

Effect of Certain Adjuvants on Enhancing the Biological Activity of Some Fungicides to Control Potato Late Blight

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ABSTRACT

A number of selected fungicides, namely chlorothalonil (Daconil 500 F), mancozeb (Dithane M 45), metalaxil (Ridomil 5 G), fluazinam (Shirlan) and fentin hydroxide (Stanex 18 Flow) were used to control late blight of potato caused by *Phytophthora infestans* in field trials. Fungicides were applied at normal and half normal application rates either alone or mixed with adjuvants. The adjuvants (0.1%) mixed with fungicides were "Aplus" 450, SCS 3338, SCS 3610 and SCS 3676. The best fungicide-adjuvant combination for controlling late blight of potato were fentin hydroxide-"Aplus" 450, followed by fluazinam-SCS 3676 and mancozeb-any of adjuvants. Chlorothalonil was equally well enhanced by addition of either Aplus" 450 or SCS 3338 adjuvant. Biochemical changes such as Chlorophyll content, total soluble sugars, total reduced sugars and phenolic compounds, due to late blight of potato in treated plants were investigated. When adjuvants were used, a pronounced improvement of the biological activity of reduced doses of tested fungicides was achieved in potato plants infected with *Phytophthora infestans*.

INTRODUCTION

Potato late blight caused by *Phytophthora infestans* (Mont.) de Bary is a serious worldwide threat for potato crop and considered one of the most prevalent and destructive diseases which cause marked losses to potato yield. Unfortunately, no resistant cultivars to the disease, in all potato areas are available to allow commercial potato cultivation without intensive use of fungicides. However, some commercial cultivars have only a moderate level of resistance and they could be protected by less amounts of fungicides than required by other cultivars (Hooker, 1981). High doses of fungicides contribute undoubtedly to environmental pollution and increase the costs of chemical control to become less cost-effective. One way to reduce the dose of fungicides is adding adjuvants to spray formulations. These adjuvants can sometimes support or improve the action of the active ingredients (Staubert, 1993). They can also have a positive or negative effect on the uptake of fungicides by the pathogen. It is well established that adjuvants, especially those of the nonionic types, can increase the foliar uptake of many other agrochemicals leading to the reduction of application rates or number of sprays (Amer *et al.*, 1992 & 1993 and Smith *et al.*, 1992). There has been, however, limited research on adjuvants use with fungicides. Moreover, few laboratory studies have been carried out to achieve maximum performance against *Phytophthora infestans* growth on Rye agar medium with minimum concentrations of the fungicide when mixed with certain adjuvants (Amer *et al.*, 1991), however, *in vivo* tests have not yet been made. Furthermore, possible activation of potato plant response to the causal agent through changing its chemical constitutions when adjuvants are applied has not previously been explored. Such knowledge might be promising for future fungicides in particular and pesticides in general in order to avoid or minimize pollution (Staubert, 1993). Therefore, the objectives of this study were to: (i) evaluate some commercial systemic fungicides and their effect on the control of potato

late blight, (ii) evaluate the biological activity of adjuvant and fungicide combinations, (iii) study the effect of fungicide and adjuvant combinations on the biochemical changes of infected potato leaves.

MATERIALS AND METHODS

Fungicides and adjuvants

Fungicides used in the present study, their chemical names, commercial name, chemical classes and recommended dose are listed in Table 1. The concentration of the commercial product recommended for agricultural use is called Normal Application Rate (NAR). Half of the normal application rate is HNAR.

Table 1: Fungicides tested to control *Phytophthora infestans* on potato:

Compound	Commercial name	Chemical class	Commercial recommended dose (g/l)
Chlorothalonil	Daconil 500 F (Chima-Agriphar)	Miscellaneous	3
Fentin hydroxide	Stanex 18 Flow (Bayer)	Tin	0.3
Fluazinam	Shirlan (Protex)	Aniline	0.2
Mancozeb	Dithane M 45 (Protex)	Dithiocarbamat	2
Metalaxyl	Ridomil 5 G (Liro)	Fenylamide	3

The adjuvants used belonging to different types are listed in Table 2. All the tested fungicides were mixed with 0.1% of the tested adjuvants. The adjuvants were obtained from I.C.I surfactants, Kortenbergh, Belgium.

