NAPHTHALENEACETIC ACID

Task 1: Review and Evaluation of Individual Studies

Contract No. 68-01-5830

Final Report

February 19, 1981

SUBMITTED TO:

Environmental Protection Agency
Arlington, Virginia 22202

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NAPHTHALENEACETIC ACID

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CASE GS0023 NAPHTHALENEACETIC ACID STUDY 1 DP Barcode: 112277

CHEM 056002 1-Naphthaleneacetic acid

BRANCH EFB DISC 2. TOPIC 051 GUIDELINE # CFR 163.62-6?

FORMULATION: AI = ACTIVE INGREDIENT

FICHE/MASTER ID: 05011554 CONTENT CAT: 1

Clifford, D.R.; Hancock, J.G. (1980) Fungal detoxication--[A-metabolism of 1-naphthaleneacetic acid by Aspergillus niger van Tieghem. Phytochemistry 7(2):1499-1502.

SUBJ. CLASS = S.

OTHER SUBJECT DESCRIPTORS
SEC: RCRP-05-1015

DIRECT EVA TIME = 9 (hr) START-DATE END DATE

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CONCLUSIONS:

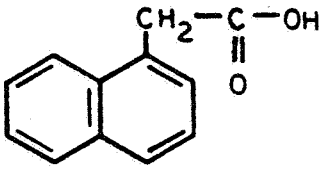
Metabolism - Effects of Microbes on Pesticides

1. This study is scientifically valid in terms of the identification of NAA degradation products but is weak due to the lack of controls.

2. Aspergillus niger was implicated as metabolizing naphthaleneacetic acid (NAA). The major product is 5-hydroxy NAA. The presence of the 4- and 6-hydroxy NAA isomers is strongly suggested.

MATERIALS AND METHODS:

Naphthaleneacetic acid, NAA 800,
Fruitone N, Rootone, Transplantone,
Tre-Hold



1-Naphthaleneacetic acid

Aspergillus niger (Mulder strain) was grown in a liquid glucose-salts medium (not described) for 3 days. The medium was poured off, the mycelial mats were rinsed with water, and a 5×10^{-4} M solution (volume not given) of naphthaleneacetic acid (NAA) (source and purity not given) in 10^{-2} M phosphate buffer was added. Cultures were incubated for 3 days at 25 C. The fungal substrate volume was reduced by evaporation followed by acidification and 16-hour continuous ether extraction.

The ether extracts were extracted with a sodium bicarbonate ($NaHCO_3$) solution. The ether fraction was subjected to thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). The $NaHCO_3$ solution extract (containing 95% of the metabolites) was esterified with acetone dimethyl acetal. The unmetabolized NAA ester could be separated, as well as fungal debris, from the hydroxy ester metabolites by partitioning with dilute NaOH solution.

The hydroxy ester metabolites were saponified and analyzed by thin-layer chromatography (TLC), paper and column chromatography, ion-exchange chromatography, gas-liquid chromatography (GLC), and fractional crystallization.

REPORTED RESULTS:

TLC analysis of the $NaHCO_3$ solution extract revealed at least six "diazo positive" (phenolic or naphtholic) compounds, several in trace amounts. The major metabolite, identified by IR spectra, TLC and GLC comparison with authentic standards, and mixed melting point determinations with authentic samples, was 5-hydroxy NAA. It was separated from two other compounds with similar TLC migrating distances by fractional crystallization. The other two compounds, although not separable by GLC, TLC, or paper-, column-, or ion-exchange chromatography, were strongly suggested as being the 4- and 6-hydroxy NAA isomers based on TLC comparisons with authentic isomers alone and in combination.

At least eight compounds, four phenolic or naphtholic, were detected, but not identified, in the ether extract.

DISCUSSION:

1. No quantitative data were given. Although qualitative analysis of NAA metabolites was the purpose of the study, the extent to which NAA can be metabolized cannot be determined.

