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Micellization and Related Behaviours of Polyoxyethylene-Type Non-ionic Surfactants on Root Elongation of *Allium cepa* L.†

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In this study, polyoxyethylenated fatty alcohol ether (POE) nonionic surfactants polyoxyethylene (23) lauryl ether ($C_{12}E_{23}$) (Brij 35); polyoxyethylene (10) cetyl ether ($C_{16}E_{10}$) (Brij 56); polyoxyethylene (10) stearyl ether ($C_{18}E_{10}$) (Brij 76); polyoxyethylene (10) lauryl ether ($C_{12}E_{10}$) (POE 10). A large proportion nonionic surfactants are made hydrophilic by the presence of a polyethylene glycol chain obtained by the polycondensation of ethylene oxide. They are called polyethoxylated nonionics. In this study the effects of polyoxyethylene-type nonionic surfactants on root elongation of *Allium cepa* L. were determined for a week under laboratory conditions in the concentration ranges of 5.00, 2.50 and 1.25 g/L (w/v) at 16 ± 0.5 °C. It was determined that the stimulatory effect on root elongation becomes stronger when the number of ethylene oxide group (C_2H_4O) was increased in the POE surfactant. The stimulatory effect decreases in the order $C_{18}E_{10} > C_{16}E_{10} > C_{12}E_{23} > C_{12}E_{10}$ due to the decreasing of the number hydrocarbons in the series.

Key Words: Allium cepa, Root, Stimulatory effect, Polyoxyethylene, Non-ionic surfactant.

INTRODUCTION

Surface active agents have an affinity for fats (hydrophobic) and water (hydrophilic) and so act as emulsifiers, e.g. soaps and detergents. Used as wetting agents to assist the reconstitution of powders, including dried foods, to clean and peel fruits and vegetables, also in baked goods and comminuted meat products. Surfactants collected on the phase boundary are able to modify interfacial properties of the liquids in which they are present. High performance detergent should simultaneously play several roles acting as wetting agent, emulsifier, dispersant and supporting laundering agent. The laundering action is the most important application of surface-active compounds and this is the place of their biggest usage. Linear alcohols in C_{12-16} are used to prepare the alkyl-ester-sulfates used as detergents or foaming agents in shampoos, tooth pastes and hand dishwashing products. Ethoxylated alcohols tend to displace ethoxylated alkylphenols, which are fading away because of their toxicity. Alcohols can be made by controlled hydrogenation of natural fatty acids. Non-ionic surfactants come as a close second with about 45 % of the overall industrial production. They do not ionize in aqueous solution, because their hydrophilic group is of a nondissociable type, such as alcohol, phenol, ether, ester or amide.

Non-ionic detergents contain uncharged, hydrophilic head groups that consist of either polyoxyethylene moieties as in BRIJ. In general, non-ionic detergents are better suited for breaking lipid-lipid and lipid-protein interactions than protein-protein interactions. Hence, they are considered nondenaturant and are widely used in the isolation of membrane proteins in their biologically active form. Unlike ionic detergents, salts have minimal effect on the micellar size of the non-ionic detergents.

Detergents with polyoxyethylene head groups may contain alkylpolyethylene ethers with the general formula $C_nH_{2n+1}(OCH_2CH_2)_xOH$, or a phenyl ring between the alkyl chain and the ether group. A large proportion of these nonionic surfactants are made hydrophilic by the presence of a polyethylene glycol chain, obtained by the polycondensation of ethylene oxide. They are called polyethoxylated non-ionics. Ethylene oxide is a very unstable gas, very dangerous to manipulate, because its triangular structure is submitted to extreme tension. PEG, PEO, or POE refers to an oligomer or polymer of ethylene oxide. Polyoxyethylene chains form random coils and are consequently farther removed from the hydrophobic core of the micelles. Detergents with shorter polyoxyethylene chains form aggregates and viscous solutions in water at room temperature, whereas those with longer chains do not aggregate.