Table 2: The code number of adjuvants, their chemical names and dose used

Adjuvant	Chemical description	Dose
"Aplus" 450	Alkylpolysaccharide based adjuvant	0.1%
SCS 3976	Alkenyl succinic anhydride condensate based (ASAC)	0.1%
SCS 3338	ASAC - PEG (Poly ethylene glycol)	0.1%
SCS 3610	ASAC - diglucamide	0.1%

Fungicide and adjuvant combination treatments:

In order to evaluate the efficacy of fungicides, adjuvants and/or their mixture in combination with fungicide were prepared in distilled water (100 ml). They were used alone or together with the fungicide at half of the normal application rate (HNAR).

Three sprays, with the tested fungicides at normal application rate (NAR) alone and at half normal application rate (HNAR) alone or in combination with tested adjuvants at 0.1% were applied at 10 and 15 days intervals. Appearance of the late blight symptoms was due to natural infection after suitable changes of the weather and rainy days. Untreated plots were sprayed with water and served as control. Early season infestation with aphids and thrips was sprayed with selecron 72% at 187.5 ml/100 liters. Results were recorded seven days after each spray. Potato late blight severity was estimated using the growth of the disease (lesions diameter) as a parameter for fungitoxicity. All treatments were replicated four times. Statgrafics package was used for analysis of variance.

Experimental design

A field experiment was conducted at the farm of the Faculty of Agriculture, Saba-Bacha, Alexandria University. Plots, 20 m² each comprised of 5 rows 80 cm apart were used as experimental units and arranged in a complete randomized block design with three replicates. Certified potato seeds of Nicola variety were obtained from Seed Production Department, Ministry of Agriculture at Giza. Seeds were sown, after irrigation and drying of plots in hills 25 cm apart, on the 30th of October. The plots were irrigated six weeks after planting at 10 days intervals for six times during potato season. Recommended fertilizers and services were carried out.

Biochemical changes associated with the application of fungicides mixed with adjuvants:

An experiment was designed in order to study physiological or biochemical changes including chlorophyll content, total soluble sugars, reduced soluble sugars and phenolic compounds in infected, adjuvant and/or fungicide treated potato plants. Samples of leaves collected from treated and untreated potato plants were analysed for their chlorophyll contents by the method described by Harbon (1973). Also, total and reduced soluble sugars were determined according to Yem & Willis (1954) and Plummer (1971). Total phenolic compounds were also colourimetrically determined using Folin-Denis reagent according to Snell and Snell (1953). All treatments were replicated four times. Statgrafics package was used for analysis of variance.

RESULTS AND DISCUSSION

Fungicide and adjuvant combinations treatment:

The diameter of necrotic lesions on diseased plants as a parameter for the biological activity of five different commercial systemic fungicides and/or four types of adjuvants on the control of potato late blight caused by *Phytophthora infestans* are given in Figure 1-3. Results presented in Figure 1 indicate that the activity of adjuvants varied from one fungicide to another depending on the adjuvant type. For instance, the best adjuvant mixed with chlorothalonil and fentin hydroxide was "Atplus" 450 as lesion diameter in both treatments reached 24.65 and 27.46 mm, respectively. While SCS 3610 and SCS 3976 were the best adjuvants for metalaxyl as the lesion diameter was 23.85 and 25.15 mm, respectively, while it was as large as 31.85 mm for SCS 3610 plus mancozeb.

Adding an adjuvant to fungicides in spray solution was significantly more effective in disease control comparing with fungicide alone at HNAR (Fig. 2). All tested adjuvants, however, were quite effective in controlling the disease and reducing the lesion diameter. It can also be observed that there are no wide variations between effect of the adjuvant types used for enhancement of the activity of the fungicides in reducing the disease severity. No significant differences were observed between NAR and HNAR plus adjuvant "Atplus" 450 and SCS 3610, which appear to be the most effective adjuvants as lesion diameter was respectively 14.89 & 15.28 mm in comparison to the two other adjuvants SCS 338 and SCS 3610, where lesion diameter was 17.52 and 18.63 mm, respectively.

