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TOWARDS RESEARCH ON ENTOMOPATHOGENIC FUNGI SPRAY QUALITY DEPOSITON AND THEIR EFFICACY WITH THE USE OF NATURAL DYES

Summary

The studies evaluated the usefulness of concentrated beetroot juice, chokeberry and beetroot powder as potential tracers of spray deposits as well as their impact on the ability of conidia germination of Isaria fumosorosea and Lecanicillium lecanii. Aqueous solutions of beetroot and chokeberry products affected the physical properties of the spray liquid. The chokeberry juice concentrate at concentration of 2% did not reduced significantly the static surface tension and can be useful as tracer in studies on the quality of spraying. Unfortunately, the chokeberry juice concentrate can be low compatible in mixtures with some adjuvants. Beetroot products lowered significantly static surface tension. Beetroot juice concentrate at a concentration of 2–4% allowed to create stable mixtures with adjuvants and did not influence significantly spreading of droplet. Conidia germination capacity of entomopathogenic fungi was not significantly reduced in solutions of juice concentrates or adjuvants. The mixture of adjuvant and juice concentrate raised the risk of a reduction in ability of conidia germination.
Key words: *spray deposition tracer, surface tension, droplet spreading, conidia germination*

W KIERUNKU BADAŃ NAD JAKOŚCIĄ OPRYSKIWANIA I SKUTECZNOŚCIĄ DZIAŁANIA GRZYBÓW ENTOMOPATOGENICZNYCH Z UŻYCIEM NATURALNYCH BARWNIKÓW

Streszczenie

W badaniach oceniono przydatność koncentratu soku z buraka ćwikłowego i aronii oraz proszku buraka ćwikłowego jako potencjalnych wskaźników jakości opryskiwania jak również ich wpływ na zdolność kiełkowania zarodników konidialnych Isaria fumosorosea i Lecanicillium lecanii. Wodne roztwory produktów z buraka ćwikłowego i aronii wpływały na właściwości fizyczne cieczy. Koncentrat soku z aronii w stężeniu 2% nie obniżał znacząco statycznego napięcia powierzchniowego i może pełnić rolę znacznika w badaniach nad jakością opryskiwania. Koncentrat soku z aronii może być jednak niekompatybilny w mieszaninach z niektórymi adiuwantami. Produkty z buraka ćwikłowego obniżały znacząco statyczne napięcie powierzchniowe. Koncentrat soku z buraka ćwikłowego w stężeniu 2–4% umożliwiał uzyskanie stabilnych mieszanin z adiuwantami i nie wpływał znacząco na rozlanie kropli. Zdolność kiełkowania zarodników konidialnych grzybów entomopatogenicznych nie ulegała znaczącemu obniżeniu w roztworach koncentratów soków ani adiuwantów. Połączenie adiuwanta i koncentratu soku podnosiło ryzyko obniżenia zdolności kiełkowania zarodników konidialnych.
Słowa kluczowe*: wskaźnik naniesienia cieczy opryskowej, napięcie powierzchniowe, rozlanie kropli, kiełkowanie zarodników konidialnych*

1. Introduction

The development of safer plant protection products for human and the environment results in an extension of the range of microbiological preparations containing bacteria, fungi, fungus-like organisms and viruses. The precision of application is an important factor in their effectiveness. When entomopathogenic fungi are applied by spraying of foliage, the biological activity is primarily dependent on direct contact between the fungus and pest. During the assessment of the spray deposits on the plant, standard dyes and fluorescent indicators of synthetic origin are used. Standardization of concentrates and plant extracts enables wider application in the economy due to the human health safety and high uniformity of the product [2]. Beetroot (*Beta vulagris* L. subsp. *vulgaris*) and chokeberry (*Aronia melanocarpa* (Michx.) *Elliott*) can be a good source of dyes of natural origin. The color of beetroot juice is determined by the presence of the substances from the group of betalains, it is purplish red betacyanins and yellow betaxanthins [4]. The color of solutions of chokeberry juice depends on the presence of anthocyanins [1]. Quantitative determination of pigments in beetroot juice and chokeberry is carried out by spectrophotometry [9, 13]. From the point of view of the usefulness of the dye as a spray deposits tracer, it is important to recognize its effects on the physical properties of the spray liquid. The linking this information with the knowledge of the influence of the substance on the biological activity of microorganisms might help in the selection of the most suitable tracer of natural origin. The aim of the study was to evaluate the juice concentrates of beetroot and chokeberry and beetroot powder as tracers of spray deposit and their effects on the germination ability of *Isaria fumosorosea* and *Lecanicillium lecanii* conidia.

