RESULTS OF BACILLUS SUBTILIS AGAINST MAJOR DISEASES ON GREENHOUSE CROPS

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ABSTRACT

Bacillus subtilis and other Bacilli have long been used in the field of agriculture as a biocontrol reagent to protect plants against soil-borne plant pathogens. Evaluation the efficacy of bio-agents, application as foliar spray against vegetables foliar diseases incidence was carried out in greenhouse conditions. The tested Russian bioagents Bacillus subtilis-26D, and Bacillus subtilis-M-22 were evaluated. The recorded foliar diseases, i.e. Powdery mildew, Angular spots of Cucumber, Early, Late blights of Tomato were significantly reduced at all treatments either alone or in combinations comparing with untreated plants. Application with either B. subtilis-26D and B.subtilis-M22 showed significant reduction in diseases incidence comparing with the untreated control.

KEY WORDS: tomato, cucumber, bioagent, fungi, bacteria

INTRODUCTION

Vegetable crops grown under protected cultivation, facing a serious problem due to diseases infection that cause about 10-15 % loss of produced yield because of favourable environment for disease incidence and development. Therefore, many control practices need to be integrated in order for minimizing this figure to occur. Powdery mildew as well as Early and Late blights are the most serious foliar diseases attacked vegetables grown in plastic houses. Powdery mildew disease is one of the most serious plant diseases, causing large yield losses in a number of crops. Powdery mildew caused by *Sphaerotheca fuliginea*, is a serious disease affecting the leaves, stems and fruits of cucumber grown in greenhouses. Late and early blights of tomato caused *Phytophthora infestans* and *Alternaria solani* were also recorded in growing greenhouse tomato. Among the biocontrol agents, species of the strain *Bacillus subtilis* is most promising and effective biocontrol agent.

The aim of present study is to evaluate the abilities of foliar spray with bio-control agents for controlling Powdery mildew and angular spot of Cucumber, Early and Late blights of Tomato under greenhouse conditions.

MATERIALS AND METHODS

We use transplants of cucumber, tomato and antagonistic bacterial strain *Bacillus subtilis 26D* and *Bacillus subtilis M-22* in this study.

Bio-agent application against vegetables foliar diseases was carried out in greenhouse, Bayanchandmani soum, Tov province.

Transplants of Cucumber and Tomato were planted in natural sandy soil as sexty plants per pot and tree replicates in each particular foliar treatment. Foliar spraying with tested bio-agent- *Bacillus subtilis,* were applied twice with tree weeks intervals starting one week from transplanting. One week after the second antagonistic foliar application, foliar artificial infestations with *Sphaerotheca fuliginea* and *Pseudomonas syringae pv. lacrymans* the causal fungi and bacteria of Cucumber, *Alternaria solani* and *Phytophthora infestans* of tomato.

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between applied treatments and disease incidence.

Determining disease prevalence: Disease prevalence have been calculated using the formula of Dementeva M.N (1970, 1985), Geshele E.E (1971).

$$P = \frac{a}{N} \cdot 100$$

P- disease prevalence, %

a- number of diseased plants involved in experiment

N- total number of plants involved in experiment

Determining marking damage scale:

0 score: no disease symptoms have shown

1 score:until 10% of leaf surface is damaged

2 score:until 11-25% of leaf surface is damaged

3 score:until 26-50% of leaf surface is damaged

4 score:more than 51% of leaf surface is damaged

Determining disease development:

When determining disease development by methods Popkova M.K, mean of disease damage is expressed by percentage. Disease development is calculated by following the formula.

$$Px = \frac{\Sigma(a * b) * 100}{n * k}$$

Px- disease development, %

a- number of infected plants

n- number of plants to be estimated

κ- highest score of disease progress

Biocontrol efficacy was calculated using the following formula of Abbot

$$A = \frac{(K - o) * 100}{O}$$

A- Biological efficacy,%

K- Disease development in control, %

O- Disease development in variant treated with biocontrol agents, %

RESULTS AND DISCUSSION

Results in Table (1) represented the species composition of tomato and cucumber diseases in the Bayanchandmani greenhouse. On cucumber we detected 5 fungal, 2 bacterial and 1 virul, on tomato 4 fungal, 2 bacterial and 1 virul diseases. We calculated disease prevalence and development before and after application bioagents and on tomato disease prevalence is 3.3-11.2% and development is 3.3-6.7%. On cucumber disease prevalence is 5.0-20.0% and disease development is 5.0-14.1%. Data showed in the table 2.

In table (3) presented data showed that two applied bioagents significantly reduced the recorded foliar diseases comparing with untreated control. Application with *B. subtilis* showed significant reduction in diseases incidence comparing with the control. The recorded percentage of Powdery mildew in bio-agents spray application ranged between 18.7-27.2%, angular spot is 6.7-10.5% in Cucumber comparing with 75.0-53.3% in control treatment, respectively. Tomato plants recorded Early and Late blights infection as 5.0-16.0% and 4.0-7.0%, comparing with unsprayed plants which showed diseases incidence calculated as 11.0% and 14.0% for both diseases, respectively.

