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EFFECT OF GROWTH REGULATORS ON SPORE GERMINATION OF DIPODASCOPSIS UNINCULATA

BY

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ABSTRACT

The effect of plant growth regulators on spore germination was variable and completely dependent on the concentration of the growth regulator. 2,4-D decreased the percentage of germination at all concentration used, while 4CPA was stimulatory especially at concentration 10^{-7}M. IAA gave the highest percentage of germination at 10^{-8}M. The stimulatory effect was also observed after treatment with GA_3 and kinetin at concentrations 10^{-5} and 10^{-8}M respectively. Partial synchronization of spore germination was induced by growth regulators at certain concentrations.

INTRODUCTION

Dipodascopsis uninucleata, Batra & Millner (Biggs) is a homothallic yeast first isolated from a dead pupa of Drosophila melanogaster (Biggs, 1937). It is regarded as an intermediate between single celled system of the yeasts

and the complex mycelial system of the Euascomycetes and was used to investigate control of growth and reproduction in a simple mycelial system (Elwy, 1981). It was found that the ellipsoid ascospores swelled and changed to spherical spores which germinated and grow to from a long sprout in which asci were formed in a regular pattern. The pattern of growth was the same in semisolid medium and in shaken liquid culture which indicates that there are internal cellular contents which govern the morphogenetic sequences which occur in D. uninucleata. The lack of synchrony between the sprouts in D. uninucleata makes it difficult to perform qualitative histochemical analyses to explore the differences in the metabolic gradient between the cells of the sprout during the life cycle. Synchronizing agents such as hydroxyurea failed to produce synchronization of spore germination and of growth of D. uninucleata. The use of different inhibitors of protein and nucleic acid synthesis in morphogenetic studies of D. uninucleata were also unsuccessful (Elwy 1981). It is hoped that some growth regulators could be used to influence the germination and growth of the fungus. Yanagishima (1963) suggested that yeast cells have a growth regulation mechanism which is sensitive to plant growth regulators (PGRs). Plant growth regulators are used for the control of growth and differentiation in higher plants (Wareing and Phillips, 1973).

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Auxins, gibberellins and cytokinins proved to have various effects on germination, growth and sporulation of some fungi (Gruen, 1959 ; Kamisaka et al., 1967). Nakamura et al. (1978) reported that the conidial germination rate in the wild type Neurospora crassa was promoted by adding auxins and gibberellic acid to the medium. The same effect was shown when auxins or gibberellic acid were added to gibberella fujikuroi and Penicillium notatum during conidial germination (Nakamura et al., 1985).

The aim of the present study is to investigate the action of auxins, gibberellins and cytokinins on spore germination of D. uninucleata in an attempt to produce synchronization of spore germination.

MATERIALS AND METHODS

The fungus Dipodascopsis uninucleata Batra & Millner (Biggs) was obtained from C.A.B. International Mycological Institute (IMI 86676). Fungal growth was maintained on MYGP medium composed of :

Malt	3 g / L
Yeast	3 g / L
Glucose	10 g / L
Peptone	5 g / L

Spore germination

Conical flasks containing 30 ml of MYGP medium were first

autoclaved then 10^{-4}, 10^{-5}, 10^{-7} or 10^{-8}M indole -3- acetic acid (IAA) or 2,4-Dichlorophenoxy acetic acid (2,4-D) or parachlorophenoxy acetic acid (4 CPA) or kinetin (Kin) are added . Gibberellic acid (GA_3) was used at the following concentrations: 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}M.

The spore inoculum was prepared by filtering 7 days old cultures through sterilized glass wool then washing the material with sterilized distilled water 0.2 ml of spore inoculum at conc. of 50- 60 spores/ field were added to each flask. The experiment was carried out in duplicate and controls with no added agents were also grown. Cultures were incubated in a rotary shaker at 180 rpm at 25 ± 1 °C. When the control cultures reached about 60 % germination, the different cultures were examined and the percentage germination estimated for each duplicate. After a further 24 h , the cultures were reexamined to study the effect of PGRs on the final percentage of germination. The experiment was repeated three times and the mean percentage germination for each treatment was estimated and compared with the controls.