2. No controls were included. It cannot be stated that the hydroxy NAA compounds were formed as a result of hydrolysis, photolysis, or microbiological metabolism. However, NAA is quite resistant to photolysis (Plant Propagation, 3rd ed., 1975, Hartmann and Kester, Prentice-Hall, Inc.) and hydrolysis above its pK (~5.6). The medium used in this study probably would be above pH 5.6, although not specified. This information, although preliminary, reveals that hydroxylation of NAA occurs, regardless of the source of degradation.

CASE 090123 NAPHTHALENEACETIC ACID STUDY 2 DP [illegible] 11/26/79

CHEM 'Sou'2 1= naphthaleneacetic acid

BRANCH EFB DISC 2: TOPIC 1.09 GUIDELINE # [illegible]

FORMULATION [illegible] = ACTIVE INGREDIENT

FICHE/MASTER ID [illegible] CONTENT CAT 11

Doi, S.; Takanashi, T.; Yanagishima, N. (1973) Auxin-induced large cell mutants in "Saccharomyces cerevisiae". I. Induction, and biochemical and genetic characters. Japanese Journal of Genetics. A translation of: Idenshaku Zasshi. 48(5):345-355.

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTIONS
PRI: PCAM=5-1005 EFF=1-35 TOX=1-15

DIRECT RVW TIME = 12 (MH) START-DATE END DATE

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CONCLUSIONS:

Metabolism - Effects of Pesticides on Microbes

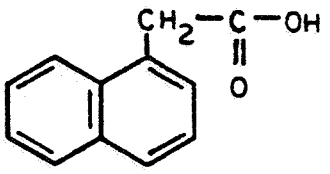
1. This study is scientifically valid.

2. α-Naphthaleneacetic acid (NAA) induces mutations in the yeast Saccharomyces cerevisiae. The mutations studied resulted in large cell size and increased nucleic acid and protein content. NAA induced a change in the ploidy of the parent strain to create diploid mutants. NAA at high concentrations also inhibited the growth of S. cerevisiae in culture; about 50% inhibition occurred with exposure at 300 ppm. Diploid mutants displayed a level of ascus production higher than the parent strain.

3. This study can be used to help fulfill microbiological metabolism data requirements in Section 163.62-8(f)(3) of EPA's Proposed Guidelines for Registering Pesticides (July 1978) because it demonstrates that NAA causes mutations in and affects the growth of a fungus commonly found in the soil or associated with plant matter.

MATERIALS AND METHODS:

Naphthaleneacetic acid, NAA 800,
Fruitone N, Rootone, Transplantone,
Tre-Hold



1-Naphthaleneacetic acid

The haploid strains of Saccharomyces cerevisiae used were H1-0 (a, ade, trp, leu_2, his_4) and H40 (α, ura_2). Four media were used: (1) YHG medium contained glucose, peptone, and yeast extract; (2) VHG-BII medium was prepared by replacing yeast extract in YHG medium by traces of vitamins plus adenine sulfate and uracil; (3) minimal medium for prototroph recovery contained mineral salts plus glucose; and (4) sporulation medium containing potassium acetate was designated KAc. Media were incubated at 26 C.

Cells of H1-0 grown for 2 days on YHG agar plates were spread at about 300 cells/plate on VHG-BII plates containing α-naphthaleneacetic acid (NAA) at 500 mg/liter. The source and purity of the NAA were not described. One hundred colonies were sampled at random after 10 days' incubation and inoculated on separate YHG plates. After 2 days' incubation, the cells of each colony were suspended in distilled water and photographed for cell volume (V), which was calculated as $V=\underline{a}x\underline{b}^2$ where $\underline{a}$ and $\underline{b}$ are the long and short axes, respectively. Control cultures, minus NAA, were run in parallel.

Large-cell mutants that were isolated were tested for respiration deficiency in YHG agar medium in which glucose was replaced by glycerol; colony formation after 2 days' incubation indicates the cells are not respiration deficient. Growth of mutants was tested in liquid VHG-BII medium supplemented with NAA at 100-500 ppm. After 3 days' incubation, cells were counted by standard techniques and compared with the counts obtained for the H1-0 strain.

