EFFICACY OF ALTERNARIA INFECTION ON RESISTANT AND SUSCEPTIBLE TOMATO CULTIVARS PRETREATED WITH SAR CHEMICAL INDUCERS

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ABSTRACT

In order to create environmentally friendly alternatives and to reduce the risk of the use of chemical pesticides, chemical inducers used for inducing systemic acquired resistance as a means of resistance to plant diseases without polluting the environment with chemicals. Tomato seedlings which pretreated with the tested SAR chemical inducers, i.e. SA, INA and thiamin, prior to inoculation with *A. solani*, significantly reduced early blight disease incidence in resistant Tezier cv, where reduction% were 68, 52 and 80%, respectively, compared with control. Reductions in disease incidence in susceptible Castle Rock cv were 62.88, 50.1 and 79.22%, respectively. Thus, highest reduction rates in disease incidence were induced by thiamine, compared with the other tested inducers. Reduction of early blight disease severity in resistant Tezier cv, 36.92, 26.33 and 61.93%, respectively, compared with the control. Reductions in disease severity in susceptible Castle Rock cv were 35.59, 23.85 and 61.55%, respectively. Thus, highest reduction rates in disease severity were induced by thiamine, compared with the other tested inducers. In detached leaf experiment the highest reductions were by thiamin in lesion frequencies and lesion diameter in both resistant Tezier cv and susceptible Castle Rock cv (52.22 and 46.7%) for lesion frequencies and (60.99 and 56.44) for lesion diameter, respectively, less than untreated control.

Keywords: Alternaria infection; Salicylic acid, 2, 6-dichloroisonicotinic acid and Thiamine (vit. B_1)

INTRODUCTION

Alternaria solani Sorauer, the causal agent of early blight disease in tomato and potato plants, is one of the best-known and most economically important members of the genus *Alternaria* (Chaerani and Voorrips, 2006). Disease symptoms are characteristic dark brown to black lesions with concentric rings, which produce a "target spot" effect (van der Waals *et al.*, 2004). Early blight may affect foliage, stems and in more severe cases, fruits (Haggag and Farghaly, 2007). Poysa and Tu (1996) tested more than 650 tomato cultivars, breeding lines, and accessions of related species, and were evaluated for resistance to early blight, caused by *A. solani*. Chaerani (2006) suggested that cultivars highly resistant to early blight are not

known in cultivated tomato. All breeding lines and released cultivars are susceptible to moderately resistant. Several wild species have been identified as potential sources of resistance. Edreva (2004) confirmed that chemical inducers of plant resistance possess quite different mode of action as compared to fungicides and pesticides. Systemic acquired resistance (SAR) inducers have been used in various field studies on several crop plants to reduce disease incidence. In all of these studies, SAR inducers led to reduced disease symptom development (Sandhu *et al.*, 2009). Chemical inducers like salicylic acid, jasmonic acid, DL-β-aminobutyric acid (BABA), oxalic acid and acibenzolar-S-methyl benzo-(1,2,3)-thiadiazole-7-carboxylic acid S-methyl ester (ASM) have been successfully employed in controlling diseases of various crop plants. The induction of resistance by chemical elicitors can form an important component in the integrated plant disease management program (Abdel Razik *et al.*, 2008, Ajay and Baby, 2009 and Martin *et al*., 2009).

MATERIALS AND METHODS

Treatment with chemical inducers: Shoot system at the five leaf stage of Tomato plants was sprayed with Salicylic acid (SA) (500 ppm), Isonicotinic acid (INA) (750 µl/L), and Thiamine (vit. B_1) (100 mM), on the upper and lower leaf surfaces. As a control treatment, water was used instead of chemical inducer solution in each case.

Inoculation of tomato: Tomato plants (45 days-old) were inoculated with spore suspension of *A. solani*. Each treatment includes 5 pots for each cultivar each containing 5 seedlings, whereas two pots left as control. A modified technique of Fritz (2005) was applied through this experiment.

Assessment of disease incidence and severity: Two weeks after inoculation by *A. solani*, disease incidence was estimated according to El-Farnawany (2006) using the following ratings: 0 = free of infection, 1= trace -25% leaf area spotted, 2= 26-50%, 3= 51-75%, 4= 76-100% leaf areas killed, the percentage of disease severity was then calculated according to Tarabulsi *et al.* (1998) as follows:

$$\%DS=\sum (n \times r) \times 100/ 4N$$

Where: DS = disease severity, n = number of seedlings of a given disease rating, r = disease severity rating, N = total number of seedlings rated.

