THE EFFECT OF THE ROOT EXTRACT OF WATER HYACINTH (EICHHORNIA SPECIOSA KUNTH), ON THE GROWTH OF MICROORGANISMS AND MASH KALAI (PHASEOLUS MUNGO VAR. ROXBURGHII), AND ON ALCOHOLIC FERMENTATION

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Roots of water hyacinth have been extracted with different solvents such as ethanol and distilled water under different conditions and their influence on the growth of Microorganisms, Mash Kalai and Alcoholic Fermentation has been studied. In all cases, the root extract enhances the growth of microorganisms and Mash Kalai and accelarates the alcoholic fermentation. Analyses of the extract showed that the ogranic substances have no effect and the inorganic constituents are responsible for the activity of enhancement of growth.

Introduction

Earlier workers [1-4] have reported that the water extract of the root of water hyacinth enhances the growth of the shoots and roots of certain plants. Sarcar and Kundu[2,3] identified aspertic acid, glutamic acid, arginine, cystine, tyrosine, glycine, amino-iso-butyric acid, lysine, valine and theonine in this extract but found that the mixture of these amino acids, gibberellic acid, and indole-3-acetic acid in water hyacinth, were not responsible for the enhancement of growth. They therefore attributed this activity to some unknown constituent. The effect of the Root Extract of Water Hyacinth (*Eichhornia speciosa* Kunth) on the growth of microorganisms and mash kalai (*Phaseolus mungo* var. *Roxburghii*) and on Alcoholic Fermentation has been studied at these laboratories and the results are presented in this paper.

Experimental

Extractions.—The fresh fleshy roots, collected from local sources, were cut into thin slices and extracted with different solvents, filtered and distilled under reduced pressure at 40°C. according to the following procedures:—

1. *Extraction with Distilled Water*: (a) At lower temperature: 20 g. of roots were extracted with 100 ml. of water for 40 hours. The filtrate (Soln. No. 1) was evaporated and 1% solution of the solid substance was prepared with sterile distilled water (Soln. No. 2). (b) At room temperature: The method was same as in (a). The filtrate was designated as solution No. 3 and the 1% solution of solid substance as solution No. 4. (c) At higher temperature: 20 g. of roots were treated with 100 ml. of water and autoclaved under 30 lbs. pressure/sq. inch for two hours. The filtrate was designated as solution No. 5 and the 1% solution of the solid substance as solution No. 6. (d) After oven drying: 20 g. of oven dried (100°C.) root of water hyacinth was extracted with 100 ml. of water for 40 hours at room temperature. The filtrate was designated as solution No. 7 and the 1% solution of the solid substance as solution No. 8.

2. *Extraction with Methyl Alcohol*: (a) At lower temperature: 20 g. of roots were extracted with 100 ml. of methanol at 4°C. for 40 hours. The solvent was removed and 1% solution of the solid substance left was prepared and this designated as solution No. 9. (b) At room temperature: The experiment 2(a) was repeated at room temperature (30-32°C.) and the 1% solution of the solid substance thus obtained, was designated as solution No. 10. (c) After oven drying: 20 g. of the oven dried roots were extracted with 100 ml. of methanol for 40 hours at room temperature. 1% solution of the solid was prepared and designated as (Soln. No. 11).

3. *Extraction with Ethyl Alcohol:* (a) At low temperature: Method was the same as in case of methyl alcohol (a) and the 1% solution of the solid was designated as (Solution No. 12). (b) At room temperature: The above experiment was repeated at room temperature and the 1% solution of the solid substance was designated as solution No. 13.

Bioassay.—The strain of *Aspergillus niger* No. 21* was used as the test organism for bioassay on the medium No. 1 in test tubes of uniform size, having the dimensions 16×1.7 cms. 5 ml. of the medium No. 1 was used in each test tube and one ml. of the each test solution was used in each experiment. In control experiment 1 ml. of distilled (sterile) water was used in place of test solution. These tubes were then inoculated with equal quantities of the spores of the test organism and

* These organisms were isolated in these laboratories from local sources.

incubated at 30-31°C. for 42 hours, and then the results were recorded. The growth of the organism, was determined on dry weight of the mycelium and results on sporulation were evaluated by visual observation. The data is presented in Table 1. In control experiment, the growth of the mycelium was considered as 100%.

volume adjusted to 20 ml. Solution No. 19: The pH of the 20 ml. portion of the solution No. 14 was adjusted to 2.0 with HCl, 0.5 g. of activated charcoal added to decolourise the solution and then filtered. The pH of the clear solution was adjusted to 7.0 with NaOH solution, and precipitate obtained was separated from the filtrate

TABLE 1.