Synchronization of spore germination using growth regulators:

Flasks were prepared as described in the spore germination experiment. To inoculated flasks, one of the following chemicals was added: 10^{-5}M GA_3; 10^{-7}M 4CPA; 10^{-8}M

IAA or 10^{-8}M Kin. Duplicates without the addition of growth regulators were used as controls. The flasks were examined after 5, 8, 11, 14, 17, 21 & 26 h of incubation and the mean percentage germination was estimated at those intervals. Ascospores were considered germinated when the length of the germ tube was nearly half the diameter of the spore and at least 500 ascospores were counted for each treatment . The experiment was carried out three times in duplicates.

Statistical analysis:

All mean germination rates were given as x ± S.D. where the standard deviation was calculated at 95% confidence limit (Bishop, 1971). The difference between the means was tested using the T test at 95 % c.1.

RESULTS

The effect of different concentrations of growth regulators on spore germination is shown in Table 1. GA_3 increased the percentage of germinated spores when used at 10^{-5} and 10^{-6}M, at which percentage germination was 79% while that of control was 65% . On the other hand, concentrations from 10^{-2} to 10^{-4}M had no significant effect on germination. 4CPA at concentration 10^{-4}M decreased the percentage of germination to 55% compared with 65% of control. However, low concentrations (10^{-5} to 10^{-7}M) significantly increased the percent germinated spores.

Application of IAA at concentrations 10^{-4} and 10^{-5}M resulted in an inhibitory effect and percentage germination was 20% less than the control. Concentrations less than 10^{-6}M induced the germination process and the highest increase was achieved by concentration 10^{-8}M (83 %) while the control was 65%. Kinetin significantly inhibited the process of germination particularly at concentrations, 10^{-4}, 10^{-5} and 10^{-6}M but had no effect at 10^{-7}M . However, 10^{-8}M of kinetin greatly increased the percentage of germination at which the percent was 79% compared to 65% of control. 2,4-D had an inhibitory effect and percentage germination was significantly lower than control. Such inhibition was found to decrease by decreasing the concentration of the auxin.

The results show clearly that growth regulators may inhibit or stimulate spore germination, such an effect is dependent upon the growth regulator and its concentration. The highest germination rate was observed after treatment with 4CPA at concentration 10^{-7}M. Also, IAA (10^{-8}M), GA_3 (10^{-5}M) and kinetin (10^{-8}M). After 24 h, the cultures were examined again it was found that all spores in the different treated cultures had germinated and formed sprouts which indicated that PGRs influenced the rate of germination of D. uninucleata and also the the final percentage germination obtained and their inhibitory effect on spore germination at certain concentrations was temporary and it only

delayed cell division but did not inhibit it completely.

Germination of spores in the presence of growth regulators at the effective concentrations was examined (Figs. 1- 4). It is clear that the addition of these growth regulators enhanced the germination process in all stages . The lag period which is the intercept of the extrapolated line of Figs. 1- 4 and which indicates the time required for the spores to start germination. After incubation this was longer for the treated spores and there was a delay in the appearance of the first germ tube from 2.45 to 3.25 h. Once the germ tube was initiated, the rate of germination increased in spores germinated in the growth regulators than in control . The arrows in Figs. 1-4 indicates G_{50} and G_{82} which is the time required for 50% and 82% of the spores to germinate. This were calculated in Table 2. It was shown that the required time for untreated spores to start germination was shorter than that of the treated spores but that the time required to reach G_{50} and G_{82} was longer in control cultures than in cultures containing growth regulators. G_{82} stage was reached in 19 h in control but the treated cultures decreased this period by 7.25-9.25h.

It was also observed that spores germinated in the presence of the chemicals grew faster and reached 3 cells long in the treated cultures after 26 h of incubation, while

in the control cultures, the percentage germination was 82%. and the sprouts were only 2 cells long.

DISCUSSION

It was demonstrated clearly that 1AA, 4CPA, Kin and GA_3 increased the rate of spore germination, produced partial synchronization and stimulated cell division and growth of the cells when used at suitable concentration. The optimum concentrations are within the ordinary cincentration range at which they act as plant regulators in higher plants. Similar promotion was observed by Nakamura et al. (1978) when 1AA, and GA_3 were added to germinating conidia of N. crassa at concentrations 10^{-6} and 10^{-4}M respectively.

