Modern Environmental Science and Engineering (ISSN 2333-2581)
May 2018, Volume 4, No. 5, pp. 421-426
Doi: 10.15341/mese(2333-2581)/05.04.2018/003
Academic Star Publishing Company, 2018
www.academicstar.us

The Effect of Dialysis Purification of Humic Substances Solutions on Mitotic Index

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Abstract: The biological activity of neutralized pyrophosphate solutions of humic substances and the same solutions that were purified by dialysis was compared. It was found that neutralized pyrophosphate solutions of humic substances (0.001 mg C per 1 ml) and their dialyzed solutions much increased the mitotic index in the apical root cells and onions (*Allium cepa* L.) and peas (*Pisum sativum* L.). Dialysis of neutralized pyrophosphate solutions of humic substances had no significant effect on the size of the mitotic index of the apical root cells of both plants.

Key words: humic substances, mitotic index, purification by dialysis, *Allium cepa* L., *Pisum sativum* L.

1. Introduction

Humic substances (HS) are dark-colored natural amphiphilic high-molecular organic nitrogen-containing randomized redox heteropolymers of aryl glycoprotein nature, which are multifunctional polyampholytes. Biological activity of HS is one of their important properties, which is determined by the versatile properties of specific compounds and is determined by the presence of a variety of functional groups, colloidal properties and biochemical composition [17]. The biological activity of HS is most often assessed on the basis of the determination of the germination of seeds, the length of stems and roots, and also the weight of plant seedlings [2, 6, 15, 22, 25, 27]. However, these methods require considerable time and strict standardization of the conditions for growing plant seedlings. One of the fastest and most reproducible methods for biotesting physiologically active substances is to determine the value of the mitotic index (MI) of cells of root apex of plants [3, 4, 9].

The purpose of this publication is to show the effect of HS, with later dialysis and without it, on the mitotic index of cells of root apexes of onion (*Allium cepa* L.) and pea (*Pisum sativum* L.).

2. Material and Methods

The HS solutions as an object of a research were used. They were isolated from the humus horizons: AU1 (0-17 cm) and AU2 (17-50 cm) of virgin Chernic Chernozems (the State Nature Reserve “Belogorye”, Belgorod region), PU1 (0-20 cm) and PU2 (20-40 cm) of arable Chernic Chernozem (Belgorod region), [AU] (0-26 cm) of buried Chernozem (Scythian fortification, urban-type settlement of Borisovka, Belgorod region), AU (0-15 cm) and AEL (15-45 cm) of Greyic

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Phaeozems (the State Nature Reserve "Belogorye", Belgorod region).

The biological activity of HS solutions was assessed by the value of the mitotic index of the root apical cells of both onion (*Allium cepa* L.) and pea (*Pisum sativum* L.). Humic substances from the soils were isolated by alkaline pyrophosphate solutions [13], after which the solutions were neutralized with sulfuric acid (up to pH ~ 7). One part of the solutions remained unchanged, the second part of the solutions before the test was purified from mineral salts by dialysis through a cellophane membrane. The resulting HS solutions were diluted with distilled water to a carbon concentration of 0.001%. In the experiments, the root apes of the seedlings were treated with neutralized pyrophosphate HS solutions and dialyzed pyrophosphate ones for 24 hours. To find the mitotic activity in the cells of the apical meristem of *Pisum sativum* L. pea, its seeds were germinated in Petri dishes in distilled water for 3 days. Before germination, all seeds and vessels were sterilized with an aqueous solution of $KMnO_4$. Bulbs (*Allium cepa* L.) were germinated in test tubes filled with distilled water, also for 3 days. The root apex of pea and onion were treated for 24 hours with both neutralized pyrophosphate HS solutions and dialyzed ones. Control was the roots of onion and pea grown on diluted neutralized pyrophosphate solution before and after dialysis, respectively. For each variant of the experiment, not less than 1500 cells were analyzed. The mitotic index (MI) was determined by the standard method, and its value was calculated by the formula [20]:

$$MI = \frac{\Sigma(P+M+A+T)}{\Sigma(I+P+M+A+T)} \times 1000$$

where *I*: interphase, *P*: prophase, *M*: metaphase, *A*: anaphase, *T*: telophase.

