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Domates seralarında Kurşuni Küf hastalığının Biyolojik ve Kimyasal Kontrolünde Borun Arttırıcı Etkisi

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Özet

Bu çalışmada, Domateste Kurşuni küf hastalığının kimyasal ve biyolojik kontrolü üzerine borun etkisini saptamak için, bor elementinin üç farklı dozunun (0, 30, 60, 90 ppm) hastalığı engelleyici etkisi test edilmiştir. In vitro'da bor-fungusit kombinasyonunun Botrytis cinerea'nın miselyal gelişimi üzerine etkisini tespit etmek için, her bir bor dozu fungusitlerden Iminoctadine (450 ppm), Fenhexamide (1 ppm) ve Cyprodinil (%37).+ Fludioxonil (%25) (600 ppm) ile birlikte kombine bir biçimde uygulanmıştır. Ayrıca borun tek başına B. cinerea'nın gelişimi üzerine etkisini belirmek amacıyla üç farklı doz denenmiş (30, 60 ve 90 ppm) ve fungusun miselyal gelişiminin 90 ppm'de kontrole göre % 36.47 oranında engellendiği tespit edilmiştir. 90 ppm Bor içeren ortamda ise Bacillus subtilis Y 1336'nin B. cinerea'ye karşı antibiyozis etkinliğinin arttığı saptanmıştır. Fenhexamide ve Cyprodinil (%37).+ Fludioxonil (%25)'in %25 azaltılmış ticari dozları ile 90 ppm bor uygulaması sonucu, doğal olarak hastalıkla bulaşık seralarda enfeksiyon oranın fungusitlerin tek başına uygulandığı kontrole göre, istatistiksel derecede önemli bir düşüş olduğu belirlenmiştir. B. subtilis Y 1336'ın 90 ppm bor ile kombine uygulanması sonucu enfeksiyonun biyolojik ajanı bakterinin tek başına uygulandığı kontrole göre daha düşük düzeyde olduğu tespit edilmiştir.

Anahtar Kelimeler: *Bor, Botrytis cinerea, fungisid, kombinasyon, kontrol*

Enhancer Effect of Boron on Biological and Chemical Control of Gray Mold Disease in Tomato Greenhouses

Abstract

Gray mold disease caused by Botyritis cinerea is a pathogen of which control is possible by frequently spraying of chemicals in tomato greenhouses. In the present study, the inhibitory effect of boron by three different doses (0, 30, 60, 90 ppm) was tested to determine the chemical and biological control of Gray Mold disease on tomato. Each boron dose was tested with iminoctadine (450 ppm), fenhexamide (1 ppm) and cyprodinil (%37).+ fludioxonil (%25) (600 ppm) in vitro conditions in order to determine the effect of boron-fungicide combination on mycelium growth of the pathogen. Furthermore, the effect of three different boron doses (30, 60 ve 90 ppm) alone was also tested to evaluate the effect on B. cinerea growth and a siginificantly inhibition by 36.47%, resulted from 90 ppm application, on mycelium growth was determined, compared to control. An ascending inhibitory effect resulted from Bacillus subtilis Y 1336 in medium containing boron by 90 ppm to pathogen was also observed. The doses decreased up to 25% of Fenhexamide and Cyprodinil (%37).+ Fludioxonil (%25) by combination with 90 ppm boron resulted in statistically significant less infection ratio in naturally infected greenhouses than control plots where the fungicides were sprayed alone. Moreover, combination of B. subtilis Y 1336 and 90 ppm of boron resulted in significantly lower infection ratio than that of control plots where the biological control agent were applied alone in greenhouses where the infection was not also observed.

Keywords: *Boron, Botrytis cinerea, fungicide, combination, control*

Introduction

Tomato is on the top of list within the protected vegetable production of Turkey. Only is being tomato in Fethiye produced, which is more than in 20.000 da area. Gray mold disease is of importance in view of diseases having been faced with in protected cultivation. The construction of greenhouses and climatic conditions result in serious crop losses on yield.