Nakamura et al. (1985) reported that auxins and gibberellins promoted conidial germination of G. fujikuroi and P. notatum. 1AA and GA_3 in concentrations 6×10^{-8}- 6×10^{-6} M and 3×10^{-8}- 1.5×10^{-3}M respectively were reported to increase the percentage of germination of akinetes in Pithophora oedogonia (Agrawal, 1985). As far as the author is aware, little is known about the action of kinetin and 4CPA on spore germination, but some work had been carried out on the effect of kinetin on growth of some microorganisms The present results show that kinetin inhibited spore germination at 10^{-4} - 10^{-6}M, while low concentration (10^{-8}M) promoted spore germination. This is in contrast to the

work done by Valiente et al. (1983) who found that Kin, GA_3 and IAA at concentration of 2×10^{-7}-2×10^{-5}M, 0.3×10^{-7}-1.3×10^{-5}M and 10^{-7}-3×10^{-5} M, respectively had no effect and neither stimulated nor inhibited growth of the cyanobacterium Anacystis montana, while the same growth regulators at a concentration of 5×10^{-4}-1.9×10^{-3}M, 3×10^{-4}- 1.2×10^{-3}M and 6×10^{-4} - 2.3×10^{-3}M respectively, led to a retardation of growth and fruiting in Lentinus legrinus, Agaricus arvensis and Morchella conica (Mayer et al. 1984).

The increase in the percentage of germination using GA_3 and cytokinins may be correlated with the effect of these substances on other plant cells. GA_3 and cytokinins e.g. (kinetin) stimulate the biosynthesis of cAMP which in turn promotes mitotic activity (Woong- Seop and Kim,1987). This agrees with the results of Tu and Malhotra (1977), who observed a shortening in the lag period of spore germination in Phycomyces blakesleeanus when cAMP was added to the germinating spores. When 80 ug/ ml cAMP was added to the cultures of D. uninucleata, it enhanced growth and reproduction (Elwy, 1981).

2,4-D temproray. inhited spore germination at all concentrations used (10^{-4}- 10^{-8}M). In contrast with the present results, Nakamura et al. (1978) found that 10^{-6}M

was an optimum concentration of 2,4-D which promoted conidial germination of N. crassa. 2,4-D had no effect on the growth of Rhizobium trifolii at a concentration of 1.1×10^{-4} M but concentrations up to 2.3×10^{-4}M gave moderate to poor growth (Fletcher,1956). 2,4-D decreased the RNA levels of wheat plants when used as an auxin-like herbicide (Chen et al. 1972). It is possible that the inhibitory action of 2, 4-D on spore germination is due to a decrease in the RNA levels during cell division which in turn led to a decrease in the rate of cell division of the cells.

The variation in the effect of the same concentrations of 2,4-D and 4CPA may be due to the difference in the number and position of the chlorine atom. The results show that there is an increase in the lag period of the treated spores, this is in contrast to the work done using a mixture of 10^{-7}M GA_3 and 5×10^{-7}M Kn in the growth medium of the diatom Thalassiosira gravida. These growth substances decreased the lag phase and increased the number of cells (Holdsworth, 1985).

It is concluded that certain concentrations of PGRs increade the rate of spore germination of D. uninucleata and induced partial synchronization of spore germination, they also stimulated cell division and growth. Therefore futher investigation could be carried out to explore the effect of those growth hormones on the growth and reproduction of D. uninucleata.

effect of those growth hormones on the growth and reproduction of D. uninucleata.

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Table 1 : The effect of growth regulators at different concentrations on spore germination of D. uninucleata.

Concentration (M)	Percentage germination				
	GA_3	4CPA	IAA	Kinetin	2,4-D
0	65± 4 (control)				
10^{-2}	66± 2*				
10^{-3}	63± 3*				
10^{-4}	65± 3*	55 ± 3	41 ± 2	49 ± 2	24 ± 3
10^{-5}	80± 2	79 ± 1.5	45 ± 3	45 ± 3	29 ± 3
10^{-6}	79± 1	90 ± 0.5	67 ± 1*	58 ± 1	32 ± 2.5
10^{-7}		90 ± 2	77 ± 3	62 ± 3 *	45 ± 3
10^{-8}		68 ± 3*	83 ± 1	79 ± 2	51 ± 2

* = No significant difference with the control.

± = St. D. at 95 % C.L.

