The Effect of Sulfobetaine on the Activity of Strobilurins and Benzimidazole Fungicides

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ABSTRACT: *Surfactants (adjuvants) are the substances often added to the spray tank, besides the pesticide formulation, whose role is to improve the performance of the pesticide or the physical properties of the spray mixture, or both. Adjuvants are not often used as fungicides themselves, however, it is interesting to investigate if they can improve the fungistatic activity of the system they are included in. Therefore the main aim of this work was to determine the effect of the addition of a newly obtained, never investigated before, sulfobetaine type surfactant to the fungicides on their final properties. In the in vitro experiment fungistatic activity of the surfactant and the system of surfactant with strobilurins and benzimidazole fungicides were evaluated. The tested surfactants were added to the liquid medium of Potato Dextrose Agar (PDA) at concentrations: 1, 10, 50, and 100 ppm. The organisms of Fusarium culmorum, Rhizoctonia solani, Microdochium nivale, and Alternaria brassicas were used as indicators. The experiments have shown that the addition of the new surfactant studied to the medium resulted in inhibition of mycelium growth of all the tested fungi. The system with the surfactant and the fungicide was much more effective against the tested fungi than individual fungicides.*

KEYWORDS: *Sulfobetaine; Fungistatic activity; Strobilurins; Benzimidazole fungicides.*

INTRODUCTION

Adjuvants are important constituents of pesticide application. Addition of an adjuvant to the pesticide can improve the efficacy of the formulation obtained[1]. It is expected that application of an adjuvant to the formulation will improve the performance of the pesticide or the physical properties of the spray mixture, or both [2]. A correctly selected adjuvant may reduce or even eliminate spray application problems, thereby increasing overall pesticide efficacy. Adjuvants are designed to perform specific functions, e.g. to increase wetting, spreading, penetrating, sticking, or to reduce evaporation and volatilization, improve buffering and dispersing. No single adjuvant can perform all these functions, but different compatible adjuvants often can be combined to perform multiple functions simultaneously [2].

Adjuvants are often used in combination with pesticides. Spray adjuvants have the potential to improve deposition by effecting uniform distribution of the active

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ingredient on plant surfaces [3]. Quantification of the effects of adjuvants on droplet behaviour on plant surfaces is needed to ameliorate pesticide spray application efficiency [4] and improve foliar spray deposition [5]. As has been shown, not only the use of proper adjuvant is important but also e.g. spray volume and nozzle type [6].

Fungal infections of crops have become a crucial issue. Diseases caused by pathogenic fungi reduce the quantity and quality of yield as well as cause economic losses. This problem concerns all type of crops. Wheat, which is one of the most cultivated crop worldwide, can be seriously affected by some fungal pathogens such as *Fusarium graminearum.* This species is a causal agent of Fusarium Head Blight (FHB), infecting root and stem-base root of cereals. Literature data indicate that FHB may cause serious economic lossess. For example, in Northern and Central America, the losses caused by FHB reached $ 2,7 billion in the period 1998 – 2002 [7]. Fungal diseases cause also significant losses in several crops worldwide such as maize, oat, barley and rice [8-10]. As *Chahal et al.* (2012) [11] have reported, fungal infection of peanut crops is an important problem and a wide range of agrochemicals have been used to manage pests and to optimize crop growth and development. Foliar and soilborne diseases are prevalent in peanut and intensive fungicide programs are often implemented to minimize yield loss [11]. Also *Gillard* and *Ranatunga* [12] have emphasized the importance of the fight against *Colletotrichum lindemuthianum* in bean production. New modern fungicides are continuously designed and investigated. Quaternary ammonium ionic liquids exhibit good antifungal and antibacterial activity and their effects are often compared with those of commercial fungicides [13]. The ionic liquid such as benzotriazolate, tebuconazole and propiconazole salts have been suggested as a new groups of anti-microbial plant protection agents [13,14]. At present research work is conducted mainly on the following two groups of problems: the first - related to the improvement of effectiveness of the formulation comprising adjuvants and the second - related to the search for new antifungal compounds. Another important question is to find out if and if yes - in which way the addition of an adjuvant affects the fungistatic activity of a given fungicide. Although many new chemicals and their antifungal activities have been tested, there is scarcity of information about the effect of an adjuvant on the antifungal activity of fungicides. A new literature reports have suggested that it is important issue and promising subject. *Gillard* and *Ranatunga* have found that the addition of a surfactant to foliar fungicides azoxystrobin has increased the seed yield and permitted return on the investment under high disease pressure, but had no effect when added to the other fungicide – pyraclostrobin [12]. Sulfobetaine surfactants are known as zwitterionic compound that find application in cosmetic industry. There can be found the literature report not only about their surface properties, but also about their antimicrobial activity. There is no literature data concerning the possibility of using sulfobetaines as adjuvants. SB4C14 surfactant is known to show good surface properties, high ability to reduce surface tension and good wetting properties. Its critical micelle concentrations are low and it has low foaming ability [15-17]. The application of sulfobetaine type surfactant as well as the lack of information about the effect of such an adjuvant on the antifungal activity of fungicides have prompted us to undertake this study. Therefore the aim of this study was to determine the activity of sulfobetaine type surfactant on pathogenic fungi as well as the effect of the addition of such a surfactant on the fungicidal activity of selected fungicides.