The gray mold disease caused by *Botrytis cinerea Pers.* was first described over 200 years ago (Bessey, 1950). It is generally considered to be of a saprophytic nature and in this capacity has a very extensive host range. This fungus can be pathogenic and destructive under favourable environmental condition. The various injury caused by *Botrytis* are seen on fruit, stems rots, neck and bulb rots, leaf spots, tuber and root decay, and blossom blight. The fungus survives in the soil and on plant debris, the main sources of inoculums. However, the spores of this fungus are airborne. The chemicals are

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frequently sprayed to control of *B. cinerea*. The resistance forming of pathogen to chemicals enforces researchers seeking of alternative methods. Therefore biological control has been selected as successful method and applied on few major crops (Elad and Freeman, 2002). Selection of only one measurement method is not able to efficiently control. Therefore, supportive or enhancer method having effect on biological control efficiency have been investigated and a successful result provided.

Certain strains of *Pseudomonas* sp. can protect plants from soil-borne fungal pathogens and have been studied as potential bio-control agents for use in commercial greenhouses and/or in the field [(Fenton *et al.* 1992); (Keel *et al.,* 1992); (Cook 1993)]. For instance, *P. fluorescens* F113 can control the extent of damping-off disease [(Fenton *et al.,*1992); (Shanahan *et al.* 1992)]. The biocontrol ability of the strain results mainly from the production of 2,4-diacetylphloroglucinol (Phl), a secondary metabolite that inhibits the damping- off agent *Pythium ultimum* under *in vitro* conditions [(Fenton *et al.,* 1992); (Keel *et al.,* 1992); (Shanahan *et al.* 1992); (Russo *et al.* 1996)]. Synthetic Phl was also found to inhibit various microorganisms that are common in soil [(Keel *et al.* 1992); (Shanahan *et al.* 1992); (Reddi *et al.,* 1969)]. Production of Phl is being modified in F113 by genetic means to develop a mutant strain with higher bio-control efficacy. A genetically modified derivative of *P. fluorescens* CHA0 that overproduced Phl and pyoluteorin *in vitro* and in the rhizosphere proved to have detrimental effects on certain host plants (Maurhofer *et al.*, 1995). Inoculation of seed/soil with Phl-producing Pseudomonads for bio-control involves the release of high numbers of cells into the environment and implies the need for risk assessment studies. Moreover, an isolate of *Pseudomonas flourescens* has been applied with low dose of fenhexamid that resulted in control of gray mold disease by up to 74% on tomato (Yıldız *et al.*, 2007). In another study a combination of *Trichoderma harzianum* T39 led to decreasing in number of chemical application (Shtienberg and Elad, 1997).

One of the contributory components to control of plant disease is plant nutrition. Although the pathogen has been controlled by tolerance and genetical resistance (Agrios, 2005), the environmental conditions and deficiency and phytotoxicity have seriously effect on disease emergence [(Marschner, 1995); (Krauss, 1999)]. Whilst some plant nutrient elements have directly effect on pathogen, systemic resistance on plants can be induce by applying the elements of less concentrations (Reuveni and Reuveni, 1998).

Boron is a semiconductor element that has intermediate properties which is between metals and non-metals and its atom small with only three valence electrons. The chemistry of boron is unique (Greenwood and Earnshaw, 1984). As an element some biochemical and physiological process which are of importance on resistance tolerance of plants can be affected by boron besides other micro nutrient elements such as Zn and Mn (Kostas *et al.*, 2006). Boron effect was informed on *Plasmodiophora brassicae*, TMV, *Fusarium solani* on bean, *Verticillium albo-atrum* on tomato and cotton, TYLCV on tomato, *G. graminis* on wheat (Graham and Webb, 1991). The recession in disease severity of *Blumeria graminis* by boron application was determined (Marschner, 1995). Different concentrations of boron resulted in efficiently control of *Alternaria brassicae on Brassica campestris* ve *B. Juncea*. Combination of 0.2% mancozeb with 0.53% boron or 0.22% boric acid application was provided a protection by 16-20% compared to control (Kolte *et al.*, 1998).

The aim of the study is to assess the role of boron on biological and chemical control of gray mold disease in protected cultivation fields of tomato.