No. of the test solution	Eye-observation after 42 hours	Dry weight of mycelium in g.	% of growth on the basis of dry weight
Control	Slight growth and no sporulation ..	0.026	100
1	Fair growth and no sporulation ..	0.049	188.4
2	Fair growth and slight sporulation ..	0.050	192.3
3	Fair growth and fair sporulation ..	0.065	250.0
4	Fair growth and no sporulation ..	0.046	176.9
5	Heavy growth and heavy sporulation ..	0.093	357.6
6	Heavy growth and heavy sporulation ..	0.116	446.1
7	Heavy growth and fair sporulation ..	0.085	326.9
8	Heavy growth and fair sporulation ..	0.088	376.9
9	Good growth and fair sporulation ..	0.082	315.38
10	Good growth and fair sporulation ..	0.087	334.6
11	Heavy growth and heavy sporulation ..	0.090	346.1
12	Fair growth and slight sporulation ..	0.03	115.38
13	Fair growth and slight sporulation ..	0.053	203.8

Experiments to Find Out the Active Substance in Water hyacinth.—It was observed from the results of the Table 1 that the maximum quantity of active substance could be obtained from the root of water hyacinth by extracting the fresh, fleshy sliced root with distilled water after autoclaving under 30 lbs. pressure/sq. inch for a period of two hours. In order to collect sufficient substance to carry out further experiments, 5 kg. of sliced root was extracted by the same method as described above and the solid substance thus obtained was designated as sample No. 1. The yield of this sample No. 1 was 1.25% on the basis of the fresh weight of the sliced roots. 500 ml. 1% solution of the sample No. 1 was prepared (Soln. No. 14) for the preparation of the following solutions:–Solution No. 15: The pH of soln. No. 14 was adjusted to 2.0 with hydrochloric acid. Solution No. 16: The pH solution No. 14 was adjusted to 10.0 with sodium hydroxide solution. Solution No. 17: 20 ml. portion of the solution No. 14 was treated with 5 ml. of 25% $ZnSO_4$ solution, pH adjusted to 7.0 with NaOH solution. The precipitate thus obtained was separated by filtration from the filtrate. Solution No. 18: The precipitate from No. 17 was dissolved in minimum quantity of hydrochloric acid and the

by filtration. Solution No. 20: The precipitate from the solution No. 19 was washed three times with distilled water and then dissolved in minimum quantity of HCl and the volume adjusted to 20 ml. Solution No. 21: The remaining charcoal from solution No. 19 was washed five times with distilled water and then extracted with 20 ml. of 2N NaOH solution, filtered and the pH adjusted to 7.0 with HCl. Solution No. 22: The pH of the 20 ml. portion of the solution No. 14 was adjusted to 10 with NaOH solution and 0.5 g. of activated charcoal added for decolourisation. These were then filtered. The colour was not removed by charcoal. The pH of the filtrate was adjusted to 7.0 with HCl solution. Solution No. 23: The charcoal from solution No. 22 was washed three times with distilled water and then extracted with 20 ml. 2N HCl. The pH of the extract was adjusted to 7.0 with NaOH solution. Solution No. 24: 20 ml. portions of the solution No. 14 was treated with .5 g. of activated charcoal and then filtered. Solution No. 25: The charcoal from solution No. 25 was washed with distilled water and then extracted with 20 ml. 2N NaOH solution. The pH of the extract was adjusted to 7.0 with HCl. Solution No. 26: 20 ml. portion of the solution No. 14 was first

treated with 5 g. Amberlite Resin IR-45 (OH) and then filtered. The filtrate was then treated with 5 g. Amberlite Resin IR-120(H), and then filtered.