EXPERIMENTAL SECTION

Surfactants

Two commercially available surfactants and one synthesized by us were chosen for the study. The commercially available surfactants are two nonionic compounds of organosilicon structure: Silwet Gold and Slippa. The surfactant synthesized by us is classified to the group of zwitterionic group. The laboratory synthesis of sulfobetaine: N,N-dimethyl-N-tetradecyl-4-ammonio-1-butanesulfonate (abbreviation-SB4C14) (Fig. 1) was carried out by the method proposed by *Cheng et al.* with some modifications [18]. The resulting crystalline products were separated from the reaction mixture by filtration and further purified by recrystallization. Finally white solids were obtained. The structure of obtained compounds was confirmed by spectroscopic methods, elemental analysis and melting point determination. This sulfobetaine has not been previously applied as an adjuvant.

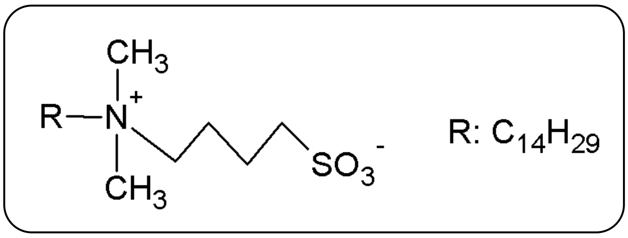

Fig. 1: Structure of N,N-dimethyl-N-tetradecyl-4-ammonio-1-butanesulfonate (SB4C14).

N,N-dimethyl-N-tetradecyl-4-ammonio-1-butanesulfonate (SB4C14)

^{1}H NMR ($CDCl_3$) δ=0.878 (t,3H,CH_3), 1.258 (m,22H,CH_2), 1.349 (m,2H,CH_2), 1.689-1.941 (m,4H,CH_2-CH_2), 2.896 (m,6H,$(CH_3)_2N^+$), 3.263 (m,2H,CH_2N^+), 3.425 (m,2H,$CH_2SO_3^-$),

^{13}C NMR ($CDCl_3$) δ=14.1, 19.7, 19.9, 20.4, 21.0, 21.2, 21,8, 22.6, 23.5, 24.1, 25.5, 26.5, 27.7, 29.5, 31.8, 48.3, 50.1, 54.3, 56.9, 59.9

IR=1035, 1193, 2850, 2919 cm^{-1}

Anal. Calcd: C 63.66, H 11.41, N 3.71, S 8.49; Found: C 63.21, H 11.05, N 3.28, S 8.86.

mp. 262-264°C,

Yield: 96%

Evaluation of antifungal activity of surfactants

Fungistatic activity assay was performed by the plate method, described earlier [13]. To the liquefied and cooled to 50ºC medium, the tested compounds were added at concentrations as above, and then the liquid medium was poured onto Petri dishes (Ø 50 mm). Next, fungal discs of 4 mm in diameter were placed at the center of the plate. Subsequently the plates were incubated at room temperature (about 21°C) until the mycelium reached the edge of the control plate. Then the diameter of the mycelium was measured subtracting the initial diameter of the disc with fungi (4 mm) from the result of measurement. The experiment was performed in two series. Both series were conducted in three replications. The results were subjected to the Student-Newman-Keuls analysis with significance level α = 0,05, and revealed a significant difference between the control and the trials with the addition of tested compounds. ARM (Agriculture Research Manager) computer program was used for statistical analysis.

