم أستخدام بعض المعاملات الحيوية التجارية الفطرية والبكترية على نباتات الخيار لمقاومة مرض البياض الزغبى وتحسين نموه ومحصوله خلال موسمى الزراعة ٢٠٠٥-٢٠٠٦

وأوضحت نتائج الصوبة الأتى:-

١ – أختلفت أصناف الخيار فى درجة حساسيتها لشدة الأصابة فكانا لصنف بينوستار أكثر الأصناف قابليةللأصابة (٨٤و٧٤%)بينماالصنف شروق يعتبر أقل قابلية للإصابة (٧١و٣٣%).

٢ - كل المعاملات الحيوية التجارية أدت إلى خفض شدةالأصابة بمرض البياض الزغبى علىالنباتات عندما أستخدمت كمعاملة وقائية فى كلا موقعى التجربة وهي طوخ و سخا .

أ – أظهر المركب الحيوى ريزو-ن أعلى كفاءة فى مقاومة مرض البياض الزغبى (١٧و٦١ – ٦٥و٦٢%)بينما كان المركب الحيوى بلانت جارد أقلهم كفاءة مقارنة بالمبيد شامبيون فى هذا الخصوص .

ب – كل المعاملات الحيوية التى أدت إلى خفض الأصابة بالبياض الزغبى أدت إلى زيادة أطوال النباتات وعدد الأزهار ومحصول الثمار وذلك عندما تم أستخدام هذه المركبات كرش ورقى قبل ظهور الإصابة (كمعاملة وقائية).

ج – أظهر المركب الحيوى ريزو-ن أحسن تأثير فى زيادة أطوال النباتات (٢٨٢ – ٢٨٦سم /نبات) وعدد الأزهار (٣٣و٩٠ – ٠٩و ٩١للنبات) وأيضا زيادة محصول الثمار (٩٩و٤٧ – ٩٤و٤٩كجم/بلوت) يليه المركب بلوت ستوب وكان المركب بلانت جارد أقلهم كفاءة مقارنه بالمبيد شامبيون وذلك عند أستخدام هده المركبات الحيويه كمعاملة وقائية .

٤- عند أستخدام المركبات الحيوية كمعاملة علاجية كانت أقل كفاءة فى مقاومة مرض البياض الزغبى وأيضا فى تحسين النمو مقارنة بالمبيد أكواجين برو .

٥ – كان النشاط الإنزيمى فى الأوراق المصابة أكثر من النشاط الإنزيمى فى الأوراق السليمةفى كلا من المعاملات بالمركبات الحيوية كوقائية وعلاجية .

٦- أنخفض النشاط الأنزيمى فى النباتات المعاملة علاجيا بمبيد الأكواجين برو مقارنة بالمركبات الحيوية المستخدمة.

Table (9): Effect of different biocides used as protected and Curated treatment on oxidative enzymes of cucumber (Beto Star cv.) in correlation with downy mildew disease under greenhouse conditions .

Bioagents	Protected treatment						Curated treatment					
	Poly phenol-oxidase		Peroxidase (expressed opticaldensity)		Catalase (mg.H_2o_2 reaction/ time)		Poly phenol-oxidase		Peroxidase (expressed opticaldensity)		Catalase (mg.H_2o_2 reaction/ time)	
	H	I	H	I	H	I	H	I	H	I	H	I
Plant Guard (2.5 ml/l)	0.228	0.396	0.403	0.728	4.052	7.123	0.231	0.512	0.623	0.903	5.214	8.312
Rhizo-N (2.5 g/l)	0.260	0.286	0.550	0.562	5.231	5.637	0.271	0.466	0.694	0.814	6.641	7.455
Blight Stop (10 ml/l)	0.254	0.302	0.542	0.601	5.019	5.890	0.259	0.495	0.679	0.871	6.230	7.908
Sham pion (2.5 g/l)	0.265	0.281	0.562	0.570	5.310	5.531	-	-	-	-	-	-
Equagen Pro (0.45 g/l)	-	-	-	-	-	-	0.274	0.301	0.721	0.762	6.820	6.998
Control	0.204	0.498	0.438	0.867	3.720	8.113	0.210	0.592	0523	0.956	3.934	8.465
L . S . D at 5%	0.010	0.095	0.018	0.116	0.194	0.104	0.008	0.041	0.012	0.052	0.169	0.415

H = Healthy **I = Infected**