Figure 3 shows a different pattern for each fungicide when used at NAR, HNAR or HNAR plus adjuvants compared to control. The disease necroses was greatly reduced by adding the adjuvants to mancozeb (8.94 mm), chlorothalonil (13.02 mm), metalaxyl (16.8 mm), fentin hydroxide (16.35 mm) and fluazinam (28.52 mm) compared to these fungicides alone at full rate. There were also significant differences between HNAR plus adjuvants, HNAR, NAR and control. A significant improvement in the efficacy of tested fungicides has occurred when adjuvants (1%) mixed with HNAR in comparison with HNAR, NAR of the fungicide alone and the control as lesion diameter reached 4.42, 6.17, 6.25, 8.94 & 12.52 mm for metalaxyl, chlorothalonil, fentin hydroxide, mancozeb and fluazinam, respectively. These results are in agreement with Amer *et al.* (1992, 1993) as they indicated that adjuvants increased the activity of fungicides. Moreover, they added that there was a positive relationship between the high efficacy of the fungicide and the concentration of the adjuvants. It was also found that growth of *Phytophthora infestans* has been inhibited on Ray agar medium by using adjuvants in combination with reduced dose of the fungicides as mancozeb+ofurace, mancozeb+oxadixyl and propienib+oxadixyl (Amer and Poppe, 1991). These results could be explained in view of the various effects of the surface active agents summarized by Holly (1964) such as increase of cuticular penetration, increase of stomatal penetration and increased wetting. Concerning the improvement of the disease control due to mixing adjuvant with fungicide, adjuvants can enhance the uptake and transport of the fungicide spray solution into the cell wall of the fungus (Steurbaut, 1989). Similar results have been obtained by Amer *et al.*, 1993 and 1994 on other foliar diseases such as Septoria leaf spot (*Septoria apiicola*) chocolate leaf spot (*Botrytis fabae*). The efficacy of the fungicides was greatly improved by all types of adjuvants. It is apparent that the addition of adjuvants decreased the rate of leave infection with more than 160% compared to fungicide at full rate (NAR) without adjuvant.

The optimum adjuvant concentration was limited between 2.0-4.0 g litre⁻¹, which gave the best performance for the fungicides (Amer *et al.*, 1994). Prediction obtaining same results in field trials with tested fungicides and adjuvant types might be possible. Remarkable is the difference in behavior of fungicides when used alone or combined with adjuvants, depending on the fungus and fungicide. Almost all adjuvants tested resulted in an enhanced uptake of the fungicide. Also, the impact of an adjuvant is perhaps more pronounced due to a better penetration of the spray solution through the fungal cell wall (Clifford & Hislop, 1975; Tomlinson & Faithfull, 1979; Steurbaut *et al.*, 1989). The influence of adjuvants on enhancement or transport of fungicides towards their site of action at cell level, however is not well known, but Steurbaut *et al.*, 1993 mentioned that this effect is probably more pronounced for locations situated at the outside of the fungal cell than for compounds more centrally situated inside the fungal cell.

Biochemical changes associated with adjuvants and fungicides treatments:

The physiological and biochemical changes occurred in tested plant leaf tissues following infection by *Phytophthora infestans* are shown in Figures 4-9. Data representing the effect of potato late blight severity on both total and reduced soluble sugar contents are also shown in Figure 4 and 5.

Data illustrated in Figure 4 show the effect of different five fungicides in combination with adjuvants on the reduction of the level of total sugars in treated leaf tissues. All tested adjuvants greatly enhanced the performance of mancozeb in decreasing the level of total sugars. The decreasing of total sugars reached 58.66, 61.13, 60.03 & 62.12 mg/g with SCS 3610, "Aplus" 450, SCS 3976 and SCS 3338, respectively, compared to healthy plants (47.8 mg/g) and control (78.68 mg/g) while other combinations varied in their effect.

Results on the effect of fungicide, adjuvant and their combinations to control potato late blight and their effect on the reducing soluble sugars are presented in Figure (5). The adjuvant SCS 3610 highly enhanced the performance of all tested fungicides in general and mancozeb in particular to decrease the reducing soluble sugar level (28.97 mg/g) in comparison to control (78.65 mg/g). In the meantime, other combinations gave similar results. Sugar synthesis had been reported to be correlated with photosynthetic rate (Livne, 1964) and consequently, with chlorophyll content (Hopkins and Hampton, 1969). In the present study, the contents of total soluble sugars and reducing sugars increased in diseased untreated plants (control), while they decreased with fungicide-adjuvant applications in all tested treatments. The reduction in total soluble sugars and reducing soluble sugars in adjuvants treated plants and its relation with plant defense mechanisms necessitates further studies.