Trypan blue staining of treated tomato leaves: Trypan blue has been used to stain non-viable cells during pathogen-induced cell death. Non-viable cells were stained blue and could

be observed with light microscopy (Barrow and Ronald, 2004 and Truenit and Jim, 2008). Leaves of chemical inducers pre-treated tomato resistant and susceptible cvs., inoculated with *A. solani*, were picked and protocol for trypan blue staining (Felix, 2005) was used.

Detached leaf technique: Tomato plants for this study were grown as described before until five to six weeks after germination. Leaves of defined leaf levels were picked, and protocol for detached-leaf technique (Takegami *et al.,* 2004 and Foolad *et al.* 2000), was used with some modifications.

Statistical analysis: data were subjected to ANOVA and statistically analyzed (Gomez and Gomez, 1984), treatment means were compared by Fisher's least significant difference (FLSD) test at 5% level of probability. Means of lesion number and diameter in detached leaf technique were compared using Duncan multiple range test (DMRT), α=0.05. The package used for analysis was NCSS and GESS version 2007.

RESULTS AND DISCUSSION

Disease incidence (DI) determinations:

According to data presented in Table (1) and illustrated in Fig. (1A), results showed that control plants exhibited the highest DI values, compared with inducer treatments. This was true for both resistant Tezier and susceptible Castle Rock cvs However, DI value was relatively higher in Castle Rock (38.6%), compared with that of Tezier cv (25%). Generally, treatment with both resistant and susceptible cvs before inoculation with SA, INA or vit.B_1 resulted in significant reduction in DI, compared with untreated inoculated control. However, DI reduction rates significantly differed according to host resistance and the applied SAR inducer. Treatment with vit.B_1 inducer resulted in the highest DI reduction rates in both resistant and susceptible cvs Moreover, DI value was lower in resistant cv (5%), compared with that of susceptible cv (8.02%). SA was significantly less effective in reducing DI% in both resistant and susceptible cvs (8% and 14.33% respectively), compared with vit.B_1. The highest DI value was realized by INA (12% and 19.26% respectively), compared with those induced by vit.B_1 and SA.

Disease severity (DS) determinations:

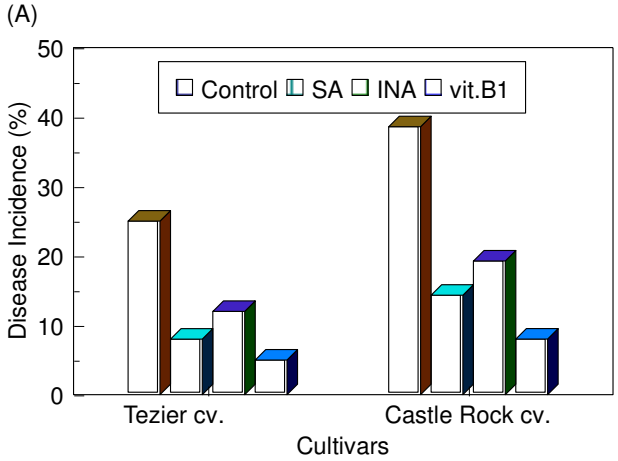
Data presented in Table (1) and Fig. (1B), revealed that inoculation of untreated tomato plants with *A. solani* resulted in significantly higher DS values, compared with the other treatments. Moreover, DS value in susceptible Castle Rock control (73.15%) was higher than that of resistant Tezier cv (27.11%). Treatment of both resistant and susceptible cvs with the

tested chemical SAR inducers, i.e. SA, INA and vit.B_1, before inoculation with *A. solani*, significantly reduced DS values, compared with control. Moreover, reduction rates were greatly affected by host resistance to early blight and by the tested chemical SAR inducer. Treatment with vit.B1 inducer before inoculation resulted in the least values of disease severity in both resistant and susceptible cvs (10.32% and 28.12%, respectively). Similar to disease incidence values, efficacy of SA to come second to vit.B_1, resulted in 17.1% and 47.11% DS values, in resistant and susceptible cvs, respectively. INA exhibited the highest DS values in both resistant and susceptible cvs (20% and 55.70%, respectively), compared with the other tested inducers.

Table (1). Effect of SAR chemical inducers on the disease incidence and severity, incited by *A. solani* in both compatible and incompatible systems.