In order to evaluate the fungistatic activity of the surfactant, fungicide and surfactant-fungicide mixtures,

Treatments	Concentration [ppm]
SB4C14	1; 10; 100
Slippa	1; 10; 100
Silwet Gold	1; 10; 100
Topsin M 500 SC	1; 10; 100
Amistar 250 SC	1; 10; 100
Topsin M 500 SC + SB4C14	1; 10; 100 + 50; 100
Amistar 250 SC + SB4C14	1; 10; 100 + 50; 100
Topsin M 500 SC + Slippa	1; 10; 100 + 50*
Topsin M 500 SC + Silwet Gold	1; 10; 100 + 100*
Amistar 250 SC + Slippa	1; 10; 100 + 50*
Amistar 250 SC + Silwet Gold	1; 10; 100 + 100*

**- concentration 10 x lower than recommended by the label's information*

two fungicides were applied: Amistar 250 SC (chemical class strobilurins - active substance: azoxystrobin) and Topsin M 500 SC (benzimidazoles - active substance: thiophanate-methyl). The comparative analyses were conducted with two nonionic organosilicon surfactants: Silwet Gold and Slippa recommended for compatibility with fungicides used. The compounds were added to the PDA medium (Potato Dextrose Agar, Difco ™) in the concentrations specified in the table below:

Indicator organisms

The following fungi were used as indicator organisms: *Fusarium culmorum* (KZF-5), *Rhizoctonia solani* (KZF-38), *Microdochium nivale* (KZF-7) and *Alternaria brassicae* (BPR-1678). These fungi come from Institute of Plant Protection-NRI collection.

RESULTS AND DISCUSSION

The diameters (F) of inhibition of mycelial growth were measured and the results obtained are presented in Tables 1 and 2. They are shown as the average of two series of measurements. Statistical analysis of the measured diameters revealed a significance of differences between the results of measurements. All the fungicide-surfactant systems were able to reduce the diameter of mycelial growth, and the difference was significant when compare with the control.

It is expected that adjuvants used as additives to fungicides will improve the effectiveness of the latter,

Table 1: The diameters of inhibition of mycelial growth of F. culmorum and R. solani.

Treatments	Diameter [cm]					
	F. culmorum			R. solani		
	1 ppm*	10 ppm*	100 ppm*	1 ppm*	10 ppm*	100 ppm*
Control	4.60a	4.60a	4.60a	4.60a	4.60a	4.60a
SB4C14	4.60a	4.60a	3.71b	4.60a	4.60a	2.22e
Slippa	4.60a	4.04b	1.93f	4.60a	4.48a	0.75h
Silwet Gold	4.60a	3.29d	2.44d	4.60a	2.34d	1.64f
Topsin M 500 SC	4.60a	4.60a	2.69c	4.60a	4.60a	3.59b
Amistar 250 SC	3.90b	3.77c	3.65b	4.60a	4.21a	3.04d
Topsin M 500 SC + SB4C14 (50 ppm)	4.47a	3.38d	1.62g	3.89b	3.76b	3.30c
Topsin M 500 SC + SB4C14 (100 ppm)	2.83c	2.04gh	1.02h	1.51d	2.50d	2.43e
Amistar 250 SC + SB4C14 (50 ppm)	2.20de	2.50e	2.82c	2.47c	3.18c	2.32e
Amistar 250 SC + SB4C14 (100 ppm)	1.58g	1.53i	1.75fg	0.36g	0.14h	0.00j
Topsin M 500 SC + Slippa (50 ppm)	1.82fg	0.99j	0.70i	0.84f	0.60g	0.47i
Topsin M 500 SC + Silwet Gold (100 ppm)	2.45d	2.39ef	1.95f	1.70d	1.64e	1.30g
Amistar 250 SC + Slippa (50 ppm)	2.00ef	1.89h	1.89f	0.38g	0.25h	0.32i
Amistar 250 SC + Silwet Gold (100 ppm)	2.40d	2.21fg	2.14e	1.11e	1.03f	0.95h
$LSD_{P=0.05}$	0.245	0.220	0.162	0.209	0.297	0.262