The influence of fungicides, adjuvants and their combinations on the changes of phenolic compounds in leaf tissues of blighted potato plants are presented in Figures 6 and 7. All fungicides induced significant reduction of phenolic compounds compared with healthy and untreated plants (Figure 6). The results indicate that healthy potato leaves contained lower amount of total phenolic compounds (4.1 mg/ml) than those of control (untreated and infected plants) (9.6 mg/ml) and other treated plants. Data also indicate that the effect of the fungicide and adjuvant combinations on phenolic compounds concentrations (mg/ml) in infected plants varied according to the fungicide tested and adjuvant type and their combinations.

Data in Figure 7 indicate that there is a reduction in phenolic compounds as a result of mancozeb, chlorothalonil and metalaxyl treatments at HNAR mixed with adjuvants as they gave 4.19, 4.8 & 5.12 mg/ml, of the phenolic compounds, respectively in comparison with check treatment (8.5 mg/ml). The level of phenolic compounds of the previous treatments (adjuvant plus fungicide at HNAR) one lower if compared with their levels for these fungicides alone at NAR as the concentrations of phenolic compounds reached 4.9, 4.15 & 4.9 mg/ml in case of respectively. All tested adjuvants gave the same trend as to the biological activity of the fungicides in relation to reducing phenolic compounds in treated plant tissues.

Concerning phenolic compounds which were found to be decreased (expressed as total phenolic compounds) after treatment of infected plants with adjuvants and fungicides, the most important factor here is probably a specific phenolic compound rather than the total phenolic compounds which could possibly be responsible for inhibiting the pathogen (Daayf, *et al.*, 2000 and Bokshi *et al.*, 2003). In this case, to be able to determine which specific phenolic compound is

responsible for inhibiting the pathogen in infected plants showing incompatible reaction could be a subject of further study.

The effect of potato late blight infection on chlorophyll content in leaf tissues is shown in figures 8 and 9. The effects of interaction between adjuvant types and tested fungicides on chlorophyll content are shown in Figure (8). Results indicate that some tested adjuvants have a negative side effect on reducing the chlorophyll content when mixed with fungicides. SCS 3610 was an active adjuvant in this respect, specially when mixed with mancozib fungicide (8.38 mg/g fresh wt.) compared to healthy (9.96 mg/g fresh wt.) and untreated infected plants (4.21 mg/g fresh wt.). The least chlorophyll content was obtained with adjuvant SCS 3338 with all fungicides in general and in particularly with fluazinam fungicide (3.38 mg/g fresh wt.).

The obtained data, however, indicate that fungicide and adjuvant and their combinations treatments caused a significant increase in the amount of chlorophyll content (a, b and total chlorophyll as a whole) in comparison with control (untreated infected potato plants) (Figure 9). Mancozeb, chlorothalonil and fentin hydroxyl treatments caused an increase in chlorophyll a of infected potato plants as they gave 4.81, 4.22 and 3.9 mg/g fresh wt., respectively in comparison to untreated infected plants which gave only 3.12 mg/g fresh wt. of chlorophyll a. In case of chlorophyll b, the increase was much more pronounced as these fungicidal treatments caused a higher increase in chlorophyll b as they gave 3.57, 3.38 and 2.9 mg/g fresh wt., respectively in comparison to only 1.8 mg/g fresh wt. for untreated infected plants. The same trend was noticed in case of total chlorophyll content as they gave 8.38, 7.6 and 6.8 mg/g fresh wt., respectively in comparison to 4.92 mg/g fresh wt. for the untreated infected potato plants. Healthy plants showed as expected higher amounts of chlorophyll a, b and total chlorophyll as they gave 10.42, 5.05 and 15.46 mg/g fresh wt. of these compounds, respectively. Remedial effect of fungicidal treatments was affected by addition of adjuvants in favor of the treated plants in most cases. Results also indicate that there is a specific adjuvant for each tested fungicide which gave better control for potato late blight and has no bad effect on chlorophyll content. The decrease in chlorophyll contents in infected plants may be due to the toxic metabolites produced by the pathogens which inhibit chlorophyll production (Fulton *et al.*, 1965 and Pero & Main, 1970). In spite of the fact that chlorophylls are responsible for the synthesis of sugars, accumulation of sugars in leaf tissues may sometimes cause a reduction in chlorophyll contents. Curtis and Clark (1950) also suggested that treatments which brought about marked accumulation of sugars were likely to result in a decrease of chlorophyll contents.

