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RESEARCH ARTICLE

IMPACT OF GROWTH PROMOTERS ON THE GROWTH OF TWO CYANOBACTERIA (*Nostoc entophytum* and *Hapalosiphon stuhlamanii*)

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ABSTRACT

The present investigation emphasizes the effects of Gibberllic Acid and Kinetin on growth of blue-green algae *Nostoc entophytum* and *Haplosiphon stuhlmanni.* Individual application of both growth promoters showed a marked stimulation of fresh weight and chlorophyll-content at all concentrations on these blue-green algae. Interaction of Gibberllic acid and Kinetin on both algae noticed with increasing concentration has synergistic response on growth of *N. entophytum* and *H. stuhlmanni.*

INTRODUCTION

Living organisms, especially micro-organisms, are exposed to various types of natural stresses, such as nutrient limitation, pesticides, pollution, drought, salinity, temperature, pH, light intensity and quality, etc. Cyanobacteria, a group of prokaryotic, oxygen-evolving, photosynthetic Gram-negative bacteria, survive in a wide variety of extreme environmental conditions (Giriyappanavar, 2012). Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no or low cost. These cyanobacteria are exposed to growth hormones which are indispensable to the modern agricultural practice. However, the use of these hormones over the years has resulted in problems caused by their interactions with the biological systems in the environment and has deleterious effects on cyanobacteria. Although considerable information exists concerning the effects of growth substances on several blue-green algae (Ahamad and Winter, 1973; Bharati and Bongale, 1975; Anand 1982; Giriyappanavar and Bharati, 1989). However, the role of growth substances in the blue-green algae is not yet completely understood so far. Therefore, the present communication describes the response of Gibberllic acid and Kinetin on the growth and Chlorophyll-a content of *Nostoc entophytum* and *Haplosiphon stuhlmanni;* blue-green algae.

MATERIALS AND METHODS

Methods of isolation and culture of *Nostoc entophytum* and *Haplosiphon stuhlmanni* is same as already have been described (Giriyappanavar and Bharati, 1989). The growth promoters Gibberllic acid (G_{19} H_{22} O_6) and Kinetin (6-Fufury lexinopurinepuriss CHR, G_{10} H_9 N_5 O) both obtained from Loba-Chemico Industrial Company, Bombay, India. These growth promoters stock solution was prepared in sterilized distilled water. The chemical dose response was obtained by treating algal suspensions with 5, 25, 50 and 100ppm concentrations. 10 ml suspension of two alga were treated with growth promoters in graded concentrations for each concentration a set of four flasks were used and also one set as control. All cultures were incubated at 28 $\pm 2^0$ C and illuminated at 2000 lux and kept facing north window for 28 days and were agitated manually twice per day. The derivations followed here are based on a modified method of Colby (1967) for calculation of interaction of Gibberllic acid and Kinetin has been used. This method facilitates calculating "Expected" response of various chemical combinations. The "Expected" response for a given combination of two compounds can be calculated using the formula E = XY/100 where, 'E' is the expected value as a percent of control: 'X' is the value as a percent of control when exposed to Gibberllic acid 'Y' is the value as a percent of control when treated with the Kinetin. When the observed response is greater than expected, the combination is synergistic: When the observed response is less than expected, the effect is antagonistic. If the observed and expected responses are equal, the combination is additive. Many workers who have used different chemicals as combination which need to be mentioned here Mc. Rae *et. a1.*, (1953), Stone and Stahler (1954) and Datta and Baines (1960). Growth of the blue-green alga was measured in terms of fresh weight and chlorophyll-a on every 7 day interval till 28th day. Chlorophyll-a was determined following the proposed formula by Talling and Driver (1963).

RESULTS

Nostoc entophytum

Gibberllic acid is found to be of significance on*N. entophytum* atvaried concentrations (Table 1). At treatment levels of 5ppm it has an effect in increasing the fresh weight from 5ppm to 100ppm on the 7th day, so also on 28th day. On 14th and 21st day it behavior on this alga seems to be quite irregular. Nevertheless the growth on the 14th day and 21st day is well marked while on 28th day the growth seems to have been reduced. The effect of Kinetin is also well marked on the growth of *N. entophytum.*

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Table 1

Effect of two growth promoters on fresh weight of *Nostoc entophytum* (gms/10ml)

Days	7th day	14th day	21st day	28th day
CONTROL	0.125	0.113	0.109	0.215
Conc.(ppm)				
Gibberlic acid				
5	085	814	106	058
25	087	779	096	096
50	110	823	899	121
100	790	088	131	069
Kinetin				
5	094	099	101	068
25	093	096	117	088
50	083	093	124	123
100	113	095	156	112
Gibberlic acid + Kinetin				
5	092	119	890	064
25	092	089	156	085
50	126	087	158	160
100	155	096	153	184

Interaction of Gibberlic acid and Kinetin on fresh weight of *Nostoc entophytum* (gms/10ml)