** Relates to a compound tested individually and the fungicide used in the mixture*

however, they are not expected to show antimicrobial activity themselves. However, the results presented in Tables 1 and 2 imply that individual adjuvants showed antifungal activity. Moreover, this activity of adjuvants can be greater than that of fungicide (e.g. Slippa and Silwet Gold showed higher inhibitory effects to fungal growth than Topsin). Sulfobetaine type surfactant showed low fungistatic activity, lower than that of the adjuvants examined for comparison. On the basis of the experiments conducted for the surfactant and surfactant-fungicide mixture, it was found that SB4C14 exerted different effects on mycelium growth of fungi tested. SB4C14 surfactant, when used alone, showed fungistatic activity when used at the concentration of 100 ppm. At lower concentrations, only a weak interaction of SB4C14 at 10 ppm against *M. nivale* was observed. Of the tested fungi, the least sensitive to the effect of this surfactant was *F. culmorum*. The inhibition of fungal growth by the comparative adjuvants: Slippa and Silwet Gold was stronger than that caused by SB4C14. An exception was found for limiting the growth of *A. brassicae* by surfactant SB4C14, which at a concentration of 100 ppm was more effective than commercially available adjuvants.

The addition of SB4C14 as well as other adjuvants to the fungicides increased their fungistatic activity, which was particularly evident for Topsin M 500 SC. With increasing the dose of an adjuvant added to the fungicide, the antifungal activity of the formulation increased. The greatest sensitivity to the tested compounds, used either alone or in mixture, was found for *M. nivale*.

On the basis of the results obtained, the adjuvant-fungicide system most effective towards the fungi tested was identified. For better visualization of the results, the indicators of fungi inhibition growth can be calculated according to the Abbot Formula (1) [19]:

$$H = \frac{K_0 - F}{K_0} \times 100\% \qquad (1)$$

where:

H – indicator of fungi inhibition growth

Table 2: The diameters of inhibition of mycelial growth of M. nivale and A.brassicae.

Treatments	Diameter [cm]					
	M. nivale			*A. brassicae*		
	1 ppm*	10 ppm*	100 ppm*	1 ppm*	10 ppm*	100 ppm*
Control	4.60a	4.6a	4.6a	4.60a	4.60a	4.60a
SB4C14	4.60a	4.16c	0.2d	4.58a	4.41a	0.67j
Slippa	3.34b	0.63d	0.00e	4.53a	4.41a	0.94i
Silwet Gold	2.37c	0.13f	0.00e	4.60a	3.60b	1.83e
Topsin M 500 SC	4.60a	4.44b	4.07b	4.60a	4.60a	4.36b
Amistar 250 SC	0.00e	0.00g	0.00e	2.45b	1.54de	1.67f
Topsin M 500 SC + SB4C14 (50 ppm)	0.46d	0.36e	0.69c	1.49e	1.26e	2.25d
Topsin M 500 SC + SB4C14 (100 ppm)	0.23e	0.29e	0.26d	1.47e	1.65de	1.51fg
Amistar 250 SC + SB4C14 (50 ppm)	0.00e	0.00g	0.00e	1.80d	1.67de	1.49g
Amistar 250 SC + SB4C14 (100 ppm)	0.00e	0.00g	0.00e	1.47e	0.38f	1.10h
Topsin M 500 SC + Slippa (50 ppm)	0.00e	0.00g	0.00e	2.48b	2.40c	2.17d
Topsin M 500 SC + Silwet Gold (100 ppm)	0.00e	0.00g	0.00e	2.57b	2.54c	2.39c
Amistar 250 SC + Slippa (50 ppm)	0.00e	0.00g	0.00e	1.83d	1.86d	1.63fg
Amistar 250 SC + Silwet Gold (100 ppm)	0.00e	0.00g	0.00e	2.09c	1.82d	1.53fg
$LSD_{P=0.05}$	0.156	0.078	0.103	0.189	0.376	0.123