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Non-ionics are especially useful because of their low sensitivity to water hardness and pH. Since they are compatible with charged molecules, they are easily used in mixtures with other ionic surfactants, which often result in beneficial associations. For instance, non-ionics can help solubilize calcium or magnesium salts of anionics. Non-ionic surfactants are characterized by particular building. They possess as a hydrophilic moiety groups, which can not dissociate in water, e.g. alcohol or ethereal groups. Washing agents are the mixtures of various ingredients responsible for the good effect. Commercial washing powders including detergents, enzymes, bleaching agents, bleaching activators and optical brighteners. Oily, fatty, carbohydrate and protein stains and soils are removed by the detergent-builder enzyme system included in the washing agent formulation. In the past decade glucoside (sugar based) head groups, have been introduced in the market, because of their low toxicity. As far as the lipophilic group is concerned, it is often of the alkyl or alkylbenzene type, the former coming from fatty acids of natural origin. The polycondensation of propylene oxide produce a polyether which (in oposition to polyethylene oxide) is slightly hydrophobic. This polyether chain is used as the lipophilic group in the so-called polyEOpolyPO block copolymers, which are most often included in a different class, e.g. polymeric surfactants, to be dealt with later. During the past three decades, a large number of non-ionic detergents have become available for the purification of membrane proteins. Among these the polyoxyethylene-octylphenols (NP-40, Triton X-100 and Triton X-114), the polyoxyethylenesorbitans (Tween series detergents), the polyoxyethylenealcohols (Lubrol series) and the polyoxyethylene fatty acid ether (Brij series) detergents are particularly popular. Polymeric surfactants are often not accounted as surfactants and consequently do not appear in statistics, their importance is growing however, because they enter in many formulated products (as dispersants, emulsifiers, foam boosters, viscosity modifiers, etc.). Abiotic and biotic stresses cause alterations in the normal physiological processes of all plant organisms, including the economically important crops.



Fig. 1. Positive effects of ethylene on growing of some fruits

There are many studies about the phytotoxic effects of surfactants on plants¹⁻³. However, in this study mostly stimulatory effects were seen. The aim of this study was to investigate the effects of polyoxyethylene-type non-ionic surfactants on *Allium cepa* L.

EXPERIMENTAL

Polyoxyethylene (23) lauryl ether ($C_{12}E_{23}$) (Brij 35); polyoxyethylene (10) cetyl ether ($C_{16}E_{10}$) (Brij 56); polyoxyethylene (10) stearyl ether ($C_{18}E_{10}$) (Brij 76); polyoxyethylene (10)

lauryl ether (C₁₂E₁₀) (POE 10) Four non-ionic surfactant were supplied from Merck and used without purification. All solutions were prepared with tap water. Allium cepa L was selected as a material for this study. Because it is a good specimen for study and has many primordial (adventitious) root that are growing rapidly. So it has got much absorption surface. All experiments were performed on primordial root of the onion. The onions used in the experiments were prepared as described by Wierzbicka to obtain equal growth of primordial root¹. Twenty-four onions composed an experimental set and four onions within each set were selected for control. At the beginning of the experiment, onions were placed individually in 60 mL vessels containing different surfactants. The temperature of the laboratory was kept at 16 °C (\pm 0.5). Three different surfactants concentrations (5.00 g/L, 2.50 g/L, 1.25 g/L) were prepared from stock solution (5 g surf/1000 mL). The control groups were treated only with tap water. Experiments were replicated 3 times. Root lengths were measured using a millimeter ruler starting at the onset of incubation, then after 1st, 2nd, 3rd, 4th, 5th, 6th and 7th days. At the end of the 7th day, the total root lengths were measured. Means and standard deviations of the length of onion root tips were determined.

Statistical analysis were performed and were given in the results section. A SPSS 19 statistical package was used to run statistical analysis. Conformance of the measurable data to the normal distribution was checked by single sample Kolmogorov Smirnov test. Kruskal-Wallis variance analysis and Mann Whitney U test were used in comparison between the groups not showing normal distribution. Median (min-max) values and arithmetical mean \pm standard deviation were provided as descriptive statistic. Significance limit was specified as (p \leq 0.05) for all statistics. After Kruskal-Wallis variance analysis, in comparison of the results of Mann Whitney U, Benferroni correction was made.