2. Materials and methods

In the study the concentrated juices of beetroot and chokeberry (Vinkon, Poland) and beetroot powder (Hortimex, Poland) in different concentrations were used. Safranine O, and sodium fluorescein were comparative substanc-

es. Tests were carried out on two species of entomopathogenic fungi: *Isaria fumosorosea* isolate Le- Mroz and *Lecanicillium lecanii* isolate derived from Bialowieża, which were obtained from the collection of the Department of Biological Methods and Quarantine of Plant Protection Institute –National Research Institute in Poznan. The experimental preparation ZW 64 at 0.5% of a proprietary composition and Ludwik (a mixture of coconut oil fatty amidopropylenobetanins less than 5%, coconut oil fatty acid diethanolamide less than 5% oil fatty acid amide.coconut less than 5% of the sodium salt of sulfated ethoxylated (2) C12-C14 alcohol less than 10% aqueous solution of alpha-(C 14-16) olefinosulfonate sodium less than 10%) at a concentration of 0.1% as adjuvants of potential application in conjunction with entomopathogenic fungi were used.

Measurement of surface tension. Static surface tension was measured by tensiometric method. The concentrations of juice and powder were 2, 4, 8 and 16% and reference substances had a concentration of 0,0125, 0,0025, 0,05 and 0,1%.. Experiment was performed in four replicates.

Measurement of droplet spread on the glass. The droplets of the volume of 2µl were applied on microscopic slide and measured after 3 minutes by using microscope with a micrometer eyepiece. The area formula of ellipse was used to describe the droplet spread. The range of concentrations of substances and preparations was the same as during measurement of surface tension. Experiment was performed in five replicates, each measured 4 droplets.

Evaluation of germination ability of conidia. The mixture of spore suspension of the fungus, the dye and adjuvant was incubated for 30 minutes in a test tube and then the droplets of volume of 50µl were put on PDA substrate on Petri dish. The droplets were covered by glass and plates were incubated at 20°C. After 20 hours, the germinated and not germinated spores were counted at 600x magnification in the number of 100 in each of the five replications. Tests were carried out on the one concentration level of the liquid solution in spore suspension.

Statistical analysis. The analysis of variance (ANOVA) and Tukey's (HSD) test were performed at $\alpha = 0.05$. for post-hoc examination. Correlation coefficients between the data were calculated and their significance was verified. The polynomial regression equations between the concentration and static surface tension or droplet spreading were calculated.

3. Results and Discussion

The solutions of chokeberry juice concentrates and beetroot as well as beetroot powder have color close to red, just as safranin O and they are clearly visible on the green tissue of a leaf surface. The sodium fluorescein solution has got the yellow- orange color. The solution of sodium fluorescein excited by blue light emits radiation in the range of 513 to 560 nm [3].

The solutions of plant concentrates that enable obtaining color of intensity similar to safranin O in concentration of 0.1% oscillated at around 8 %. However, the spray droplets were clearly visible on the green leaves at the both concentration of 4 % and 2 %. Visibility of trace of droplet was better in concentrations higher than 2%.