Solution No. 27: 20 g. of sample No. 1 was first extracted with dry methanol. The extract was evaporated to dryness and the solid substance thus obtained was dissolved in 2000 ml. of distilled water. Solution No. 28: After extraction with methanol the solid residue was extracted with acetone. The acetone extract was evaporated to dryness and the solid substance thus obtained was dissolved in 2000 ml. of distilled water. Solution No. 29: After extraction with acetone the remaining solid residue was designated as sample No. 2. 1% solution of this sample No. 2 was prepared. Solution No. 30: 1 g. of the sample No. 2 was extracted with dry methanol and the extract was evaporated to dryness. The solution of this solid substance thus obtained was prepared in 100 ml. of distilled water. Solution No. 31: After extraction with methanol the residue was extracted with petroleum ether (40-60°) and the extract was evaporated to dryness and the solid substance obtained was dissolved in 100 ml. of distilled water. Solution No. 32: After extraction with petroleum ether, the residue was extracted with ether. After evaporating ether, the residue was dissolved in 100 ml. of distilled water. Solution No. 33: After extraction with ether the residue was extracted with xylene. After evaporating xylene the residue was dissolved in 100 ml. of distilled water. Solution No. 34: After extraction with xylene the residue was extracted with benzene and the benzene extract was evaporated to dryness. The dried substance was dissolved in 100 ml. of distilled water. Solution No. 35: After extraction with benzene the residue was then extracted with chloroform. After evaporating the chloroform, the residue was dissolved in 100 ml. of distilled water. Solution No. 36: After extraction with chloroform, the residue was extracted with ethyl acetate. After evaporating ethyl acetate the residue was dissolved in 100 ml. of distilled water. Solution No. 37: After extraction with ethyl acetate, the residue was again extracted with acetone. The acetone extract was evaporated to dryness and the residue thus obtained was dissolved in 100 ml. of distilled water. Solution No. 38: After extraction with acetone, from the residue, 0.1 g. was dissolved in 10 ml. of distilled water. Solution No. 39: 0.5 g. of the residue, from the remaining after extraction with acetone, was burnt into ashes and the ash was dissolved in minimum quantity of HCl and volume adjusted to 50 ml.

Analysis of the Sample No. 2.—The sample No. 2 contained carbon 19.2%, ash 61.2%, nitrogen 3.447%, silica 0.233%, Ca 0.612%, Mg 1.19%K 23.80%, P 1.07%, Na 1.21%, & Fe 0.275%.

Solution No. 40: This solution contained exactly the same quantity of metallic ions, nitrogen and phosphorous as contained by the sample No. 2. This solution contained sodium silicate 0.0506%, $CaCl_2$ 0.0842%, $MgSO_4$, H_2O, 0.346%, K_2SO_4 0.92%, KH_2PO_4 0.228% $NaNO_3$ 0.156%, NH_4NO_3 0.42%, and $FeCl_3$ 0.004%. Solution No. 41: This solution contained exactly the same amount of metallic ions and phosphorous and the salts were added in the form of chlorides and phosphates. This solution contained Na-silicate 0.0506%, $CaCl_2$ 0.0842%, NaCl 0.108%, KH_2PO_4 0.228%, KCl 0.506%, FeCl3 0.004%, MgCl2, $6H_2O$ 0.512%. Solution No. 42: 100 p.p.m. gibberellic acid solution. Solution No. 43: 100 p.p.m. indole-3-acetic acid solution. Solution No. 44: 10 mg./l of each of the following amino acids:- aspertic acid, glutamic acid, arginine, cystine, tyrosine, glycine, amino-iso-butyric acid, lysine, valine and threonine. Solution No. 45: Mixture of the solutions Nos. 42, 43 and 44 having the same percentage of substance as in their respective solutions. Solution No. 46: Solution No. 45 plus 1 % ash solution of sample No. 2. Solution No. 47: Mixture of the solution No. 40 and 45.

Using 1 ml. of the test solution in each case for each experiment, the bioassays were carried out exactly by the same method as described earlier. The experimental results were recorded as in Table 2.