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Table 2 : The effect of certain concentrations of 4CPA, GA_3 , IAA and Kin. on different stages of spore germination of D. uninucleata.

Growth regulators		Period of stage (in hour)			
		Lag period	G_{50}	G_{82}	G_{82}-Iag period
Control		7.00	19.75	26.00	19.00
+ 4CPA	10^{-7}	10.25	16.50	20.00	9.75
+ GA_3	10^{-5}	10.00	17.00	21.50	11.50
+ IAA	10^{-8}	10.25	16.75	20.75	10.50
+ Kin	10^{-8}	9.75	16.75	21.50	11.75

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Effect of Growth Regulators

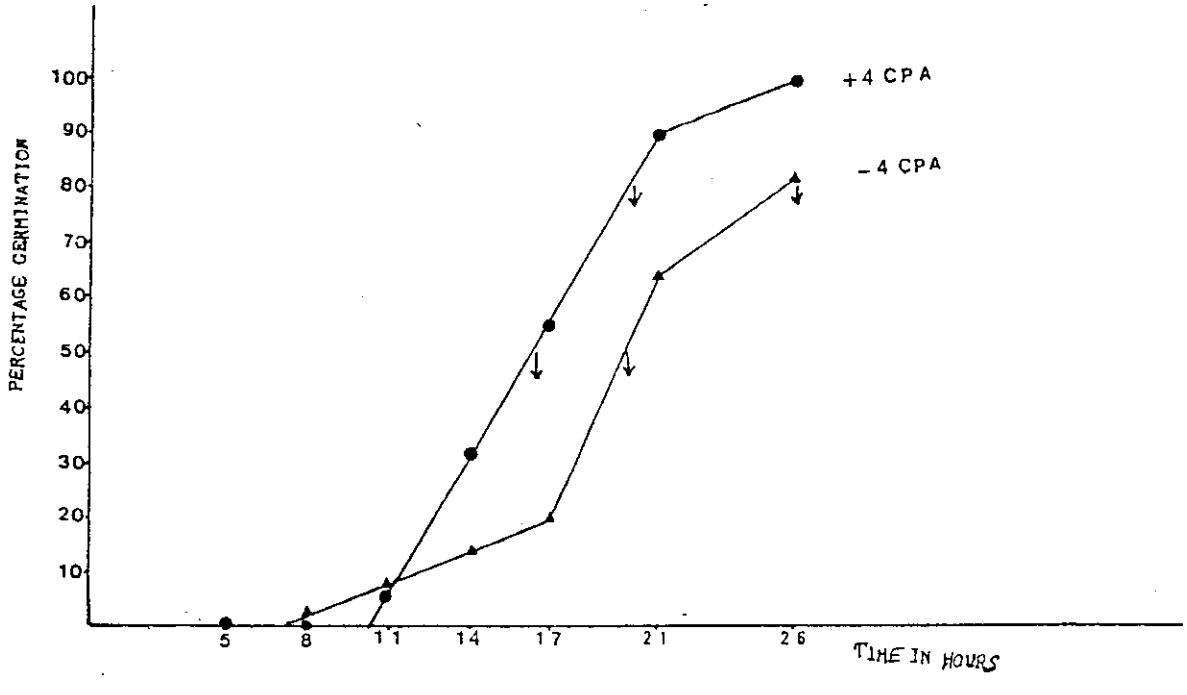

Fig.1. Effect of 10^{-7} M para-chlorophenoxy acetic acid on germination of *D. uninucleata*. Arrows indicates G_{50} and G_{82}