The juices intensity and color tone depend on the ratio of the dyes and conditions of the processing and storage [1, 4]. The color depends on the pH. This parameter could be important for color stability in case of juice concentrate or powder mixture with some adjuvants. The purplish red betacyanins content is less dependent on pH than the yellow betaxanthins, and a purplish–red color is the most stable at pH approx. 5 [1]. pH has a significant impact on the form of anthocyanins and the color of the chokeberry juice. With the increase in the pH the changes in structure and quantity of pigments from flavylium cation, which staining red color to colorless hemiacetal and yellow or colorless chalcone forms are observed [2, 5].

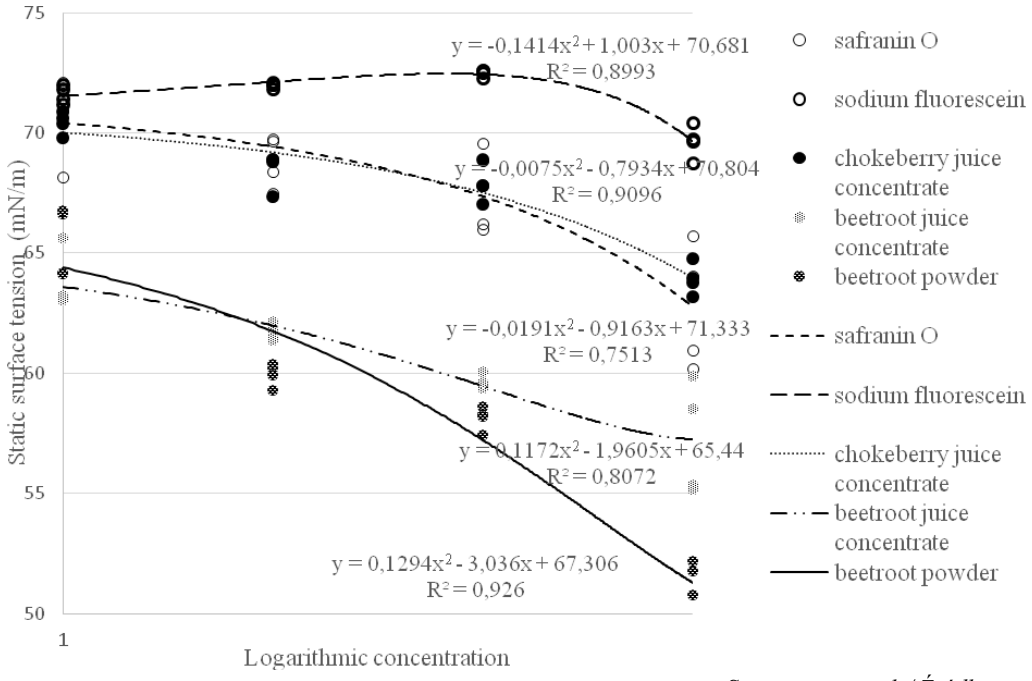
In studies on the suitability of dyes for evaluation of the quality of spraying, the attention is paid to the physical properties of a solution, especially to the surface tension [10]. Our studies showed that the dyes concentration was negatively correlated ($p < 0.05$) to the static surface tension (Fig. 1). R ranged from – 0.72 (sodium fluorescein) trough – 0.87 (safranin O and beetroot concentrate) to – 0.95 (beetroot powder and chokeberry juice concentrate). The solutions of beetroot juice concentrate, beetroot powder and chokeberry juice concentrate at a concentration of 2–16%, 2–16% and 4–16% , respectively, significantly reduced the surface tension in comparison to water alone. The smallest changes caused chokeberry juice concentrate, which just like safranin O decreased surface tension from 2 to 10 units in the tested concentrations. Fortunately, the chokeberry juice concentrate at concentration of 2% did not reduce significantly the surface tension. The sodium fluorescein did not affect significantly the surface tension in the range of 0.01–0.1%.