Application of Water hyacinth Extract.—A. Effect on mash kalai (*Phaseolus mungo* Var. *Roxburghii*):—(a) Preparation of the solutions of water extract of the root of water hyacinth: 1 g. of the sample No. 1 was extracted with methanol. 1% solution of the residue was designated as fraction No. 1. The methanol extract was evaporated to dryness and extracted with ether. The ether extract was evaporated to dryness and the residue obtained from it was dissolved in 100 ml. of water. This solution was designated as fraction No. 2. After extraction with ether the remaining residue was extracted with acetone. The acetone extract was evaporated to dryness and this residue was dissolved in 100 ml. of distilled water. This solution was designated as fraction No. 3. After extraction with acetone 0.1 g. of the residue was dissolved in 10 ml. of distilled water. This solution was designated as fraction No. 4. The remaining portion of the residue was dissolved in 5 ml. of methanol, chromatographed,

through a column (45×1.7 cm.) of activated neutral alumina. The column was eluted with methanol. Methanol elute was evaporated to dryness and the residue thus obtained was dissolved in 90 ml. of distilled water. This fraction was designated as fraction No. 5. (b) Experiments

TABLE 2.

No. of the solution	Dry weight of the mycelium in g.	% of growth on the basis of dry weight
Control	0.027	100
14	0.112	415
15	0.030	111
16	0.031	115
17	0.065	241
18	No growth	0
19	0.110	410
20	0.068	252
21	0.024	89
22	0.099	367
23	0.025	92
24	0.109	408
25	0.026	96
26	0.028	104
27	0.060	222
28	0.022	81
29	0.108	400
30	0.026	96
31	0.027	100
32	0.025	92
33	0.027	100
34	0.026	96
35	0.028	104
36	0.027	100
37	0.026	96
38	0.107	396
39	0.099	367
40	0.086	318
41	0.088	326
42	0.023	85
43	0.020	74
44	0.021	78
45	0.023	85
46	0.096	355
47	0.085	315

on mash kalai: The above five fractions were used to study their effect on mash kalai. The mash kalai seeds were germinated on filter paper in petri-dishes using distilled water in the plate to moisten the filter paper. After germination, 1 ml. from each test solution was given at the base of each germinating seed. After two days, the length of the roots and shoots were measured and their dry weights were taken. The results are recorded in table No. 3 and the growths are shown in Fig. 1. (B-control)

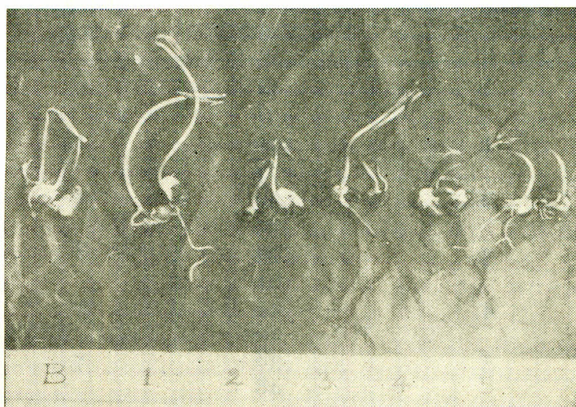

Fig. 1.

B. Effect on *Rhizopus arrhizus* ATCC 11145 and *Rhizopus* R-3 : 1 ml. of the 1% solution of sample No. 2 was used in this experiment and the experimental procedure was exactly the same as described earlier for bioassay. The results ware recorded in Table 4.

C. Effect on growth of yeast: This experiment was carried out on the yeast *Torulopsis utilis* NCYC 359 and on *Saccharomyces cerevisiae ellipsoideus* NCYC 506 by aerobic method of fermentation using the medium No. 2, 1 ml. of the 1% solution of the sample No. 2 was used per 5 ml. of the fermentation solution. For control experiments, corresponding volume of distilled water was used. To see the effect on the growth of yeast, at different interval of times, 5 ml. of fermentation solution were taken out, centrifuged and washed with distilled water three times and then suspended in 0.1% of sodium bicarbonate solution and the volume adjusted to 50 ml. The optical density of these solutions were determined by spectrophotometer using a wave length setting of 550 mμ. The experimental results were recorded as in Table 5 and Fig. 2.