The finding that reducing sugar and total soluble sugars decreased when adjuvant was added to the fungicides may be interpreted in view of the fact that this reduction may have affected the pathogen growth and its development and consequently the disease severity. As an attempt for explaining the role of adjuvants in decreasing either the reducing or the total soluble sugars in infected plants one could think of this effect on disease development on view of the traditional theory about plant mechanisms for defense i.e. the nutrition theory which had previously been mentioned by many authors (Heitefuss, 1976 and Agrios, 1997).

Finally, the increase of chlorophyll content in adjuvants treatments mixed with fungicides shows an agreeable effect of the tested adjuvant as it may indicate that it increased the uptake of the fungicide to the level which caused inhibition to the plant pathogen itself and stopped, reduced its effects on chlorophyll content.

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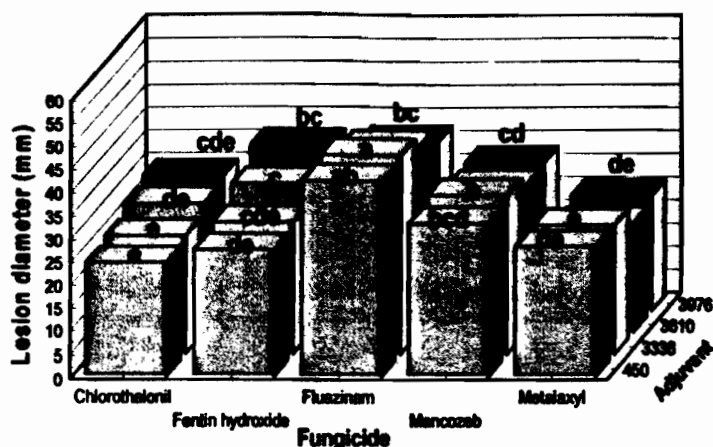


Fig. 1: Effect of adjuvants (0.1%) on the efficacy of certain fungicides for controlling late blight of potato (over all mean of fungicides treatments). Means followed by the same letter are not significantly different

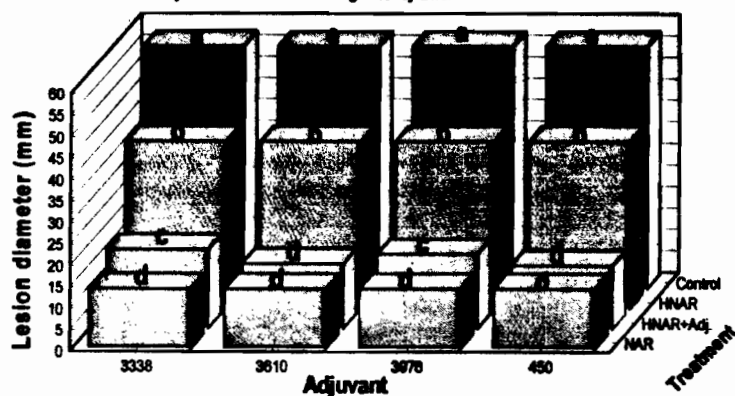


Fig. 2: Effect of different adjuvants (0.1%) on the efficacy of certain fungicides at NAR, HNAR and HNAR in combination with adjuvant to control potato late blight (mean of tested fungicides). Means followed by the same letter are not significantly different

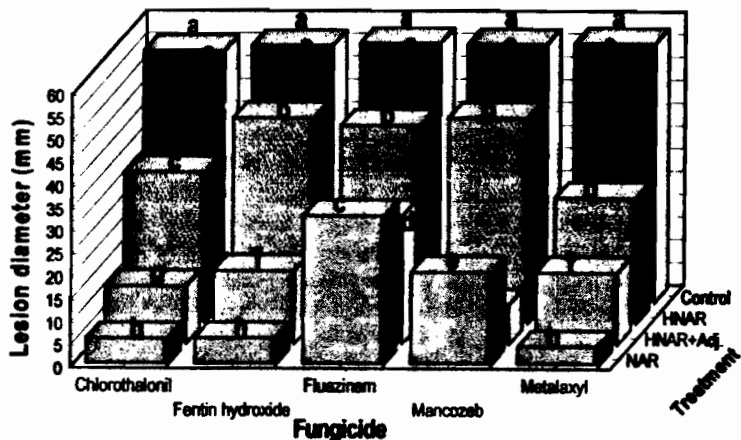


Fig. 3: Effect of certain fungicides at NAR, HNAR and HNAR in combination with adjuvants (0.1) on the control of potato late blight (mean of adjuvants types). Means followed by the same letter are not significantly different