Little is known about the characteristics of substances used as a tracers in terms of spreading a droplet on the surface. The spreading of the droplet on the glass surface amounted to 6.25 mm^2 and was positively correlated ($p < 0.05$) to the concentration of sodium fluorescein, powder and juice concentrate of beetroot (Fig. 2) and R was of 0.47, 0.4 and 0.25, respectively. However, only the beetroot juice concentrate and beetroot powder at the concentration of 16% and sodium fluorescein at concentration of 0.1% increased significantly the spreading of droplets as compared to water alone. It is interesting to note that as the concentration of dyes increased the highest droplets spreading increase caused sodium fluorescein, although the surface tension did not decrease. The addition of surfactants caused considerable changes in the spreading of droplet (Fig. 3 and 4). The changes were dependent on the dye type.

In case of Ludwik (Fig. 3) spreading of the droplet amounted to 9.13 mm2 and was negatively correlated ($p < 0.05$) to the concentration of beetroot powder (R= – 0.57), safranin O (R= – 0.51) and juice concentrate of beetroot (R= – 0.33) whilst positive correlation only to the of sodium fluorescein (R=0.62) was obtained. However, statistic test showed significant increase of droplet spreading only in case of sodium fluorescein at concentration of 0.05 and 0.1% as compared to Ludwik solution without dye. On the other hand, any of the examined dyes did not cause significant ($p < 0.05$) decrease in droplet spreading of Ludwik solution. Observations showed that the concentrate of chokeberry juice was incompatible with the Ludwik due to flocculation and precipitation. The safranin O was incompatible with the Ludwik, as well. The weakening of these mixtures color was observed. Despite of low droplet spreading only very small precipitation was observed for mixture of Ludwik with beetroot powder. Chokeberry juice contains many different components. Pigments are hydrophilic and sensi-

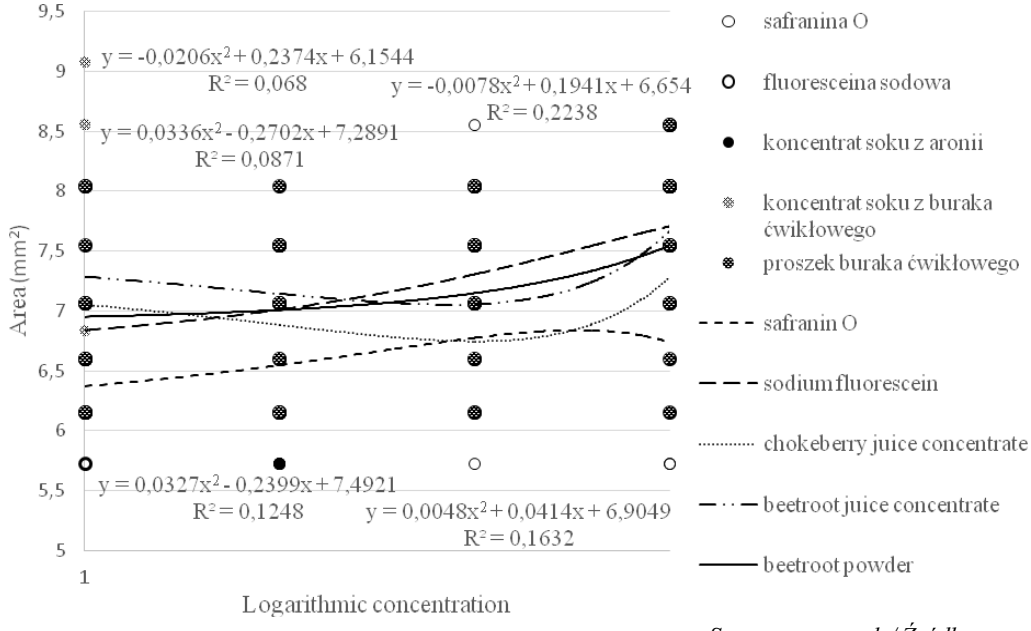
tive to pH [5]. However, some of the Ludwik surfactants have got a relatively low HLB. We suppose that interaction between these components may adversely affect the mixture stability.