RESULTS AND DISCUSSION

In this study, stimulatory effects were observed all Brij-35, 56, 76 and POE concentrations. The effect of surfactant type and concentrations on *A. cepa* root elongations after 7-days according to various surfactant treatments were given. It was observed that because of ethylene oxide in the surfactants stimulatory effects were determined instead of phytotoxic effects. As it was seen in Table-1 stimulatory effects were seen at all concentrations in doses especially at the dose 5,00 g/L of Brij 56 and Brij 76.

Stimulatory effects were seen in Brij series while phytotoxic effects were seen in Triton series. There are many studies about stimulatory effects of surfactants. However there isn't any study similar to our study. This is an original research about stimulatory effects of BRIJ series. It was found that the toxicity of surfactants is about the number of the ethylene oxide groups and the hydrophobic state of the surfactant⁴. A large proportion of these non-ionic surfactants are made hydrophilic by the presence of a polyethylene glycol chain, obtained by the polycondensation of ethylene oxide. They are called polyethoxylated non-ionics. PEG, PEO or POE refers to an oligomer polymer of ethylene oxide. Ethylene oxide is a hormone

TABLE-1

EACH ARITHMETICAL MEAN VALUE REPRESENTS THE MEAN OF ROOT ELONGATIONS AFTER A WEEK TREATED WITH DIFFERENT FOUR SURFACTANTS WERE GIVEN. (BRIJ 35); (BRIJ 56); (BRIJ 76); (POE 10) (*) INDICATES SIGNIFICANT DIFFERENCES AT THE 5% LEVEL BETWEEN VALUES OBTAINED UNDER CONTROL AND TREATED PLANTS (P ≤ 0.05).

	Root length
Group	Arithmetical mean ± standart
	deviation, median, (min-max)
Control	$13.30 \pm 9.22, 11, (1-55)$
BRIJ 35 (5.00 g/L)	$13.05 \pm 8.89, 12, (1-38)$
BRIJ 35 (2.50 g/L)	(*) 11.41± 10.93, 8, (1-55)
BRIJ 35 (1.25 g/L)	$14.74 \pm 13.35, 11, (1-60)$
BRIJ 56 (5.00 g/L)	(*) 24.04 ± 16.3, 20, (1-67)
BRIJ 56 (2.50 g/L)	(*) 7.31 ± 6.38, 5.5, (1-44)
BRIJ 56 (1.25 g/L)	$12.81 \pm 10.5, 9, (1-49)$
BRIJ 76 (5.00 g/L)	(*) 25.27 ± 17.73, 20, (1-60)
BRIJ 76 (2.50 g/L)	$15.51 \pm 10.33, 15, (1-41)$
BRIJ 76 (1.25 g/L)	(*) 27.43 ± 22.02, 19 (1-73)
POE 10 (5.00 g/L)	$13.91 \pm 10.96, 11, (1-47)$
POE 10 (2.50 g/L)	$15.18 \pm 13.18, 10, (1-53)$
POE 10 (1.25 g/L)	$17.52 \pm 13.19, 15, (1-50)$

in plants that helps to grow plant to be mature. So in this study, it could be effective on the root elongation of *Allium cepa* L positively. Present results are in agree with the following other studies, which have stimulatory effects too. Tween-20 increased the hormonal activity in apple^{5,6} Tween-20, Tween-80 and Surfax 505 increased the growth of leaves and roots in barley⁷; Tween-20, Tween-80 and fatty acid esters increased growth of pea stem sections^{8,9}; polyvinyl alcohol increased

the rate of growth and chlorophyll content in peas¹⁰ and Tween-20 increased growth and nicotine synthesis in tobacco¹¹. Also phytotoxic effects were seen because of non-ionic Triton surfactant series on *Allium cepa* L.¹².

Future investigation in this area will increase the knowledge about the reaction mechanisms of plants imposed to different unfavourable conditions. Additionally, new approaches to the problem of induction of a cross-adaptation or synergism will be revealed and the specificity of the multiple-stress interaction will be better elucidated. Such information will open new possibilities for a deliberate and predictable approach for improvement of the adaptation capacity of plant organisms.

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