D. Effect on alcoholic fermentation: To study this effect, *Saccharomyces cerevisiae ellipsoideus* ATCC 11145 was used in anaerobic method of fermentation. 1 ml. of the 1% solution of the sample No. 2 was used per 5 ml. of the medium No. 2 for this experiment, the control experiment was done using the same volume of distilled water in place of test solution. The speed of the fermentation was determined by recording the volume of CO_2 evolved at different

TABLE 3.

Fraction No.	Shoot length mm.	Dry weight (Mean) mg.	% (Mean) mg.	Root length mm.	Dry weight (Mean) mg.	% (Mean)
B	42	6	100	12	2	100
	31			15		
1	70	12	200	40	4	200
	77			55		
2	30	5	87	22	3	150
	31			17		
3	60	8	137	15	2	100
	61			19		
4	21	3	50	10	1	50
	20			13		
5	38	7	117	45	3	150
	48			38		

TABLE 4.

Name of the organism	Dry weight of mycelium	% of growth on the basis of dry weight
Rhizopus arrhizus ATCC No. 11145	0.036 g.	124
Rhizopus R.-3*	0.038 g.	131
Control	0.029 g.	100

TABLE 5

Name of the yeast	Experiment with	Readings taken after	Optical density against 550 mu.
Torulopsis utilis	Control	0 hour	0.04
	W.H.		0.04
	Control	4 hours	0.0645
	W.H.		0.067
	Control	20 ,,	0.196
	W.H.		1.33
	Control	24 ,,	0.2425
	W.H.		1.4
	Control	28 ,,	0.25
	W.H.		1.5
Saccharomyces cerevisiae ellipsoideus	Control	0 hour	0.025
	W.H.		0.025
	Control	3 hours	0.0255
	W.H.		0.032
	Control	6 ,,	0.0445
	W.H.		0.0625
	Control	22 ,,	0.1925
	W.H.		0.525
	Control	26 ,,	0.275
	W.H.		0.675

W.H. means water hyacinth

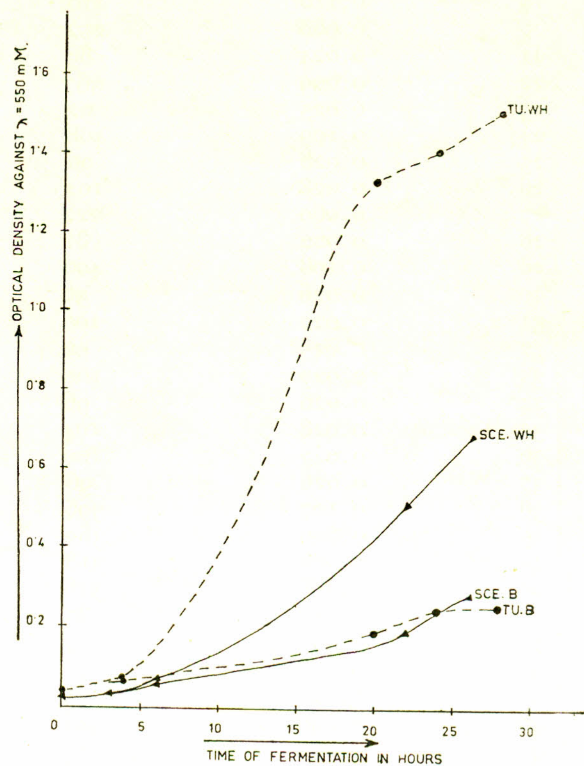

Fig. 2.—TU.B=Control with *Torulopsis utilis*. SCE. B = Control with *Torulopsis saccharomyces cerevisiae ellipsoidus*. TU.WH.=Experiment with water hyacinth extract on *Torulopsis utilis*. SCE. WH.=Experiment with water hyacinth extract on *Saccharomyces cerevisiae ellipsoidus*.

intervals of time and by estimating the quantity of ethanol produced. The volume of CO_2 was measured by collecting the gas by displacement of water in cylindrical glass tube and adjusting

the level of water inside and outside the same tube. Ethanol was estimated by Micro diffussion method.[5] The experimental results were recorded in Table 6 and Fig. 3.