In case of ZW 64 (Fig. 4) spreading of the droplets amounted to 15.55 mm^2 and was positively correlated to the concentration (p <0.05) of chokeberry juice concentrate, sodium fluorescein and beetroot juice concentrate and R was 0.48, 0.35 and 0.30, respectively. However, Tukey's test did not show significant increase of droplet spreading. Concentrations of beetroot powder was significantly negatively correlated to the spreading of a droplet (R= – 0.38). The statistic test showed that beetroot powder at all concentrations significantly reduced the surface of droplet spreading (p <0.05). Despite of low spreading of droplet only very small visible changes in this mixture were observed.



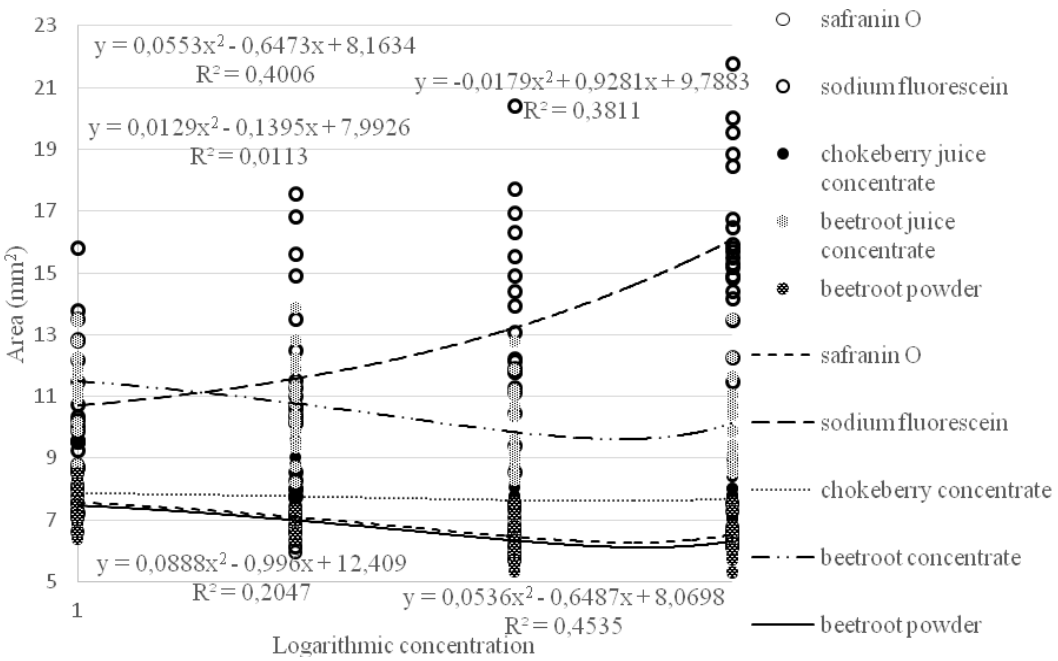
Source: own work / Źródło: opracowanie własne

Fig. 1. The static surface tension of the tested mixtures as a function of concentration
Rys. 1. Statyczne napięcie powierzchniowe badanych mieszanin w funkcji stężenia