Material and Methods

Four different protected fields where gray mold disease was observed in previous season were selected to conduct the experiments. Boron element (containing 18%) was supplied from National Boron Research Institute and *Bacillus subtilis* Y 1336 were isolated from commercial Biobac WP (BIOTECH INC.).The fungicides assayed were fenhexamid (Teldor SC 500 Bayer, 500 g l^{-1}), cyprodinil + fludioxonil (Switch 65.5 WG, Syngenta, %37.5 + %25) and iminoctadine (Bellkute 40 WP, Sumitoma %40).

The effect of boron on mycelia growth of B. cinerea

B. cinerea was cultured on PDA for 3 days and from this culture, one mycelial plug was removed using a flame sterilized borer from the outer margins of each fungal mycelium mass, and placed on the center of a fresh PDA containing 0, 30, 60 and 90 ppm B to assess *B. cinerea* sensitivity and plates were incubated in the dark at 25^{o}C, until the fungi on the control plates (containing no boron) had grown together. Percent inhibition was calculated using the following formula: % inhibition = (1-(Fungal growth/Control growth)) X 100. Each treatment had five replicates.

The effect of boron-fungicide combination on mycelia growth of B. cinerea in vitro

Additionally, to assess the boron- fungicides combinations iminoctadin (450 ppm) fenhexamid (1 ppm and cyprodinil + fludioxonil (600 ppm) were added into PDA containing 0, 30, 60 ve 90 ppm B and mycelia growth was measured. Each treatment was done by 5 replicates. Percent inhibition was calculated using the following formula: % inhibition = (1- (Fungal growth/Control growth)) X 100. Each treatment had five replicates.

The antagonistic effect of biological agent by combination with boron on mycelia growth of B. cinerea in vitro

B. cinerea was cultured on PDA for 3 days and from this culture and 0.1 ml *B. subtilis* suspension was dispersed on PDA containing 0, 30, 60 and 90 ppm B and one mycelial plug was removed using a flame sterilized borer from the outer margins of each fungal mycelium mass and placed on the centre of PDA to inoculate with *B. cinerea*. The petri dishes were incubated at 25 ^{0}C. Inhibition was calculated according to growth mycelium of control. Each treatment had four replicates.

Testing of boron-fungicide and boron-BA combinations in greenhouse conditions

The most efficient dose by 90 ppm boron determined *in vitro* studies has been tested and sprayed with 25% reduced iminoctadin (33.75 g/100 L), fenhexamid (75 ml/100 L) and cyprodinil + fludioxonil (45 g/100 L). In each greenhouse 100 plants were selected for a treatment. Each fungicide+ boron and BA+ boron combinations were sprayed two times by 30 days interval in growing season of 2008-2009. Control plants were treated with only water.

Experimental design and statistical analysis

In vitro assays percent inhibition was calculated using the following formula: % inhibition = (1- (Fungal growth/Control growth)) X 100. Each treatment had five replicates.

In greenhouse experiment, each greenhouse has been considered as a replication and sum of the recorded total lesion was subjected to Duncan test and protection (% value) was calculated.

Results

Within the tested doses of different boron concentrations 60 and 90 ppm were the most effective on mycelia growth of *B. cinerea*. Mycelial growth was decreased 8.6% by 60 ppm and 36.47% by 90 ppm. Further studies showed that this inhibitory effect, which is not stable, can be affected from temperature and light intensity. However, 90 ppm is particularly an efficient dose to control aerial mycelium of the pathogen showing less growth than control (Table 1). Fungicide+ boron combination inhibited the mycelial growth by all treatments except for imoctadine. However, the pathogen showed 9.62% recession in PDA containing 90 ppm B + 450 ppm iminoctadine compared to control. Iminoctadine was less effective than other registered fungicides. In further studies, 90 ppm B has been used with 25% lower fungicide doses in greenhouse experiments since this dose is most effective that results in successful control of the pathogen (Tab. 1).

Tab. 1: The effect of fungicide-boron combination on disease emergence.