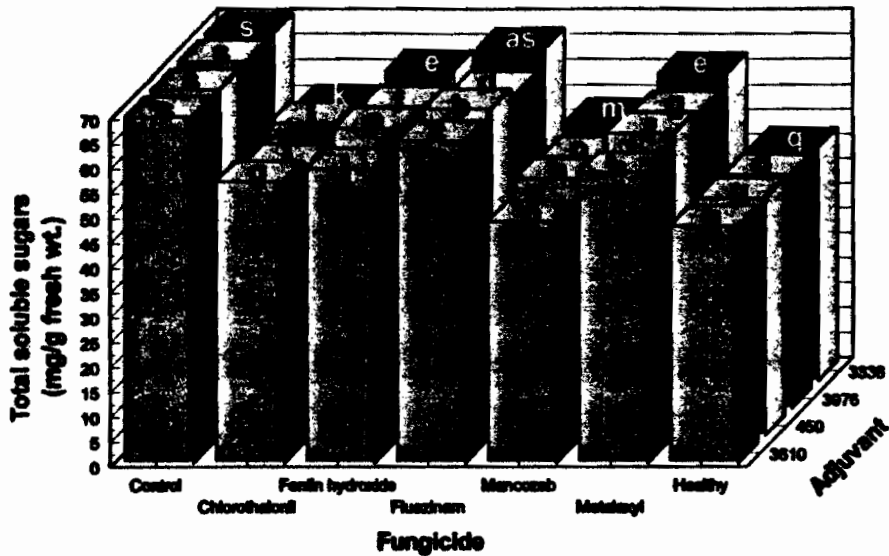


Fig. 4: Effect of certain fungicides at HNAR in combination with adjuvant types on the concentration of total soluble sugars of healthy and infected potato with late blight.

Means followed by the same letter are not significantly different.

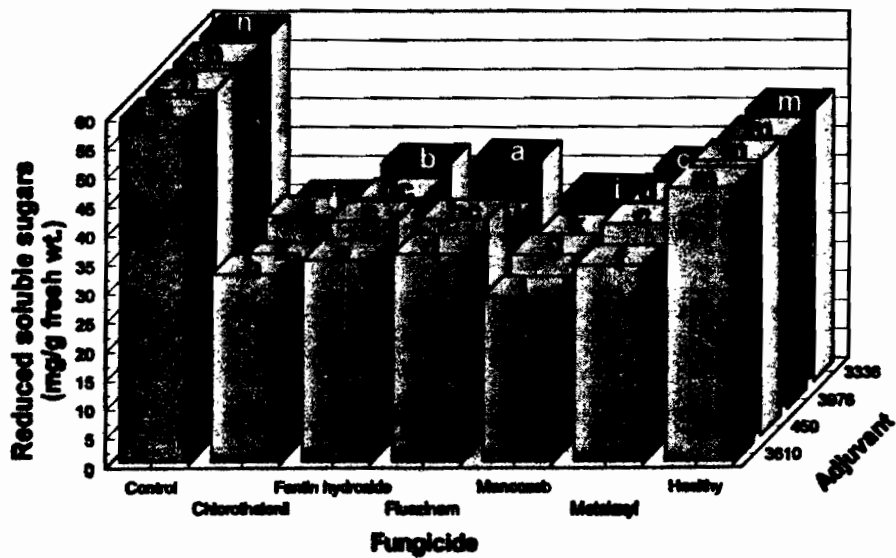


Fig. 5: Effect of certain fungicides at HNAR in combination with adjuvant types on the concentration of reduced soluble sugars of healthy and infected potato with late blight.

Means followed by the same letter are not significantly different.