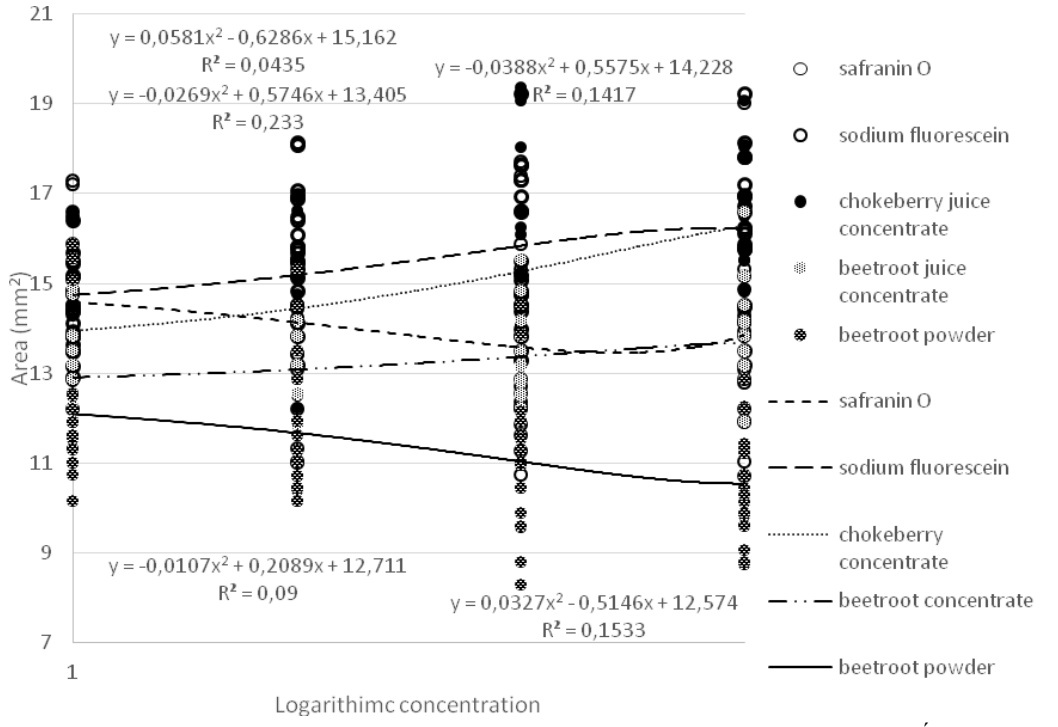
Source: own work / Źródło: opracowanie własne

Fig. 2. The dependence between the droplet spreading and concentrations of the juice concentrates, beetroot powder and reference substances (formula are vertically ordered according to the legend)
Rys. 2. Zależność pomiędzy rozlaniem kropli na szkle a stężeniem koncentratów soku, proszkiem buraka ćwikłowego i substancjami referencyjnymi (kolejność równań w układzie wertykalnym zgodna z legendą)



Source: own work / Źródło: opracowanie własne

Fig. 3. The dependence between the surface of droplet spreading on glass and concentrations of the examined mixtures with Ludwik (formula are vertically ordered according to the legend)
Rys. 3. Zależność pomiędzy rozlaniem kropli na szkle a stężeniem badanych mieszanin z Ludwik (kolejność równań w układzie wertykalnym zgodna z legendą)



Source: own work / Źródło: opracowanie własne

Fig. 4. The dependence between the surface of droplet spreading on glass and concentrations of the examined mixtures with ZW 64 (formula are vertically ordered according to the legend)
Rys. 4. Zależność pomiędzy rozlaniem kropli na szkle a stężeniem badanych mieszanin z ZW 64 (kolejność równań w układzie wertykalnym zgodna z legendą)

The beetroot powder was not taken into account to assess the ability of conidia germination due to the better physical properties obtained with beetroot juice concentrate. Studies on the germination of spores have shown that aqueous solutions of juice concentrates, fluorescein sodium, safranin O, Ludwik and ZW 64 did not decrease the germination of conidia (Tab. 1). However the mixing of adjuvant and dye increased the risk of a reduction of spore germination.