Cultivars	Treatments	Disease Incidence (DI)		Disease severity (DS)	
		%	Reduction %	%	Reduction %
Tezier	Control	25^{a}	-	27.11^{a}	-
	SA	8^{b}	68	17.1^{b}	36.92
	INA	12^{c}	52	20^{b}	26.23
	Vit.B1	5^{d}	80	10.32^{c}	61.93
Castle Rock	Control	38.60^{e}	-	73.15^{d}	-
	SA	14.33^{c}	62.88	47.11^{e}	35.59
	INA	19.26^{f}	50.10	55.70^{f}	23.85
	Vit.B1	8.02^{b}	79.22	28.12^{a}	61.55
FLSD α = 0.05		2.821		4.910	

Values followed by the same letter(s) in each column don't differ significantly ($P \leq 0.05$) according to Fisher's LSD Test



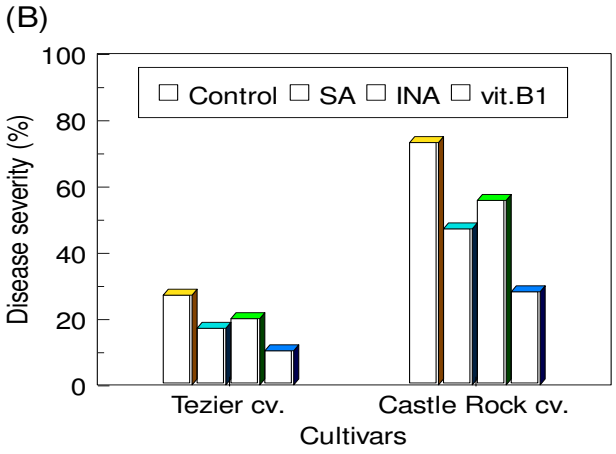


Fig. (1). Effect of treatment with the tested SAR chemical inducers on early blight disease incidence (A) and disease severity (B) in both incompatible and compatible systems.

Determination of the infected area using the trypan blue stain technique:

Trypan stain technique was applied to evaluate the efficacy of the tested inducers in inducing SAR against early blight disease caused by *A. solani*. According to this method inducer-treated and inoculated leaves were exposed over night to trypan blue stain, then distained with chloral hydrate and examined under binocular microscope, photographed by digital camera. Non-viable leaf cells were stained blue. Lesion size measurement was carried out, using digital image system. Results were statistically analyzed and presented in Table (2) and Fig. (2) then obtained results revealed that inoculation of untreated plant shoots with *A. solani* resulted in significantly damage of leaves. Moreover, the damaged area of leaves was higher in susceptible Castle Rock cv, compared with that of the resistant Tezier cv (52.15 and 30.11%, respectively, of the total leaf area). Treatment of plant shoots with the tested SAR inducers SA, INA and vit.B_1 a week before inoculation with *A. solani*, significantly reduced the damaged area of leaves, compared with untreated inoculated control. Reduction of infected leaf area significantly differed according to host resistance and the tested SAR inducer. The highest reduction in the damaged leaf area in both resistant and susceptible cvs was induced by vit.B_1 inducer. Moreover, damaged area was significantly lower in resistant than susceptible cvs (4.794% and 26.62%, respectively, of the total leaf area, i.e. 84.04 and 48.35% less than control). Treatment with SA induced also considerable reduction in lesion area, compared with control, however, effect was more pronounced in resistant than susceptible cv (56.39 and 36.68% less than control). The least reduction rates were induced, in both resistant and susceptible cvs, by INA inducer, where reduction rates, compared with control were 40.75% and 25.23%, respectively. Understanding the infection biology of fungi is the key step in devising suitable control strategies for plant diseases. Trypan blue staining

was employed to scan the infection structures of the tested pathogens and host response in tomato leaf tissues; this was endorsed by Bhadauria *et al*. (2010) who he reported that trypan blue staining technique was applied recently in demonstration and detection of host-pathogen interaction in different pathosystems.

Table (2). Effect of SAR chemical inducers on leaf lesions area ratio in both resistant and susceptible tomato cultivars, inoculated with *A. solani*.

	Lesion Area Ratio (%)			
	Tezier cv		Castle Rock cv	
	%	Reduction %	%	Reduction %
Control	30.106^{a}	-	52.152^{a}	-
SA	13.067^{b}	56.39	33.02^{b}	36.68
INA	17.838^{b}	40.75	38.99^{c}	25.23
Vit.B1	4.794^{c}	84.04	26.62^{d}	48.35

Values followed by same letters in the same column within cultivar don't differ significantly according to Duncan multiple range test (DMRT) at α=0.05.