Days	Concentration (ppm)	Observed values	Expected values	Difference
		(%)	(%)	
7th day	5	92	80	-12
	25	92	81	-11
	50	126	91	-35
	100	155	893	738
14th day	5	119	806	687
	25	89	748	695
	50	87	765	678
	100	96	083	-13
21st day	5	890	107	-783
	25	156	112	-44
	50	158	114	-44
	100	153	204	051
28th day	5	064	039	-25
	25	086	084	-01
	50	160	149	-11
	100	184	077	-107

All values are expressed as percent of control ***** = Values above thousand***

Table 2

Effect of two Growth promoters on the Chl-a of *Nostoc entophytum* (ugl^{-1})

Days	7th day	14th day	21st day	28th day
CONTROL	0.322	0.462	0.392	1.505
Conc.(ppm)				
Gibberlic acid				
5	107	080	113	122
25	696	012	094	131
50	105	012	097	124
100	686	086	095	131
Kinetin				
5	882	012	101	144
25	882	012	115	113
50	100	011	115	108
100	113	013	123	148
Gibberlic acid + Kinetin				
5	833	012	116	131
25	110	012	118	145
50	637	012	096	113
100	125	012	213	195

Interaction of Gibberllic acid and Kinetin on the Chl-a of *Nostoc entophytum* (ugl^{-1})

Days	Concentration (ppm)	Observed values	Expected values	Difference
		(%)	(%)	
7th day	5	089	078	-11
	25	059	070	+11
	50	089	178	+89
	100	111	043	-68
14th day	5	147	077	- 70
	25	130	030	- 100
	50	108	092	- 16
	100	092	071	- 21
21st day	5	057	126	+ 69
	25	100	210	+ 110
	50	266	234	- 32
	100	213	347	+ 134
28th day	5	045	045	0
	25	090	093	+ 03
	50	218	237	+ 19
	100	195	077	- 118

All values are expressed as percent of control

Table 3

Effect of two growth promoters on fresh weight of *Hapalosiphon stuhlmanii* (gms/10ml)

Days	7th day	14th day	21st day	28th day
CONTROL	0.102	0.95	0.110	0.130
Conc.(ppm)				
Gibberlic acid				
5	107	080	113	122
25	696	012	094	131
50	105	012	097	124
100	686	086	095	131
Kinetin				
5	882	012	101	144
25	882	012	115	113
50	100	011	115	108
100	113	013	123	148
Gibberlic acid + Kinetin				
5	833	012	116	131
25	110	012	118	145
50	637	012	096	113
100	125	012	129	152

Interaction of Gibberlic acid and Kinetin on fresh weight of *Hapalosiphon stuhlmanii*(gms/10ml)

Days	Concentration (ppm)	Observed values	Expected values	Difference
		(%)	(%)	
	5	833	944	+111
	25	110	**	+**
7th day	50	637	105	-532
	100	125	775	+650
	5	12	10	-02
	25	12	01	-11
14th day	50	12	01	-11
	100	12	11	-01
	5	116	114	-02
	25	118	108	-10
21st day	50	96	112	+16
	100	129	117	-12
	5	131	176	+45
	25	145	148	+03
28th day	50	113	134	+21
	100	152	194	+52

All values are expressed as percent of control ***** = Values above thousand***

Table-4

Effect of two Growth promoters on the Chl-a Of *Hapalosiphon stuhlmanii*(ugl^{-1})

Days	7th day	14th day	21st day	28th day
CONTROL	0.254	0.357	0.322	0.497
Conc.(ppm)				
Gibberlic acid				
5	088	090	143	086
25	061	129	143	137
50	113	129	143	121
100	074	080	176	100
Kinetin				
5	061	063	122	144
25	061	090	165	107
50	088	080	089	130
100	100	100	165	168
Gibberlic acid + Kinetin				
5	074	100	122	114
25	061	071	165	168
50	100	149	122	114
100	100	099	187	093

All values are expressed as percent of control

Interaction of Gibberllic acid and Kinetin on the Chl-a of *Hapalosiphon stuhlmanii*(ugl^{-1})

Days	Concentration (ppm)	Observed values	Expected values	Difference
		(%)	(%)	
7th day	5	74	53	-21
	25	61	37	-24
	50	100	99	-01
	100	100	74	-26
14th day	5	100	57	-43
	25	71	116	+45
	50	149	103	-46
	100	99	80	-19
21st day	5	122	174	+52
	25	165	236	+71
	50	122	127	+05
	100	187	290	+103
28th day	5	114	124	+10
	25	168	147	-21
	50	114	157	+43
	100	93	168	+75