** Relates to a compound tested individually and the fungicide used in the mixture*

K_0 – the diameter of colonies on the control plate

F – the diameter of colonies on the sample plate

From this equation it can be easily ascertained which fungicide-surfactant system is the most effective. The order of surfactants added to Topsin according to the increasing effect on its activity against *F. culmorum* was as follows Slippa < Silwet Gold < SB4C14, whereas their order according to the increasing effect of Amistar activity against the same fungi was as follows SB4C14 < Slippa < Silwet Gold. For all fungi as well as concentration establishment of such an ordering can be the simplest method for evaluation of the efficacy of the system. However these results do not show the increase in fungistatic activity of fungicide system caused by the addition of surfactant. The absolute increment, defined as the difference between the size of the phenomenon in a position considered, and the magnitude of this phenomenon in the ground state seem to be the simplest and accurate method for measurement of the dynamics of changes in the antifungal activity. Therefore it can be easily calculated from Tables 1 and 2 in appointed values. Although it must be emphasized that the fungistatic activity of the surfactant-fungicide system is not the sum of the individual activities of the adjuvant and fungicide. Therefore it has been suggested to define two dimensionless indexes according to the Equations (2) and (3).

$$I_1 = \frac{H_m}{H_f} \quad (2)$$

$$I_2 = \frac{H_m - H_s}{H_f} \quad (3)$$

Where:

H_m – indicator of fungi inhibition growth caused by the mixture (surfactant + fungicide)

H_s – indicator of fungi inhibition growth caused by the surfactant

H_f – indicator of fungi inhibition growth caused by the fungicide

The first index I_1 is the relative increment and shows the effect of both surfactant and fungicide on the fungistatic activity of the surfactant-fungicide mixture. It can be said that it shows the increase in activity against certain fungi with respect to the antifungal activity of a given fungicide used alone. The information inferred from the value of I_1 is very valuable and indicates the practical possibilities of using surfactant-fungicide mixtures in plant protection. As mentioned above, the fungistatic activity of the mixture is not a sum of the individual activities of the mixture components. That is why it is reasonable to introduce index I_2 that shows only the effect of surfactant on the fungistatic activity of surfactant-fungicide mixture. Therefore in the numerator of the formula defining I_2 the indicator of fungi inhibition growth caused by the surfactant (H_s) is subtracted. It is also important to notice that instead of H_m, H_s and H_f in both equations can the raw measurements in 'cm' be applied, i.e. $(K_0–F_m)$, $(K_0–F_s)$, $(K_0–F_f)$, respectively. The values of both indexes calculated using raw measurements or indicators will be identical.

The mathematical limitation is that in some cases the equation is logically contradictory. Therefore the indexes can be calculated only if the denominator takes a value greater than zero. Another assumption made is that, if the result of subtraction in the numerator is negative, the index I_2 is equal to 0, which indicates that the surfactant has no influence on the activity of the surfactant-fungicide mixture. Tables 3 and 4 shows the indexes obtained for the surfactant-fungicide systems studied.

As it can be seen in Tab. 3, the activity against fungi depends on fungicide, surfactant and their concentration. According to the I_1 index values, the surfactant that effects the most the fungicidal activity of Topsin is Slippa. It causes a twice (2.02) increase in the antifungal properties of this fungicide. The effect of SB4C14 on the Topsin's activity was lower than of commercial adjuvants, but still high. Analysis of the values of indexes characterizing Amistar fungicide revealed that in all concentrations of this fungicide the most efficacious was the newly proposed SB4C14 and the Amistar-SB4C14 system was the most effective against all fungi examined.

Index I_2 more precisely than I_1 illustrates the effect of the surfactant on the activity against the fungi studied. Results of calculations presented in Table 4 change the inference about the impact of the surfactant on the efficiency of inhibition of mycelial growth. On the basis of I_1 index, the most effective adjuvant in the system with Topsin fungicide was Slippa. However analysis of the fungistatic activity of a given surfactant proved that that the most effective mixture was that with SB4C14. As follows from analysis of I_2 index values obtained for Amistar fungicide, SB4C14 surfactant was the most effective against all fungi examined and in all concentrations applied, e.g. SB4C14 at the concentration 100ppm increased the activity of 1ppm Amistar fungicide against *F. culmorum* over 4-times. When establishing the optimal concentrations of SB4C14 within the Amistar mixture it was especially active at the concentration of 100 ppm. Nevertheless the lower concentration of sulfobetaine yielded also satisfactory results, comparable to that of addition of Slippa.