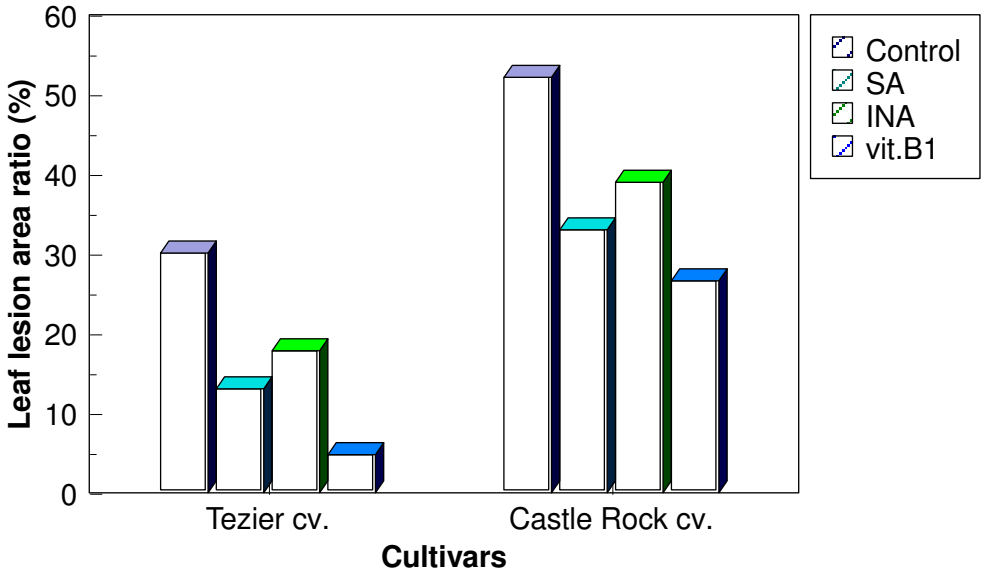


Fig. (2). Effect of treatment with the tested SAR chemical inducers on leaf lesion area ratio in both resistant Tezier and susceptible Castle Rock cvs, inoculated with *A. solani*, the early blight agent.

Determination of lesion frequencies and diameter using detached leaf technique:

The aim of this experiment was to determine the effect of treatment with the tested SAR inducers on the frequency of lesions and lesion diameter. Frequency and diameter of lesions were determined, presented in table (3) and Figs. (3), (4).

- **Frequencies of leaf lesions:** Inoculation of untreated plants with *A. solani* resulted in higher frequencies of lesions in both resistant and susceptible cvs. In addition, lesion frequency was higher in susceptible Castle Rock cv than resistant Tezier cv (10.00 and 7.66 lesion/leaf, respectively). The lowest numbers of lesions per leaf in both resistant and susceptible cvs were induced by vit.B_1. Moreover, lesion number/leaf was higher in susceptible than resistant cv (5.33 and 3.66 lesion/leaf, respectively). Treatment with SA came second to vit.B_1 and gave also pronounced reduction in lesion numbers per leaf, compared with control. Moreover, lesion number/leaf in SA treatment was higher in susceptible Castle Rock cv (9.00 lesions/leaf) than that of resistant Tezier cv (4.66 lesions/leaf). The least reduction rates among the other tested SAR inducers was exhibited by INA in both resistant and susceptible cvs, where number of lesions/leaf were 6.33 and 9.33 lesion/leaf, respectively (Table (3) and Fig. 3).

Generally, treatment with the tested inducers, i.e. SA, INA and vit.B_1 realized significant reduction rates in no of lesions per leaf in both resistant (39.16%, 17.36% and 52.22%, respectively less than lesion numbers in control) and susceptible cv (10%, 11.45% and 56.44% less than control).

Lesion diameter: Data in Table (3) and Fig. (4) showed that inoculated untreated control exhibited the highest mean lesion diameter values in both resistant and susceptible cvs Differences in lesion diameter between resistant and susceptible cvs were not pronounced (4.41 and 4.89 mm, respectively). Treatment with vit.B_1 resulted in the lowest values of lesion diameter in both resistant Tezier and susceptible cvs however lesion diameters were smaller in resistant than those of susceptible (1.72 and 2.13 mm, respectively). SA treatment gave also significant reduction in lesion diameter, compared with control, but still less than those obtained in vit.B_1 treatment. Reduction in lesion diameter was obtained in both Tezier and Castle Rock cvs, pre-treated with SA and inoculated with *A. solani*, however, lesion diameters in resistant cv were smaller than those of susceptible cv (2.20 and 3.77 mm, respectively). INA treatment resulted in the highest lesion diameter values in both resistant and susceptible cvs, compared with those induced by the other tested inducers, where lesion diameters were 2.49 and 4.33 mm, respectively. It could be concluded that treatment with the