The fresh weight at all concentrations on 21st to 28th day indicates that there is gradual increase from 5 to 100ppm, while on the earlier period of the growth (7th and 14thday) there is gradual decrease from 5 to 100 ppm. The growth at all concentrations generally increases from the 7th day to 21st day while on the 28th day the growth decreases. The interaction of Gibberllic acid and Kinetin on the growth of *N. entophytum* shows that on the 7th day the effect is antagonistic upto 50ppm, while at 100ppm there is synergistic effect on the 28th day at all concentrations of both the growth promoters. There is also an antagonistic effect which is true on the 21st day. It is only on the 14th day the interaction seems to be synergistic. Probably 25ppm concentration on the 28th day indicates an additional effect is very meager. In general Gibberllic acid and Kinetin interact to produce more antagonism on *N. entophytum.* The effect of both growth promoters on percent Chl-a of*N. entophytum* showsthat at all concentrations on day 7 and day 21 the Chl-a decreases from 5 to 100 ppm, while on 28th day the pigment gradually increases at 50 ppm. On the 14th day there seems to be no definite relation at all concentrations. Kinetin shows a marked behavior on 21st day where in percent Chl-a of this alga gradually increases from 5 to 100 ppm. On all other days, at all concentrations the growth promoters do not have significant effect. However, at certain concentrations it does show a slight decrease from 5 to 50 ppm concentrations. Interaction of these growth promoters on the percent Chl-a content of *N. entophytum* shows that, on day 14 at all concentrations the effect is antagonistic. 21st day to 28th day the effect is synergistic with additive effect on 28th day at 5 ppm. The 7th day however, shows antagonism at 5 and 100ppm while at 25 and 50 ppm it shows synergistic effect. In general the interaction of these two growth promoters on percent Chl-a of*N. entophytum*shows more synergistic effect.

Haplosiphon stuhlmannii

The individual effect of Gibberllic acid on the growth of *H. stuhlmannii* is not definite, although on 7th day at 25 and 100 ppm there is an excess growth but at most of the observation days during the experiment, the fresh weight does not follow the regular pattern of growth. Kinetin influences the growth of the alga *N. entophytum*on 14th, 21st, and 28th day at all concentrations and shows no meager change on fresh weight of *H. stuhlmannii.*

A noteworthy observation is that on 7th day the growth is more which gradually reduces on 28th day. It is interesting to note that when both the growth promoters are treated simultaneously the effect is synergistic where at nearly all concentrations on 7th,21st and 28th day the effect is synergistic it is only on the 14th day the effect is slightly antagonistic which values are closer to additive effect. It may be generalized that individual effect of Gibberllic acid and Kinetin on *H. stuhlmannii*is not significant, where as their interaction has synergistic effect. The individual effect of Gibberllic acid on the percent Chl-a content of this alga shows similar behavior as that observed for its effect on fresh weight. The only difference observed here is that the Chl-a content remains almost constant at all concentrations on 21st day. Kinetin also behaves in a similar way as that of Gibberllic acid on Chl-a content of this alga. At all concentrations of Kinetin the percent Chl-a content gradually increases on all the days except at 25 ppm where a slight reduction in the amount of Chl-a is observed. It is evident from Table-4that the interaction of two growth promoters on the Chl-a content of *H. stuhlmannii*shows that the effect becomes synergistic on 21st day onwards till the end of experiment except at 25 ppm where the effect is partially antagonistic. It may therefore be said that the overall effect of the interactions of these growth promoters on percent Chl-a content of *H. stuhlmannii* is partially synergistic and more antagonistic.

DISCUSSION

The studies already made with IAA, there is considerable increase in the growth of *Nostocmuscorum*(Ahamad and winter1971). *ChlorogloeaFristschii, Tolypothrixtenuis, Anacystis,* inoculants and *Phormidiumfeveloarum* (Ahamad and winter1968) at lower concentrations and at higher concentrations, it was inhibitory. The present work showed that individual application of Gibberllic acid and kinetin appears to enhance fresh weight and Chl-a at all concentrations in *C. membrancea*and *A. fertilissima*(except on day 14 onwards at 50 ppm, at high concentrations it inhibited Chl-a content in *C. membrane*). Whereas in case of *A. fertilissima* stationary phase was attained soon after the treatment with these growth promoters. On day 21 onwards the exponential phase continued at 25 to 50 ppm concentrations till the last day similar to this Giriyappanavar and Bharati (1989) have observed the increase fresh weight and productions of

heterocyst number along with the beneficial effect on total nitrogen fixation of blue-green algae at various doses of 3-indole butyric acid and vipul treatment. Bharati and Bongale (1975) have reported that Adenosine triphosphate (ATP) enhances the growth of *Hapalosiphonwelwitschii* at 5, 10 and 15 ppm. In the present study the combined action of Gibberllic acid and Kinetin with increasing concentration has synergistic effect on percent fresh weight and Ch1-a in *N. entophytum*(except in *H. stuhlmannii*whichshows antagonistic effect on the Chl-a content). Adhikary and Pattnaik (1979) reported that chlorophyll content of Kinetin treated *Westiellopsis*sps. was found to be much less on algal growth. However, work of Bharati and Bongale (1975) demonstrated that at lower concentrations (0.1-0.3%) of glucose, sucrose and lactose favour the growth of *Hapalosiphonwelwitschil*. It is evident from these findings that the differential physiological response of the hormones may be due to the ecophysiology of algal growth conditions and hormones specificity of the algae.

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