DNA, RNA, and protein determinations were performed on mutant and parent strains with 3-day-old VHG-BII cultures. RNA was extracted with 0.3 N KOH at 37 C for 20 hours, and DNA was then extracted with 5% perchloric acid at 90 C for 15 minutes. The residue was treated twice with 1 N NaOH at 50 C for 30 minutes to extract protein. DNA and RNA were determined colorimetrically by using diphenylamine and resorcinol, respectively. Protein was determined through the Lowry method.

Auxin-induced mutants were mated with H40 and hybrids were obtained by prototroph recovery. The sporulation of the hybrids was tested in liquid KAc medium after 2 days' preculture on YHG plates. Liquid KAc was inoculated with 10^7 cells/ml and sporulating cells were counted 1, 2, 3, and 4 days after inoculation.

Tetrads of hybrids were isolated by treatment of asci with snail digestive enzyme and dissection using a micromanipulator. Genetic analysis of segregants was performed using standard techniques.

REPORTED RESULTS:

Only 48% of the H1-0 cells could form colonies on NAA plates. Four typical large-cell mutant strains were selected for further analysis. The strains were designated H1-L5, H1-L13, H1-L26, and H1-L31.

None of the mutants were respiration deficient.

In the presence of NAA at 100, 200, and 300 ppm, H1-0 cell numbers were reduced after 3 days by about 13, 40, and 55%, respectively, and little additional effect occurred at 400 and 500 ppm. Growth of the mutants in medium with or without NAA was about the same as that of the parent H1-0 strain. DNA, RNA, and protein contents of three of the mutants were markedly higher than those in the H1-0 strain and approached that expected for a diploid strain. Results for H1-L31 were about the same as those for H1-0, and cultures of this mutant consistently contained a mixed population of small and large cells.

The mutant strains and H1-0 were mated with H40 strain in liquid YHG medium for 1 day. Cells were plated on minimal agar, and prototrophic diploid colonies were used as hybrids for the sporulation test. The designation of hybrids is shown in Table 1. All hybrids derived from large-cell mutants gave a higher yield of asci than the hybrid from the control strain, ranging from about 50 to 100% of the control strain yield after 4 days' incubation in KAc medium.

The hybrids were then subjected to tetrad analysis to determine the segregation of genetic markers. The hybrids used were those in which germination rates for spores were >90%. These were HD62 (H1-0 x H40), HD63 (H1-L5 x H40), and HD66 (H1-L31 x H40). For the other two hybrids, HD64 (H1-L13 x H40) and HD65 (H1-L26 x H40), random spore analysis was used because the germination rates were 37.5 and 15.5%, respectively. The results of the control cross showed regular segregation of each genetic marker (Table 2). Genetic markers tested in HD63 showed the 4(+): 0(-) segregation with high frequency, and a high frequency of nonmaters were derived (Table 3). The segregation patterns for HD64 and HD65 are shown in Table 4, and those for HD66 are shown in Table 5.

It was concluded that NAA induced changes in the ploidy, resulting in the auxin-induced large-cell mutants. The segregation patterns for ura_2 in HD64 and HD65, coupled with the DNA contents for the parent mutant strains, suggest that H1-L13 and H1-L26 are diploids. The mutant H1-L5 mated normally with α-type haploid and did not sporulate; thus it would appear to be an aa-type diploid. However, HD63, the hybrid of H1-L5 and H40, had segregation patterns like those of a tetraploid rather than a triploid as would be expected if H1-L5 were nearly diploid. It was postulated that some mechanism in H1-L5 may have been responsible for duplicating the genome of its mating partner. The irregular segregation of his_4 in the hybrid HD63 (H40 x H1-L31) was probably due to a histidine-dependent mutation at another locus.

DISCUSSION:

1. Although some procedural details were omitted, they do not offset the fact that excellent protocols were used in this study, and the conclusions were properly derived from the results obtained.