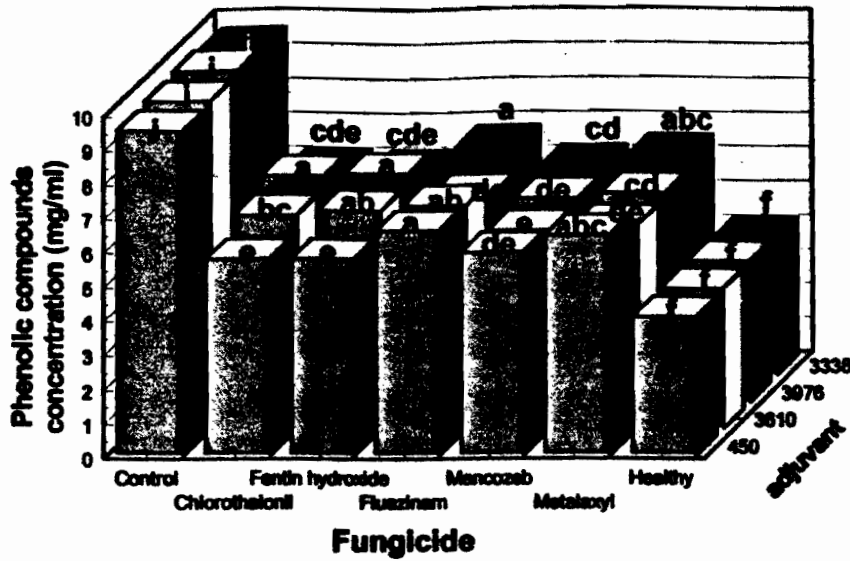


Fig. 6: Effect of certain fungicides at HNAR in combination with adjuvant types on the concentration of phenolic compounds in cell free extract of infected potato with late blight.

Means followed by the same letter are not significantly different.

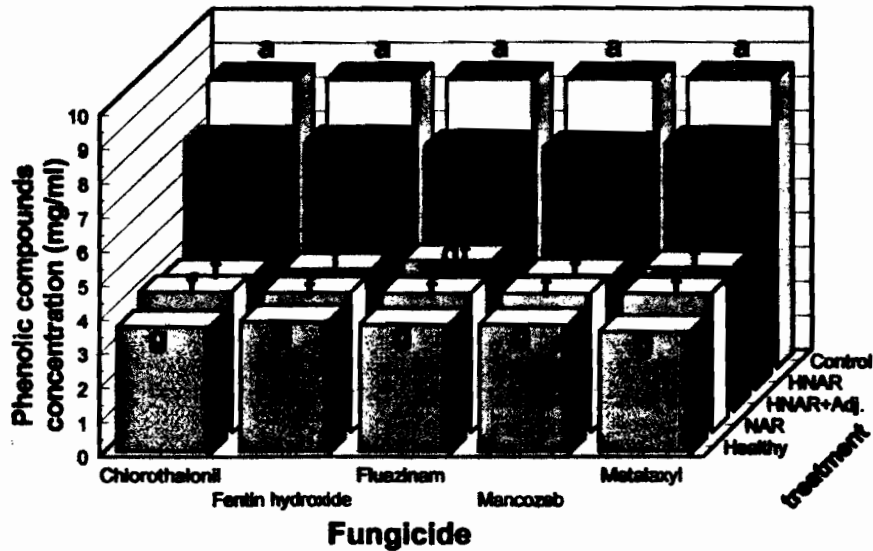


Fig. 7: Effect of certain fungicides at NAR, HNAR alone and HNAR in combination with adjuvant types on the concentration of phenolic compounds in cell-free extract of infected potato with late blight.

Means followed by the same letter are not significantly different.

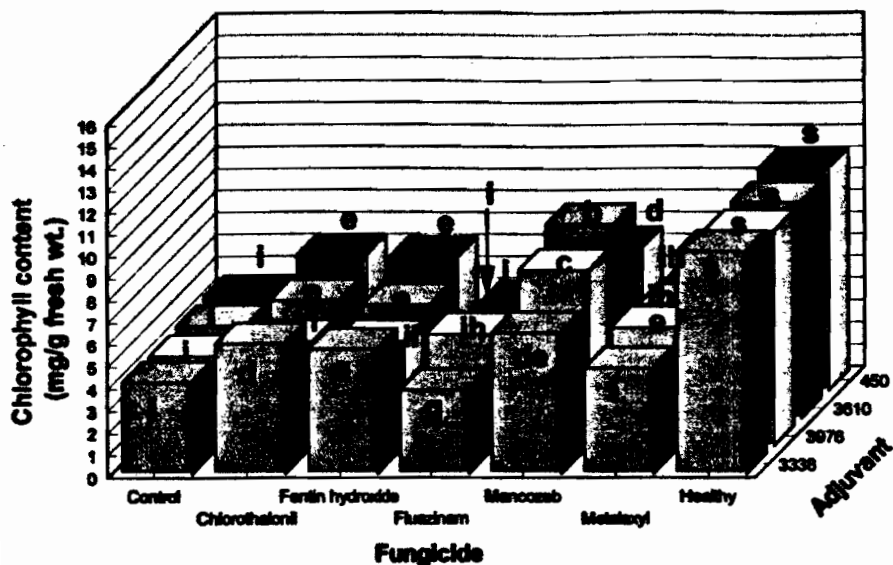


Fig. 8: The effect of different adjuvants mixed with certain fungicides at HNAR on the changes in chlorophyll content of healthy and infected potato leaves (mean of total chlorophyll).