Table 1

The species composition of diseases in the greenhouse in Byaynchandmani soum, 2014

Phylum	Class	Family	Genus	Species
		Tomato		
Heterokontophyta	*Oomycota*	*Pythiaceae*	*Phytophthora*	*Phytophthora infestans*
Ascomycota	*Dothideomycetes*	*Pleosporaceae*	*Alternaria*	*Alternaria solani*
Ascomycota	*Dothideomycetes*	*Mycosphaerellaceae*	*Septoria*	*Septoria lycopersici*
Ascomycota	*Dothideomycetes*	*Davidiellaceae*	*Cladosporium*	*Cladosporium fulvum*
Ascomycota	*Sordariomycetes*	*Nectriaceae*	*Fusarium*	*Fusarium oxysporum f.lycopersici*
Actinobacteria	*Actinomycetales*	*Microbacteriaceae*	*Clavibacter*	*Corynebacterium michiganense*
Proteobacteria	*Gamma proteobacteria*	*Xanthomonaceae*	*Xanthomonas*	*Xanthomonas campestris pv.vesicatoria*
Croup V	*Unassigned*	*Bunyaviridae*	*Tospovirus*	*Tomato virus L*
		Cucumber		
Ascomycota	*Leotiomycetes*	*Erysiphales*	*Sphaerotheca*	*Sphaerotheca fuliginea*
Ascomycota	*Leotiomycetes*	*Sclerotiniaceae*	*Sclerotinia*	*Sclerotinia sclerotiorum*
Ascomycota	*Sordariomycetes*	*Glomerellaceae*	*Colletotrichum*	*Colletotrichum orbiculare*
Ascomycota	*Dothideomycetes*	*Pleosporaceae*	*Alternaria*	*Alternaria cucumerinum*
Heterokontophyta	*Oomycetes*	*Peronosporaceae*	*Pseudoperonospora*	*Pseudoperonospora cubensis*
Proteobacteria	*Gamma proteobacteria*	*Pseudomonaceae*	*Pseudomonas*	*Pseudomonas syringaepv.lachrymans*
Croup IV	*Unassigned*	*Bromoviridae*	*Cucumovirus*	*Cucumber mosaic virus*

Table 2

Marking damage scale of dominant crop disease in greenhouse. 2014.

Date	Pathogen	Number of plants to be estimated	Score					Disease prevalence, %	Disease development,%
			0	1	2	3	4		
			No spot	Few spots	No more than 1/3-	Until 2/3	Leaf dried		
		Cucumber							
8/15	*Sphaerotheca fuliginea*	60	-	3	-	-	-	5.0	5.0
8/15	*Pseudomonas syringae pv.lachrymans*	60	-	7	5	-	-	20.0	14.1
8/15	*Colletotrichum orbiculare*	60	-	5	1	-	-	10.0	5.8
		Tomato							
8/15	*Cladosporium fulvum*	60	-	3	-	-	-	11.2	6.7
8/15	*Phytophthora infestans*	60	-	2	1	-	-	5.0	5.0
8/15	*Alternaria solani*	60	-	2	-	-	-	3.3	3.3

Table 3

Effect of spraying antagonistic bio-agents against vegetables foliar diseases in greenhouse conditions, 2014

Bioagents	Foliar diseases, %					
	Cucumber			Tomato		
	Sphaerotheca fuliginea	*Pseudomonas syringae pv.lachrymans*	*Colletotrichum orbiculare*	*Phytophthora infestans*	*Alternaria solani*	*Cladosporium fulvum*
Bacillus subtilis 26D	18.7	6.7	-	4.0	5.0	6.5
Bacillus subtilis M-22	27.2	10.5	-	7.0	16.0	-
Control	75.0	53.3	45.0	14.0	11.0	15.0

Table 4

Economic effectiveness of bio-agent application. 2014

Dominont crop disease	Experiment variants	Biological efficacy , %	Mean yield, кg/m^2	Additional yield centre/ha	%
	Cucumber				
Pseudomonas syringae pv.lachrymans,	Control	-	7.6	-	-
	Bacillus subtilis 26D	81.0	11.0	3.4	30.9
	Bacillus subtilis M-22	80.0	9.5	1.9	20.0
	Tomato				
Phytophthora infestans, Alternaria solani,	Control	-	4.2	-	-
	Bacillus subtilis 26D	83.4	5.3	1.1	20.7
	Bacillus subtilis M-22	79.1	5.7	1.5	26.3

CONCLUSION

1. Based on our research study tomato disease prevalence is 3.3-11.2% and development is 3.3-6.7%, cucumber disease prevalence is 5.0-20.0% and disease development is 5.0-14.1%.
2. As a result, *Bacillus subtilis 26D*, *Bacillus subtilis M-22* have been sprayed 2-3 times in order to prevent against dominant crop disease of greenhouse. Biologycal efficacy of *Bacillus subtilis 26D* were in cucumber 81.0%, on tomato 83.4% and *Bacillus subtilis M-22* 80.0%, 79.1% respectively.
3. Biological product *Bacillus subtilis 26D* 2.5kg/ha, *Bacillus subtilis M-22* 10таb/10l is increasing tomato yield by 20.7-26.3%, and cucumber yield by 20.0-30.9%.

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