All obtained experimental data were mathematically processed using variational statistics [19]. Repeat was fivefold. Estimation of the significance of the difference between sample means (between sample means of variants) was carried out using the variance analysis. In addition, the values of the particular and general values of the desirability function of E. Harrington [10] were calculated. The generalized desirability function is one of the most convenient ways of constructing a generalized response [12, 23, 28]. The construction of this generalized function (*D*) is based on the idea of converting the natural values of particular responses (d_i) into a dimensionless scale of desirability or preference.

The biological activity of HS solutions was estimated from the value of the mitotic index (MI) of root apical cells both pea (*Pisum sativum* L.) and onion (*Allium cepa* L.). Humic substances were isolated by alkaline pyrophosphate solutions [13], after which solutions were neutralized (before pH ~ 7) by 50% solution of H_2SO_4. One part of solutions was left without change, the second part of solutions before test was purified from mineral salts by dialysis through a cellophane membrane.

The biological activity of neutralized pyrophosphate HS solutions and the same solutions that were purified by dialysis was compared. In the course of the work, it was found that neutralized pyrophosphate extracts of HS of optimal concentration (0.001 mg C per 1 ml) from all horizons of investigated soils and their dialyzed solutions, much increased the mitotic index in the root apical cells of onion (*Allium cepa* L.), and pea (*Pisum sativum* L.).

3. Results and Discussion

From the experiment it was revealed that the neutralized sodium pyrophosphate HS solutions (before and after dialysis) much increased the MI value of both onion and pea (Tables 1 and 2).

In case of onion (*Allium cepa* L.), HS solutions without dialysis much increased the MI value of the apical root cells relative to control (Table 1). In which connection the MI of the cells of the root apex of onion increased from 10 to 50% (1.1-1.5 times). In all variants HS solutions with dialysis raise the value of

MI of root onion cells compared with the control much (Table 1) too. The MI of root apex cells under the action of dialyzed HS solutions increased from 30 to 220% (by 1.3-3.2 times).

Humic substances of neutralized sodium pyrophosphate solutions without dialysis increased the MI value of apical root pea cells (*Pisum sativum* L.) relative to control (Table 1) as well. In this case the MI of root apex cells grown from 40 to 280% (1.4-3.8 times). The dialysed HS solution (Table 1) in all variants much increased the MI value of root apex pea cells as compared to the control. The mitotic index of root apex cells the by HS action increased from 65 to 290% (1.7-3.9 times).

Table 1 The values of root apex cells mitotic index of onion and pea as a response to HS solutions before and after dialysis.

Objects	Onion		Pea	
	HS solution before dialysis	HS solution after dialysis	HS solution before dialysis	HS solution after dialysis
Control	33	24	27	23
AU1–CC	37	50	103	89
AU2–CC	44	77	43	66
PU1–ACC	51	32	61	65
PU2–ACC	45	51	39	38
[AU]–BC	42	31	48	90
AU–GP	38	47	95	69
AEL–GP	48	52	70	46
$F_{act.}$	3.85	22.76	61.98	48.31
F_{05}	2.32	2.32	2.32	2.32
SED_{05}	3.4	5.0	5.5	6.2
$F_{act.}$	14.34		55.98	
F_{05}	1.80		1.80	
SED_{05}	4.1		5.0	
The influence of factor A (objects)				
$F_{act.}$	17.21		99.88	
F_{05}	2.19		2.19	
SED_{05}	2.9		3.6	
The influence of factor B (dialysis)				
$F_{act.}$	3.77		0.02	
F_{05}	4.01		4.01	
SED_{05}	1.5		1.8	
The combined effect of AB factors				
$F_{act.}$	12.97		20.08	
F_{05}	2.19		2.19	
SED_{05}	1.0		1.3	

Notes: here and below: AU1–CC and AU2–CC — humus horizons of virgin Chernic Chernozem, PU1–ACC and PU2–ACC— humus horizons of arable Chernic Chernozem, [AU]–BC — humus horizon of buried Chernozem, AU–GP and AEL–GP — humus horizon and humus-eluvial one of Greyic Phaeozems; $F_{act.}$— Fisher's actual criterion. F_{05} — Fisher's theoretical criterion at 95% probability level; SED_{05} — the smallest essential difference at 95% probability level.