2. Although only one microbe was studied, the results may be extrapolated somewhat. S. cerevisiae is commonly found in the soil or associated with plant matter. It belongs to the large phylum Ascomycota, which primarily includes soil saprophytes. Although S. cerevisiae has a yeast morphology, its physiological and genetic characteristics are very similar to those of other Ascomycetes. Therefore, the conclusions from this study are probably applicable to many other fungi commonly found in the soil.

Table 1. Hybrids obtained from crosses of α-type standard haploid strain H40 and large-cell mutants derived from the a-type strain H1-0.

Cross	Designation of hybrid
H40 x H1-0 (parent)	HD62
H40 x H1-L5	HD63
H40 x H1-L13	HD64
H40 x H1-L26	HD65
H40 x H1-L31	HD66

Table 2. Segregation of genetic markers in the hybrid HD62 obtained from the cross of H1-0 and H40.

Segregation in ascus		Number of tetrads				
+ : - a : α	Mating type	ade_1	ura_2	leu_2	his_4	trp_1
4 : 0	0	0	0	0	0	0
3 : 1	1	2	1	3	4	1
2 : 2	57	54	56	53	52	57
1 : 3	0	2	1	1	2	0
0 : 4	0	0	0	0	0	0

Table 3. Segregation of genetic markers in the hybrid HD63 obtained from the cross of H1-L5 and H40.

Segregation in ascus	Number of tetrads						Mating type
+ : -	*ade*$_1$	*ura*$_2$	*leu*$_2$	*his*$_4$	*trp*$_1$	sporulation	*a* : α : *n*[a]
4 : 0	39	22	34	25	30	10	
3 : 1	5	17	9	11	4	9	
2 : 2	3	8	4	9	13	15	
1 : 3	0	0	0	3	0	11	
0 : 4	0	0	0	0	0	3	
							41 : 24 : 87

[a] *n*: nonmater.

Table 4. Segregation of genetic markers in the hybrids HD64 and HD65 obtained from crosses between H40 and H1-L13, and H40 and H1-L26, respectively.

Genetic marker	Segregation pattern in hybrid	
	HD64	HD65
Mating type		
a : α : n[a]	16 : 2 : 10	11 : 2 : 0
$\frac{ade_1}{+ : -}$	15 : 15	5 : 6
$\frac{ura_2}{+ : -}$	25 : 5	11 : 3
$\frac{leu_2}{+ : -}$	16 : 14	11 : 2
$\frac{his_4}{+ : -}$	12 : 18	8 : 5
$\frac{trp_1}{+ : -}$	16 : 14	7 : 6

[a] n: nonmater.

Table 5. Segregation of genetic markers in the hybrid HD66 obtained from the cross of H1-L31 and H40.

Segregation in ascus	Number of tetrads					
+ : - a : α	mating type	ade_1	ura_2	leu_2	his_4	trp_1
4 : 0	0	0	0	0	0	0
3 : 1	0	0	4	6	1	1
2 : 2	30	17	26	20	9	27
1 : 3	0	0	1	5	18	3
0 : 4	1	0	0	0	4	0

TOX/ORM [illegible] DATA EVALUATION RECORD [illegible]

CASE 05[illegible]23 NAPHTHALENEACETIC ACID STUDY 3 [illegible]

CHEM [illegible] Ethyl 1-naphthaleneacetate

BRANCH DISC IN TOPIC IN 5 GUIDELINE # OPP 163.61-1/

FORMULATION TYP = ACTIVE INGREDIENT

FICHE/MASTER ID 05[illegible]797 CONTENT CAT 01

Ukita, T.; Tamemasa, O.; Motomatsu, M. (1951) Antibacterial action of fatty acids. VIII. Syntheses of fatty acids with naphthyl, naphthoethyl and benzyl groups in alpha-position and their antibacterial action. Yakugaku Zasshi. [Journal of Pharmaceutical Society of Japan.] 71(1):263-267.