According to I_2 index, the surfactant-fungicide mixtures most effective against *A. brassicae* was SB4C14 with any of the fungicides used, while the inhibition of *F. culmorum* and *R. solani* growth was most efficiently inhibited by Amistar 250 SC + SB4C14 (100 ppm). The ranking of surfactants according to the increasing values of I_2 index characterizing the activities of Amistar and Topsin against all fungi was as follows SB4C14 < Slippa < Silwet Gold. This ordering is much different than that obtained on the basis of percentage of mycelial growth inhibition (H). Considering the concentration of fungicide in the mixture applied, it can be noted that indicator of fungi inhibition growth assigned to the fungicides increased with the increasing concentrations. A completely different situation can be observed for the surfactant-fungicide mixtures for which the percentage of mycelial growth inhibition was comparable for all concentrations and is highly dependent on the type of fungi. For such analysis the proposed indexes are very useful because a comparison of fungi inhibition growth shown only final results, giving no information on the influence of individual surfactant on the activity of adjuvant-fungicide mixture at different concentrations. Analysis of the indexes also showed that the effectiveness of the adjuvant addition on the fungistatic activity of a given fungicide decreased with the increasing concentration. It is strongly associated with the fact that the ability to inhibit mycelial growth increase with increasing fungicide concentration. That is why the most pronounced increase in activity against fungi is observed at the lowest concentration of fungicide.

Table 3: Index I_1 values for all tested fungi and surfactant-fungicide systems studied.

Treatments	F. culmorum			R. solani		M. nivale		A. brassicae		
	1 ppm*	10 ppm*	100 ppm*	10 ppm*	100 ppm*	10 ppm*	100 ppm*	1 ppm*	10 ppm*	100 ppm*
Topsin M 500 SC + SB4C14 (50 ppm)			1.55		1.27	30.67	7.08			10.20
Topsin M 500 SC + SB4C14 (100 ppm)			1.86		2.14	31.33	7.83			13.40
Topsin M 500 SC + Slippa (50 ppm)			2.02		4.09	33.33	8.33			10.60
Topsin M 500 SC + Silwet Gold (100 ppm)			1.38		3.27	33.33	8.33			9.60
Amistar 250 SC + SB4C14 (50 ppm)	3.47	2.56	1.86	3.44	1.47	1.00	1.00	1.30	0.97	1.06
Amistar 250 SC + SB4C14 (100 ppm)	4.40	3.72	2.95	10.78	2.94	1.00	1.00	1.45	1.39	1.19
Amistar 250 SC + Slippa (50 ppm)	3.80	2.61	0.05	10.56	2.74	1.00	1.00	1.28	0.91	1.02
Amistar 250 SC + Silwet Gold (100 ppm)	3.20	2.89	2.57	8.67	2.32	1.00	1.00	1.17	0.91	1.05

** Relates to a compound tested individually and the fungicide used in the mixture*

Table 4: Index I_2 values for all tested fungi and surfactant-fungicide systems studied.

Treatments	F. culmorum			R. solani		M. nivale			A. brassicae	
	1 ppm*	10 ppm*	100 ppm*	10 ppm*	100 ppm*	1 ppm*	10 ppm*	100 ppm*	1 ppm*	10 ppm*
Topsin M 500 SC + SB4C14 (50 ppm)			1.10		0.00		27.33			
Topsin M 500 SC + SB4C14 (100 ppm)			1.40		0.00		28.00			
Topsin M 500 SC + Slippa (50 ppm)			0.64		0.27		4.67			
Topsin M 500 SC + Silwet Gold (100 ppm)			0.26		0.36		1.00			
Amistar 250 SC + SB4C14 (50 ppm)	3.47	2.56	0.95	3.44	0.00	1.00	0.90	0.04	1.28	0.91
Amistar 250 SC + SB4C14 (100 ppm)	4.40	3.72	2.05	10.78	1.41	1.00	0.90	0.04	1.43	1.33
Amistar 250 SC + Slippa (50 ppm)	3.80	2.61	0.05	10.22	0.26	0.72	0.14		1.26	0.85
Amistar 250 SC + Silwet Gold (100 ppm)	3.20	1.33	0.33	3.22	0.44	0.51	0.03		1.15	0.58