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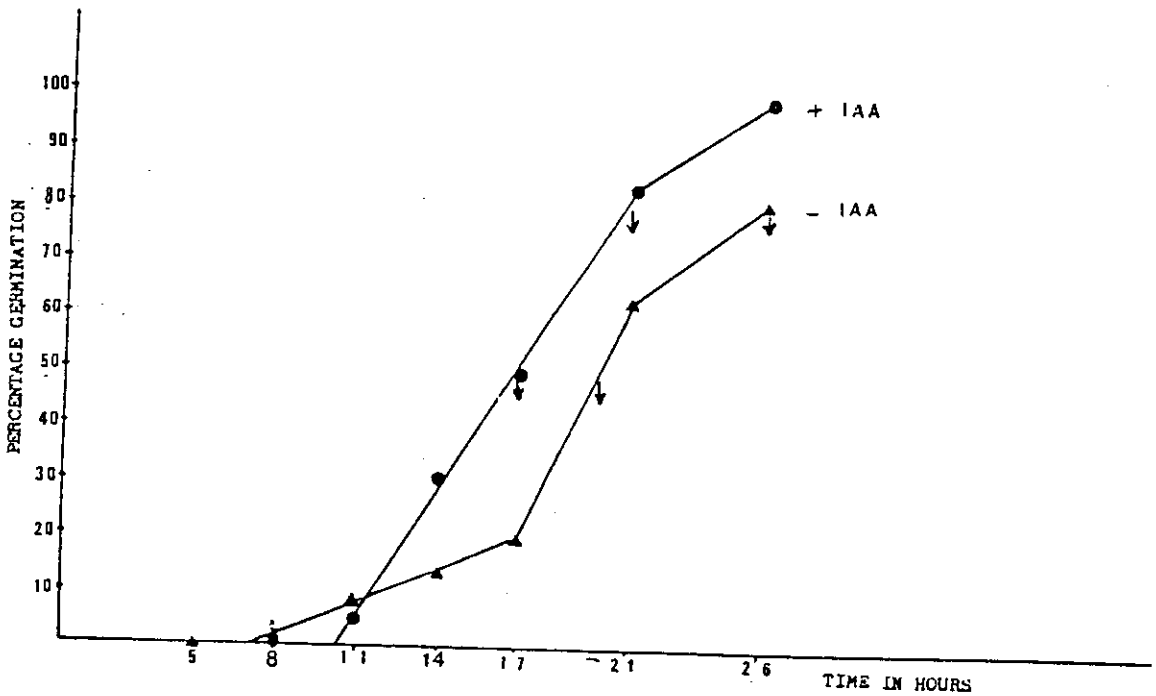

Fig.2. Effect of 10^{-8} M indole-3-acetic acid on germination of D. uninucleate. Arrows indicates G_{50} and G_{82}

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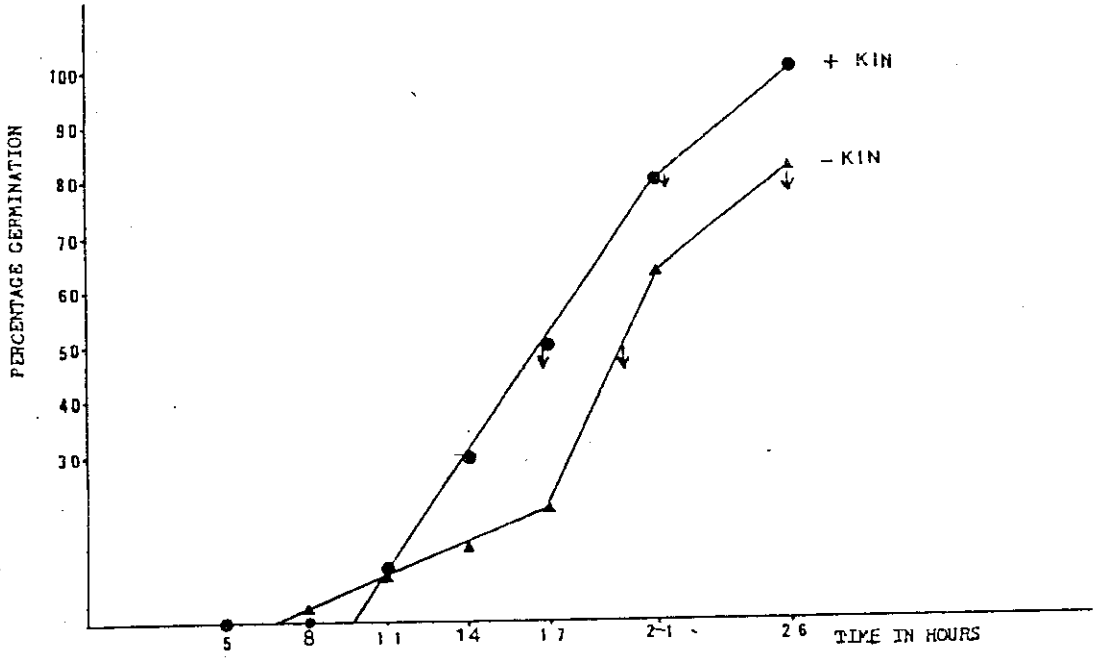