	Doz (/100 Litre)	I. GH	2. GH	3. GH	4. GH	Moderate	% Control
Kontrol	0	16	20	12	7	13,75 a	
Iminoctadine (B)*	45 g	8	11	8	5	8 ab	41.81
Fenhexamid (T)	100 ml	3	2	2	2	2,25 cd	83.63
%37.5 Cyprodinil+ %25 fludioxanil (S)	60 g	2	3	1	2	2 cd	85.45
B+Bor	33.75 g + 9 g B	7	9	7	4	6,75 bc	50.90
T+Bor	75 ml + 9 g B	2	2	1	1	1,5 cd	89.09
S+Bor	45 g + 9 g B	1	2	1	1	1,25 d	90.90
Biobac WP (*Bacillus subtilis*)	125 g	14	15	11	5	11.25 ab	18.18
B. subtilis + B	125 g + 90 ppm B	12	13	9	4	9.5 ab	30.90

**B, T ve S: The first letters of fungicides. $P \leq 0.05$*

Average inhibition zone values were 6 mm in PDA containing 30 ppm boron, 9 mm by 60 ppm and 12 mm by 90 ppm compared to control plates in studies carried out to assess the antagonistic effect of *B. subtilis* by combination with boron. The most effect was observed by 90 ppm (Fig. 1). It has been observed that the BA efficiency is increased by boron resulting in fungistatic effect.

Fenhexamide and cyprodinile + fludioxanile alone controlled the disease by 83.63 and 84.45% in greenhouse conditions while iminoctadine resulted in 41.81% control. Combination of the fungicides with boron resulted in an additive effect that can be accounted for 5.45-

12.72% more protection than fungicide application alone. *B. subtilis* alone resulted in 18.18% control but its combination with boron increased the ratio up to 30.9 %.

Fig. 1. The antagonistic effect of *B. subtilis* Y 1336 to *B. cinerea* in PDA containing 60 and 90 ppm boron

Discussion

This study shows the positive effect of boron on biological and chemical control of the gray mold disease. Boron has resulted in significant increase on protection effect of fungicides and biological control agents. Although 90 ppm is efficient dose in controlling of the disease the less concentration than 90 ppm will reduce toxicity risk in view of boron application. In another studies carried out on vineyards, boron has been shown to be affected the *Eutypa lata* disease progress and boric acid has been recommended as alternative by 22 and 83 $\mu g\ l^{-1}$ doses, resulting decrease on mycelial growth and spore germination (Rolshausen and Gulber, 2005). Boron-fungicide applications have given similar results on different plant and pathogens. Moreover, boron has also enhanced the effects of 2-methoxyethylmercury chloride, quintozone, chloroneb and carboxin sprayed on coffee to *Rhizoctonia solani* (Hans and Shyam, 2006). Similar results on *Brassica campestris* and *B. juncea* to *Alternaria solani* by 0.53% boron or 0.22% boric acid with mancozeb (0.2%) and it resulted in 16-20% more effectively control than spraying of the fungicide alone (Kolte *et al.*, 1998). These results were also confirmed on banana to *Mycosphaerella musicola* and on wheat to *Drechslera tritici-repentis* (Died) Shoem (Gerald *et al.*, 2003; Kostas *et al.*, 2006).

Our studies showed that the efficiency of BA is increased by combination with boron (Fig.1). The reason for successful effect of boron on disease control is associated with dynamic interaction plant- pathogen and plant nutrition besides its fungistatic effect alone (Huber, 1996). Boron enhances stability of cell wall and thickness, resulting in resistance (Matoh, 1997). It also induces local and systemic resistance of plants [(Graham and Webb, 1991), (Moshe *et al.*, 1997)]. The recent findings showed that boron is required for cross-linking the pectin component RG-II in plant cell walls (O'Neill *et al.*, 2001), for vesicle targeting and transmembrane transport in symbiosomes (Bolanos *et al.*, 2001). It is of importance as a ligand in the cyclic furanosyl bacterial quorum sensing signal AI-2, it has function as a "molecular linker" (Chen *et al.*, 2002). The enhancer effect of boron on BA efficacious can be associated with molecular linker role of boron, affecting bacterial quorum sensing signal.

Under the shed of these findings, it can be concluded that boron combination with lower doses than commonly used quantity and biological control agents can be recommended in control of gray mold disease. Furthermore this combination can also be applied in integrated pest control strategies in all protected fields where the gray mold control is getting more difficult.

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