Using a two-factor analysis of variance, no significant differences were found between the values of the root apex cells MI of both onion and pea (Table 1) as a response to HS solutions before and after dialysis (factor B).

Estimating the biological effect of HS solutions without dialysis, it was found that in most cases the effect of HS neutralized sodium pyrophosphate solution on MI apex cells of pea was higher than that cells of onion (see Table 2). This difference can be

explained by the different structure of the transport system of dicots and monocots [8].

Based on the fact that there were no significant differences between the biological activity of HS solutions before and after dialysis, we calculated particular and generalized Harrington's desirability functions (Table 3). These characterizations indicated that the biological effect of HS, isolated from all the investigated objects, was "very good", while the control — "satisfactory".

Table 2 Influence HS solution before and after dialysis on values of root apex cells mitotic index of onion and pea.

Objects	HS solution before dialysis		HS solution after dialysis	
	Onion	Pea	Onion	Pea
Control	33	27	24	23
AU1–CC	37	103	50	89
AU2–CC	44	43	77	66
PU1–ACC	51	61	32	65
PU2–ACC	45	39	51	38
[AU]–BC	42	48	31	90
AU–GP	38	95	47	69
AEL–GP	48	70	52	46
$F_{act.}$	44.56		44.63	
F_{05}	1.80		1.80	
SED_{05}	4.6		3.3	
The influence of factor A (objects)				
$F_{act.}$	36.60		47.65	
F_{05}	2.19		2.19	
SED_{05}	3.3		3.3	
The influence of factor B (plants)				
$F_{act.}$	129.81		97.69	
F_{05}	4.01		4.01	
SED_{05}	1.6		1.7	
The combined effect of AB factors				
$F_{act.}$	40.34		34.04	
F_{05}	2.19		2.19	
SED_{05}	1.2		1.2	

Table 3 Values of particular and generalized Harrington's desirability functions, calculated on the basis of the mitotic index

Objects	Particular desirability functions				Generalized desirability function, D	Qualitative characteristics of generalized desirability function
	Onion	Pea	Onion	Pea		
	Before dialysis of HS solution		After dialysis of HS solution			
	d_1	d_2	d_3	d_4		
Control	0.62	0.62	0.62	0.62	0.62	Satisfactory
AU1–CC	0.91	1.00	1.00	1.00	0.98	Very good
AU2–CC	0.99	1.00	1.00	1.00	1.00	Very good
PU1–ACC	1.00	1.00	0.98	1.00	0.99	Very good
PU2–ACC	1.00	0.99	1.00	1.00	1.00	Very good
[AU]–BC	0.99	1.00	0.97	1.00	0.99	Very good
AU–GP	0.90	1.00	1.00	1.00	0.97	Very good
AEL–GP	1.00	1.00	1.00	1.00	1.00	Very good

Humic substances by the nature of action on plants are nonspecific growth regulators [16]. The biological effect of HS on the vascular green plants is connected with penetration of these organic substances into plants (but it isn't obligatory in cytoplasm) and with theirs taking part in various biochemical and biophysical processes [17]. The possibility the penetration of large organic molecules in plants through the root system and their further assimilation are well a fact in evidence [1, 5, 7, 11, 14, 17, 21, 24, 26]. As follows from the scientific literature, HS positively affected all phases of the mytotic cycle in 1.5-2.0 times [9], and certain fractions of HS in 8 times [18].

4. Conclusion

Humic substances of neutralized sodium pyrophosphate solutions without and with dialysis increased the mitotic index value of apical root cells of both pea, and onion.

Dialysis of neutralized pyrophosphate extracts of humic substances did not much effect on the mitotic index value of the apical root cells either in the case of onion or in the case of pea.

The mitotic index values of apical root pea cells were larger than those of onions: 1.4-3.9 and 1.1-3.2 times higher than controls, respectively.

Values of generalized Harrington's desirability function indicated that the biological effect of humic substances, isolated from all the investigated objects, was "very good", while the control — "satisfactory".

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