tested inducers, i.e. SA, INA and vit.B_1 resulted in significant reduction in lesion diameter values in both resistant cv (50.11%, 43.54% and 61%, respectively, less than control) and susceptible cv (22.9%, 11.45% and 56.44%, respectively, less than control). The detached leaf assay has the advantage of enabling more insight into complex polygenic nature of resistance than can be distinguished using whole plant evaluation alone (Browne *et al.*, 2006). A detached-leaf method suitable for use with large mapping populations (Twizeyimana *et al.* 2007 and Jackson *et al.* 2008). Detached leaflets assays used for evaluation of early blight (EB) resistance, as a means to circumvent the influence of growth habit, which may affect the reaction of plants in the field or glasshouse, although Lynch *et al.* (1991) and Foolad *et al.* (2000) concluded that detached leaflet assays did not correlate well with field and glasshouse screenings. Laboratory assays on detached leaflets therefore show promise for studying particular aspects of resistance and for eliminating confounding influences of whole-plant physiology. However, these methods need to be carefully tuned for the research question in hand, and cannot be relied on as a replacement for field or glasshouse tests. In some tested treatments, results obtained by detached leaf technique seem to be of a low impact to present considerable resistance expression between chemical treatments and control and within chemical treatments itself, this could be due to the high concentration of *A. solani* spores applied to leaflets or because the resistance of these accessions expresses only at the whole plant level as already mentioned by Foolad *et al.* (2000). The latter may be another reason for the inadequacy of the detached leaf method for evaluating tomato germ plasm for early blight resistance. There might be another explanation with regard to increasing the lesion number in the chemical treatment is that many of these lesions, especially small-scale can be the reaction of plant to the chemical inducer, so called “hypersensitive reaction” or necrosis which considered one of the distinctive characteristics of these plant inducers to stimulate the reaction of hypersensitivity which is an important indication of the emergence of systemic

acquired resistance. Chaerani *et al.* (2007) reported similar findings in which some tomato species developed severe necrosis in the glasshouse experiment even without inoculation. After spray inoculation, this necrosis was often indistinguishable from early blight lesions.

Table (3). Effect of treatment with the tested SAR inducers on lesion frequencies and diameter in both resistant and susceptible cvs, inoculated with *A. solani*.

Treatment	Mean values of lesion frequency and diameter			
	Tezier cv		Castle Rock cv	
	Frequency/leaf	Diameter (mm)	Frequency/leaf	Diameter
Control	7.66^{a}	4.41^{a}	10.0^{a}	4.89^{a}
SA	4.66^{b}	2.20^{b}	9.00^{b}	3.77^{b}
INA	6.33^{c}	2.49^{b}	9.33^{b}	4.33^{ab}
Vit.B_1	3.66^{b}	1.72^{b}	5.33^{c}	2.13^{c}

Values followed by same letters in the same column within cultivar don't differ significantly according to Duncan multiple range test (DMRT) at α=0.05.

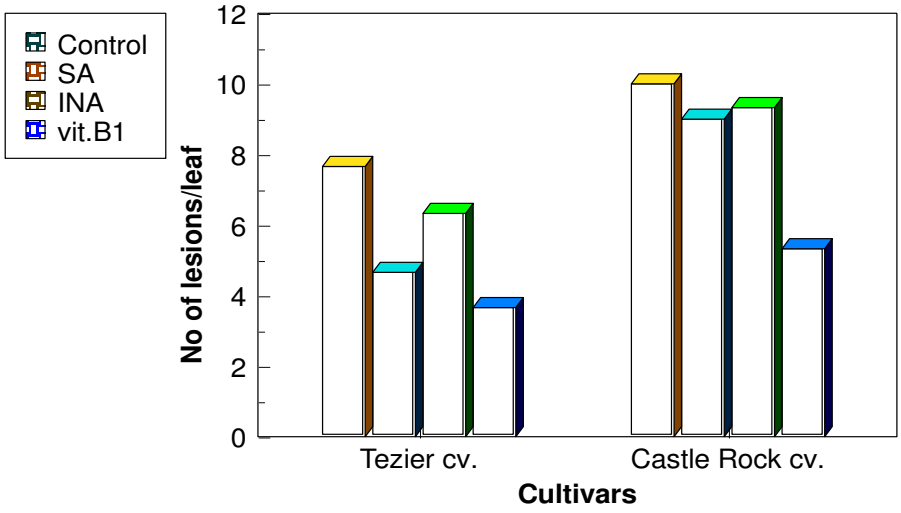


Fig. (3). Effect of treatment with the tested SAR inducers on the number of lesions per leaf in both resistant Tezier and susceptible Castle Rock cvs, inoculated with *A. solani*.