** Relates to a compound tested individually and the fungicide used in the mixture*

All results obtained in our study showed that the addition of an adjuvant, especially SB4C14, to a fungicide improved its antifungal activity, but there are reports claiming the opposite observations for other surfactants. *Khan* [20] has investigated many commercial adjuvants and their role in improving fungistatic activity of fungicides against *Cercospora beticola* that occurred in sugar beet production. They concluded that the adjuvants in combination with pyraclostrobin did not result in a significant improvement in disease control, yield, and quality when compared to the effect of pyraclostrobin applied alone [20]. Absolutely different observations have been made by *Gent et al.* [21]. These authors have reported that the addition of an appropriate adjuvant to foliar improving the effects of pesticide formulations, fungicide can significantly improve the coverage, absorption, and efficacy of the preparation applied. In laboratory and field studies they have evaluated the coverage, absorption, and efficacy of commercial adjuvants with diverse chemistries on diversity of host-pathogen systems. Organosilicon-based adjuvants have been reported to improve the coverage by 26 to 38% [21]. On the basis of experiments and literature it must be emphasized that the most important issue it to choose the appropriate surfactant to be added to a particular fungicide. As it was concluded by us and some other authors this choice is highly specific.

CONCLUSIONS

Although adjuvants have been established as agents by increasing efficacy of foliar transportations of active compounds, it seems important to find out if and if yes, in which way they can affect the activity of fungicides. As can be concluded on the basis of the experiments and calculations, the addition of a surfactant to a fungicide enhances the antifungal properties of the mixture, however the force of impact depends on the type of surfactant, fungicide, their concentration and fungi which is to be eliminated. So, the activity of surfactant-fungicide mixtures is very individual and specific. All fungi considered were found to be the most resistant to SB4C14 from all the adjuvants examined, however, this resistance was not so obvious in the results obtained for the mixtures. SB4C14 surfactant showed slight fungistatic activity against all fungi tested, but mycelial growth inhibition rate depended on the concentration and the fungus species. Addition of SB4C14 increased antifungal activity of fungicides and nominally this activity was the greatest in combination with Amistar. By comparing the percentage of mycelial growth inhibition of SB4C14 with those of the commercial adjuvants it can be noted that the tested compound proved to increase the antifungal activity against *A. brassicae* in all analyzed concentrations. *A. brassicae* was the most resistant fungi and the use of adjuvants: Slippa and Silwet Gold of Amistar formulation had no effect on improving performance of the fungicide, and in some concentrations, even worsen them.

Our experiment has shown that sulfobetaine increases the fungistatic activity of the fungicides based on strobilurins and benzimidazoles. These arguments lead to conclusions that SB4C14 surfactant after further study, could potentially find practical application for protection of plants as an addition to the fungicides.

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REFERENCES

[1] Rinella J.V., Workman R.F., Hermodson M.A., White J.L., Hem S.L., Elutability of Proteins from Aluminum-Containing Vaccine Adjuvants by Treatment with Surfactants, *Journal of Colloid and Interface Science*, **197**:48-56 (1998).

[2] Heremans B., Isebaert S., Verhorven R., Ryckaert B., Spanoghe P., Steurbaut W., Haesaert G., "Effect of Adjuvants on the Efficiency of Dimethomorph Plus Mancozeb (Acrobat 2 Kg/Ha) on the Control of Late Blight In Potato", *Presented at the Proceedings of the Tenth Workshop of an European Network for Development of an Integrated Control Strategy of Potato Late Blight*, Bologna (Italy), 269-274 (2008).

[3] Van Zyl S.A., Brink J.C., Calitz F.J., Coertze S., Fourie P.H., The Use of Adjuvants to Improve Spray Deposition and *Botrytis Cinerea* Control on Chardonnay Grapevine Leaves, *Crop Protection*, **29**: 58-67 (2010).

[4] Gimenes M.J., Zhu H., Raetano C.G., Oliveira R.B., Dispersion and Evaporation of Droplets Amended with Adjuvants on Soybeans, *Crop Protection*, **44**: 84-90 (2013).

[5] Van Zyl S.A., Brink J.C., Calitz F.J., Fourie P.H., Effects of Adjuvants on Deposition Efficiency of Fenhexamid Sprays Applied to Chardonnay Grapevine Foliage, *Crop Protection*, **29**: 843-852 (2010).