Table 1. The ability of germination of conidia of *L. lecaniii* and *I. fumosorosea* in mixture with various dyes and surfactants
Tab. 1. Zdolność kiełkowania zarodników konidialnych L. lecaniii i I. fumosorosea w mieszaninie z różnymi barwnikami i surfaktantami

Preparation	Colorant	Concentration [%]	*L. lecanii*	*I. fumosorosea*
			germination ability [%]	
Water	safranin O	0.1	91.0c	92.8c
	fluorescein sodium	0.025	92.5c	89.2bc
	chokeberry – juice concentrate	8	87.0bc	81.4abc
	beetroot – juice concentrate	8	89.2bc	79.0abc
	–		91.6c	85.2abc
Ludwik (0.1%)	safranin O	0.1	83.6abc	78.4bc
	fluorescein sodium	0.025	85.0abc	84.4abc
	chokeberry – juice concentrate	8	84.6abc	83.2abc
	beetroot – juice concentrate	8	85.8bc	80.6abc
	–		91.6c	90.4bc
ZW 64 (0.5%)	safranin O	0.1	75.0a	82.2abc
	fluorescein sodium	0.025	83.0abc	84.2abc
	chokeberry – juice concentrate	8	81.6abc	84.8abc
	beetroot – juice concentrate	8	79.4ab	74.8a
	–		87.8bc	91.8bc

*the means designed by the same letter at column do not differ significantly at α=0.05

Source: own work / Źródło: opracowanie własne

Eventually only in mixtures ZW 64 with safranin O and *L. lecanii* as well as ZW 64 with beetroot juice concentrate and *I. fumosorosea* the ability of conidia germination was reduced. The sodium fluorescein and chokeberry juice concentrate in all the analyzed cases did not limit germination of spores. The negative impact of certain mixtures on the germination of fungi indicates on usefulness of this parameter in the case of using other adjuvants, dyes and fungi. It is likely that a significant reduction in the concentration of beetroot juice concentrate or safranin O in mixture with ZW 64 would not reduce germination of tested fungi. The essential condition would be to keep concentrations at least at the level of 0.025% (safranin O) and 2% (beetroot juice concentrate), which could allow for good visualization of spray droplets.

Choosing the right dye for simultaneous studies on the spray quality deposition and effectiveness of the entomopathogenic fungi requires further reflection. Determining the impact of dye on mortality of specific insect species and their stages seems to be valuable. Different studies [6, 8] revealed that certain dyes in mixture with or without the entomopathogenic fungi had a toxic impact on insects. There is no literature data on negative impact on insects of juice concentrates or powders used in the research. The solutions of juice concentrates lowered surface tension of the liquid far less than the surfactants. This premise indicates that the juice concentrates may have a minor influence on the mortality of insects. Many adjuvants, in particular oils and surfactants have got potential insecticidal and acaricidal characteristics [11, 12]. Some oils used in the adjuvants and surfactants are used as physical insecticides. From the agrotechnological point of view, combining entomopathogenic fungi with adjuvants gives tangible benefits and is often practiced. In this case the evaluation of the spray deposits on insects can help drawing conclusions about effectiveness of a mixture ingredients. However potential synergy of action, the dynamics of spray droplets and size of objects under study caused difficulties during deduction. The researchers [7] see synergistic interaction, surfactant can cause destruction of the structure of epicuticular waxes and proteins, contributing to their enzymatic degradation, infection by the fungus and increased mortality of insects. Own observations indicate that an adjuvant can be main cause of mortality, when a single droplet of spray covers the whole insect body. The insecticidal effects of the adjuvant can increase along with the reduction in the size of the object. Partial coverage of the insect by the spray liquid may indicate that mortality was appeared after infection by the fungus. The accurate separation of two mechanisms of insecticidal action coming from mixture of adjuvant and entomopathogenic fungi may ultimately be difficult, if not impossible.

4. Conclusions

Among the studied concentrates of juices and powder cannot indicate the universal products as a model tracer to evaluation of spray deposit. The solution of chokeberry concentrate at the concentration of 2% without addition of adjuvant can be useful as tracer in studies on the quality of spraying with the use of entomopathogenic fungi. Concentrate of beetroot juice at a concentration of 2–4% can be used as tracer in the studies on the quality of spraying with addition of adjuvants.

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