TABLE 6.

Experiment with	Determinations were made after	Volume of CO_2 in ml.	% alcohol produced on the basis of solution
Control	0 hour	0	0
W.H.		0	0
Control	2 hours	2	
W.H.		3	
Control	6 ,,	5	
W.H.		41	
Control	22.5 ,,	44	
W.H.		460	
Control	25 ,,	48	
W.H.		494	
Control	28 ,,	55	
W.H.		518	
Control	30 ,,	62	
W.H.		528	
Control	46 ,,	88	0.336
W.H.		503	1.6
Control	49 hours	96	
W.H.		453	
Control	52 hours	106	
W.H.		450	
Control	54 hours	113	
W.H.		446	
Control	70 hours	152	0.65
W.H.		402	1.78

W.H. means water extract of the root of water hyacinth

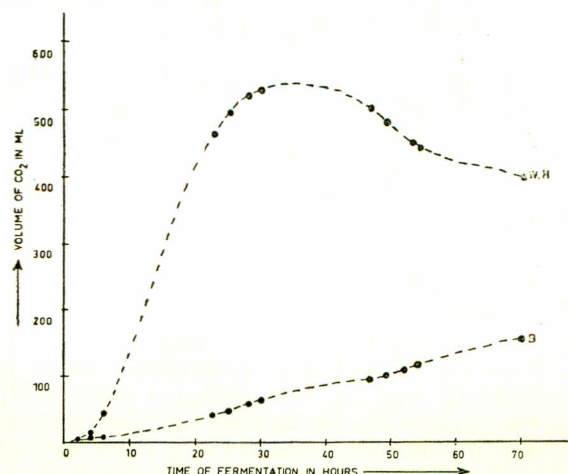

Fig. 3.—B.=Control Experiment. WH=Experiment using water hyacinth extract.

Compositions of the media: Medium No. 1: Sucrose 40 g./l $MgSO_4H_2O$ 0.12 g./l KH_2PO_4 0.30 g./l NH_4NO_3 2.25 g./l autoclaved at 15 lbs. psi for 30 minutes.

Medium No. 2: NH_4Cl 0.188% K_2HPO_4 0.100% Sucrose 5.0%; pH adjusted to 6.2 and autoclaved at 20 lbs. psi for 20 minutes.

Discussion

From the results of the Table 1, it is observed that the root of water hyacinth contains a growth promoting substance and this can be extracted from the root either with distilled water, methanol or ethanol, at any temperature such as at low, room, higher temperature or even after oven drying. This means that the substance is thermostable. The maximum quantity of the active substance can be obtained by autoclaving the fresh, fleshy, thinly sliced root with distilled water at 30 lbs. psi for two hours.

From the results of the Table 2, it is observed that the inorganic substances in the extract of the root of the water hyacinth is responsible for the enhancement of the growth of the mycelium of *Aspergillus niger* No. 21*. The colouring matters, amino acids, gibberellic acid or indole-3-acetic acid was not responsible for this type of effect.

From the results of the Table 3 and Fig. 1 it is proved that the substances insoluble in dry methanol or acetone ether was responsible for the enhancement of the growth of roots of mash kalai. The shoot and root growth

of mash kalai was double that of the corresponding growths in controlled condition.

From the results of the Table 4, it is observed that the growths of *Rhizopus arrhizus* and *Rhizopus R-3* were also enhanced by the presence of root extract of water hyacinth.

From the results of the Tables 5 and 6 and Figs. 2 and 3, it is observed that the multiplication of yeast cells were enhanced by the presence of water hyacinth extract and the speed of the evolution of CO_2 and production of alcohol were also accelerated. From these results, it is observed that the root extract of water hyacinth can successfully be applied in yeast production and in alcoholic fermentation. From the results of the effect on mash kalai, it is observed that the extract can be applied on plants. Beside the effect of solution No. 14 on the growth of mycelium, the effec t of solution Nos. 39, 40 & 41 were also studied. A comparison of growth confirms that the inorganic salts from the water hyacinth root has the same effect as the synthetic salts of similar composition. So it may be concluded that the inorganic salts are these substances responsible for the growth increase.

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