[6] Karolewski Z., Wachowiak M., Ratajkiewicz H., Kierzek R., Effect of Adjuvants, Spray Volume and Nozzle Type on Metconazole Activity Against *Leptosphaeria Biglobosa* and *L. Maculans* During Late Spring Treatments in Winter Oilseed Rape, *Journal of Plant Protection Research*, **49**: 113-118 (2009).

[7] Nganje I.B., Bangsund D.A., Leistritz F.L., Wilson W.W., Tiapo NM.," Estimating the Economic Impact of Crop Disease: the Case of *Fusarium* Head Blight in U.S. Wheat and Barley", In: *National Fusariul Head Blight Forum Proceedings*, East Lansing, Michigan State University, 275-281 (2002).

[8] Parry DW., Jenkinson P., Mcleod L., *Fusarium* Ear Blight (Scab) in Small Grain Cereals-A Review, *Plant Pathology*, 44: 207-238 (1995).

[9] Pereyra S.A., Dill-Macky R., Colonization of the Residues of Diverse Plant Species By *Gibberella Zeae* and Their Contribution to *Fusarium* Head Blight Inoculum, *Plant Disease*, **92**: 800-807 (2008).

[10] Trail F., Xu J.R., San Miguel P., Halgren R.G., Kistler HC., Analysis of Expressed Sequence Tags from *Gibberella Zeae* (Anamorph *Fusarium Graminearum*), *Fungal Genetics and Biology*, **38**: 187-197 (2003).

[11] Chahal G.S., Jordan D.L., Shew B.B., Brandenburg R.L., Burton J.D., Danehower D., York A.C., Interactions of Agrochemicals Applied to Peanut, Part 2: Effects on Fungicides, *Crop Protection*, **41**: 143-149 (2012).

[12] Gillard C.L., Ranatunga N.K., Interaction Between Seed Treatments, Surfactants and Foliar Fungicides on Controlling Dry Bean Anthracnose (*Colletotrichum Lindemuthianum*), *Crop Protection*, **45**: 22-28 (2013).

[13] Walkiewicz F., Materna K., Kropacz A., Michalczyk A., Gwiazdowski R., Praczyk T., Pernak J., Multifunctional Long-Alkyl-Chain Quaternary Ammonium Azolate Based Ionic Liquids, *New Journal of Chemisty*, **34**: 2281-2289 (2010).

[14] Markiewicz B., Łęgosz B., Kordala-Markiewicz R., Wakiewicz F., Gwiazdowski R., Kubiak K., Praczyk T., Tebuconazole and Propiconazole Salts, *Przemysł Chemiczny*, **92**: 1636-1639 (2013).

[15] Qu G., Cheng J., Wei J., Yu T., Ding W., Luan H., Synthesis, Characterization and Surface Properties of Series Sulfobetaine Surfactants, *Journal of Surfactants and Detergents*, **14**: 31-35 (2011).

[16] Wieczorek D., Staszak K., Surface Activity of Selected Sulfobetaines, *Polish Journal of Commodity Science*, **36**: 122-130 (2013).

[17] Wieczorek D., Gwiazdowska D., Staszak K., Chen Y., Shen T., Surface and Antimicrobial Activity of Sulfobetaines, *Journal of Surfactants and Detergents*, **19**: 813–822 (2016).

[18] Cheng C., Qu G., Wei J., Yu T., Ding W., Thermodynamics of Micellization of Sulfobetaine Surfactants in Aqueous Solution, *Journal of Surfactants and Detergents*, **15**: 757-763 (2012).

[19] Glen K., Boligłowa E., Evaluation of the Fungistatic Activity of Plant Extracts *In Vitro* Tests, *Journal of Research and Applications in Agricultural Engineering*, **57**: 104-109 (2012).

[20] Khan M.F.R., Khan G.R.J., Harikrishnan R., Nelson R., Bradley C.A., Effect of Adjuvants on the Performance of Pyraclostrobin for Controlling Cercospora Leaf Spot on Sugarbeet, *Journal of Sugar Beet Research*, **44**: 71-81 (2007).

[21] Gent D.H., Schwartz H.F., Nissen S.J., Effect of Commercial Adjuvants on Vegetable Crop Fungicide Coverage, Absorption, and Efficacy, *Plant Disease*, **87**: 591-597 (2003).