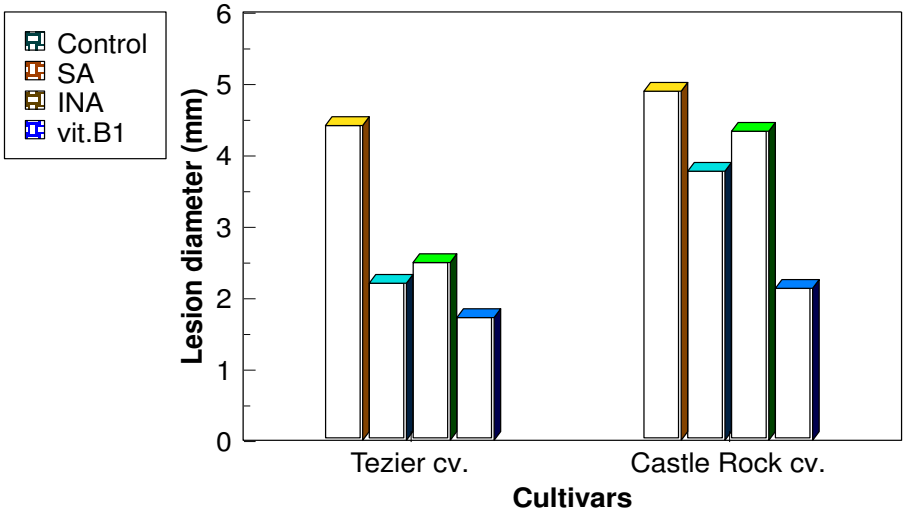


Fig. (4). Effect of treatment with the tested SAR inducers on lesions diameters in both resistant Tezier and susceptible Castle Rock cvs, inoculated with *A. solani*.

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الملخص العربي

تأثير الإصابة بفطر Alternaria على صنفي طماطم حساسة ومقاومة المعاملة مسبقا بمنشطات المقاومة الجهازية المكتسبة

الملخص

من أجل خلق بدائل صديقة للبيئة وللتقليل من خطر استخدام المبيدات الكيماوية تستخدم المنشطات الكيماوية لإستحثاث المقاومة الجهازية المكتسبة كوسيلة لمقاومة أمراض النبات بدون تلويث البيئة بالكيماويات فقد عوملت شتلات الطماطم مسبقا بمنشطات المقاومة الجهازية المكتسبة الكيميائية مثل حمض السالسيلك وحمض الأيزونيكوتينك والثيامين ثم حقنت بفطر *Alternaria solani* فقللت وبشكل معنوي ظهور مرض اللفحة المبكرة في الصنف المقاوم "تيزر" حيث قلت الإصابة بنسبة 68% ، 52% ، 80% على التوالي ، مقارنة بالكنترول. أما ظهور الإصابة في الصنف الحساس "كاستل روك" فقد قلت بنسبة 62.88% ، 50.1% ، 79.22% على التوالي. لذا فإن أعلى معدل في قلة ظهور المرض تم إستحثاثه بواسطة الثيامين مقارنة بباقي المنشطات الأخرى. تقليل الشدة المرضية لمرض اللفحة المبكرة في الصنف المقاوم "تيزر" كانت 36.92% ، 26.33% ، 61.93% على التوالي مقارنة بالكنترول ، وفي الصنف الحساس "كاستل روك" كانت 35.59% ، 23.85% ، 61.55% على التوالي ، لذا فأعلى معدل لتقليل شدة المرض تم إستحثاثه بواسطة الثيامين مقارنة ببقية المنشطات الأخرى. في تجربة الورقة المنزوعة قللت المعاملة بالثيامين عدد التبقعات وأبعادها في كل من الصنف المقاوم "تيزر" والحساس "كاستل روك" حيث كانت (52.22% ، 46.7%) لعدد التبقعات و (60.99% ، 56.44%) لأبعاد البقعة على التوالي وكلاهما أقل من الكنترول.