Fig.3. Effect of 10^{-8}M kinetin on germination of D. uninucleata.
Arrows indicates G_{50} and G_{82}

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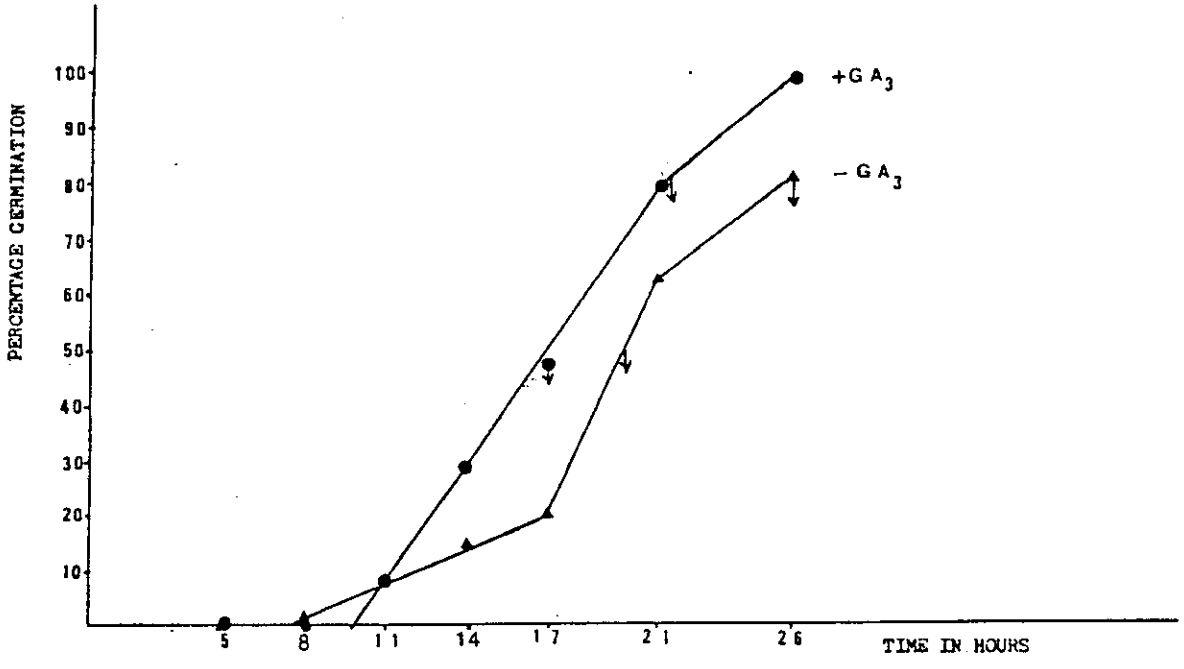

Fig. 4. Effect of 10^{-5} M Gibberellic acid on germination of D. uninucleata

Arrows indicates G_{50} and G_{82}

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تأثير منظمات النمو على انبات جراثيم فطره دايبودا سكوبسس يونينيوكلياتا

عصمت علوى على علوى
قسم النبات ـ كلية العلوم ـ جامعة القاهرة ـ الجيزة

فى هذه الدراسة تم فحص تأثير منظمات النمو على انبات الجراثيم فى فطرة "دايبودا سكوبسس يونينيو كلياتا" . وقد بينت الدراسة أن تأثير منظمات النمو كان مختلفا ويعتمد أعتمات كلى على تركيز منظم النمو المستخدم . وقد أظهر 4 C P A تأثيرا مثبطا على عملية الانبات فى جميع التركيزات المستخدمه بينما أظهر 4 C P A تأثيرا عكسيا حيث زادت نسبة الانبات فى الجراثيم خاصه عند التكيز 10^{-7} جزيئ . I A A أعطى نسبه للانبات عند التركيز 10^{-8} جزيئ . هذا وقد كانت نسبة النمو أعلى فى حالة G A 3 ، الكينتين عند تركيز 10^{-5} ، 10^{-8} جزيئ على التوالى. بينت الدراسة أن بعض منظمات النمو فى تركيزات معينه قد أحدثت مزامنه جزئيه فى عملية الانبات .