Means followed by the same letter are not significantly different

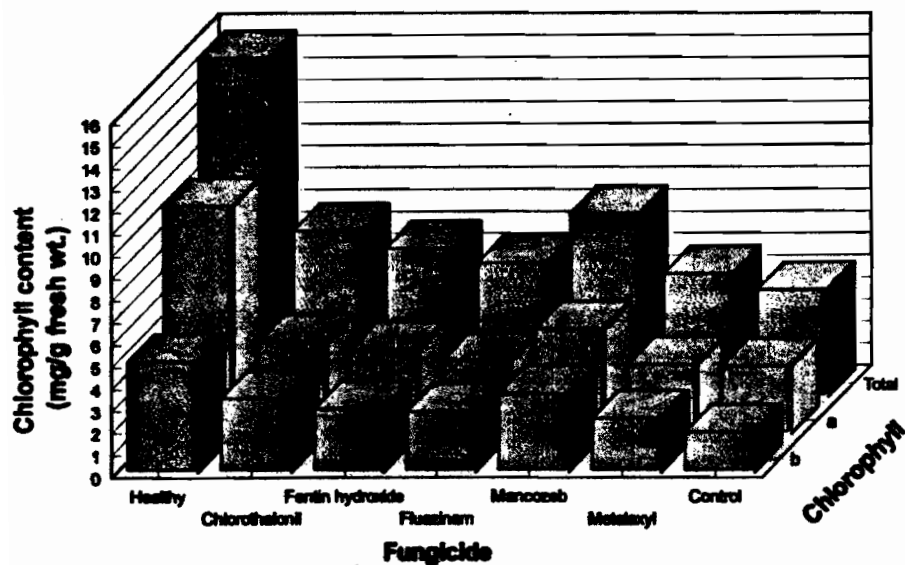


Fig. 9: The effect of different adjuvants mixed with certain fungicides at HNAR on the changes in chlorophyll a, b and total chlorophyll of healthy and infected potato leaves (mean of all adjuvants).

Means followed by the same letter are not significantly different

الملخص العربي

تأثير بعض الإضافات لتحسين الكفاءة البيولوجية لبعض المبيدات الفطرية المستخدمة لمقاومة مرض اللبحة المتأخرة في البطاطس

مصطفى عبد العظيم عمر

قسم النبات الزراعي - كلية الزراعة (سابقاً) - جامعة الإسكندرية

يستخدم في هذه التجارب التي أجريت حقلياً المبيدات الفطرية: كلوروثالونيل، مانكوزيب، ميتالاكسيل ، فلوزينام و فينتين هيدروكسيد وتمت المعاملة بالمبيدات الفطرية عند التركيز الموصى به وكذلك عند نصف التركيز الموصى به سواء بمفردها أو مخلوطة مع المواد المضافة. وكانت المضيفات المستخدمة في هذه التجارب هي "Atplus 450, SCS 3338, SCS 3610 & SCS 3976" (0.1%). وأظهرت النتائج المتحصل عليها أن خلط المبيد الفطري فينتين هيدروكسيد مع Atplus.450 و فلوزينام مع SCS 3976 و مانكوزيب مع أي من الإضافات المستخدمة أدت إلى تحسين فاعلية المبيد لمقاومة مرض اللبحة المتأخرة في البطاطس. وأن المبيد الفطري الكلوروثالونيل أظهر نتائج جيدة في مقاومة المرض بإضافته إلى أي من Atplus.450 أو SCS 3338. تم تقدير التغيرات الكيموحيوية مثل المحتوى للكلوروفيل ، السكريات الذائبة الكلية، السكريات المختزلة الكلية و الفينولات ، وأظهرت النتائج قلة محتوى الأوراق المصابة من السكريات والمواد الفينولية عند استخدام الجرعة المختزلة من المبيد الفطري مخلوطة بالمضيفات المختبرة وتحسن نسبي في محتوى الكلوروفيل عند إضافتها للمبيدات المستخدمة مقارنة بالنباتات المصابة غير المعاملة.