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS
PRIM: EFF 41 35

DIRECT RVW TIME = 8 (MH) START-DATE END DATE

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CONCLUSIONS:

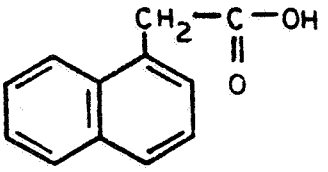
Metabolism - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Naphthaleneacetic acid (NAA) inhibits the growth of Staphylococcus aureus (a human pathogen) and human type tubercle bacilli at concentrations in excess of 50 and 63 ppm, respectively. NAA has no effect on avian type tubercle bacilli at concentrations of up to 50 ppm.

MATERIALS AND METHODS:

Naphthaleneacetic acid, NAA 800,
Fruitone N, Rootone, Transplantone,
Tre-Hold



1-Naphthaleneacetic acid

The sodium salt of naphthaleneacetic acid (NAA) (source and purity not reported) was dissolved in broth (pH 7.2) at 50 ppm, diluted to 6.3 ppm, and sterilized (method not given). Knight's protein-free media broth was inoculated with 0.5 ml Staphylococcus aureus in saline solution and incubated for 24 hours (unspecified conditions). The growth was estimated by the method described in Late et al. (1949).

Lockemann Block's medium was inoculated with avian type tubercle bacilli and treated with NAA. The cultures were incubated for 5 days and the population was estimated by the method described in Late et al. (1949).

Human type tubercle bacilli were added to 10 ml of Long's media (pH 7.8) containing NAA and incubated for 3 weeks (unspecified conditions). After incubation the mass of tubercle bacilli was sterilized, centrifuged, washed with water, dried at 100 C and weighed.

Controls were included for each microorganism (presumably treated as above but omitting NAA).

REPORTED RESULTS:

NAA reduced the growth of S. aureus (as compared with controls) at 50 ppm but had no effect at 1.6-25 ppm. NAA had no effect on the growth of avian type tubercle bacilli (as compared with controls) at concentrations of up to 50 ppm. NAA at 63-125 ppm inhibited the growth (dry weight) of human type tubercle bacilli by 60-90%. Growth was completely inhibited by NAA at 250 ppm or above.

DISCUSSION:

The cultures of human type tubercle bacilli were incubated in pH 7.8 medium. This is above the optimum growth range (pH 7.6, Zinsser, Microbiology 15th ed.) for tubercle bacilli and may have affected the growth rate of the organism.

CHEM 50042 1= naphthaleneacetic acid

BRANCH REF DISC 2 TOPIC 1015 GUIDELINE 41-2 (163.62-8(f)(3))

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05019427 CURRENT CAT 1

Granlich, J.V.; Frans, R.E. (1964) Kinetics of "Chlorella" inhibition by herbicides. Weeds 12(5):375-379.

SUBST. CLASS = S.

DIRECT RVW TIME = 15 (MH) START-DATE END DATE

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CONCLUSIONS:

Metabolism - Effects of Pesticides on Microbes

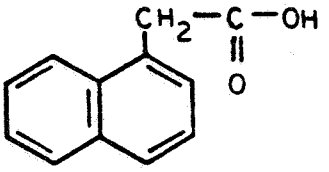
1. This study is scientifically valid.

2. When NAA was applied at 46 and 372 ppm, it inhibited the growth of Chlorella pyrenoidosa, a common soil alga, by approximately 40 and 80%, respectively.

3. This study helps to fulfill microbial metabolism data requirements in Section 163.62-8(f)(3) of EPA's Proposed Guidelines for Registering Pesticides (July 1978) by providing information on the effect of NAA on C. pyrenoidosa.

MATERIALS AND METHODS:

Naphthaleneacetic acid, NAA 800,
Fruitone N, Rootone, Transplantone,
Tre-Hold



1-Naphthaleneacetic acid

Cultures of Chlorella pyrenoidosa were grown in nutrient media (Knops' macroelement solution and Hutners' microelement solution chelated with ethylenediamine tetraacetic acid [concentration not given]) as modified for Chlorella by Meyers (1950) and incubated in a water bath (25 C) with continuous light. The growth was determined turbidimetrically.

Experimental cultures were grown in flasks containing 10 ml of nutrient media and 10 ml of an aqueous solution of naphthaleneacetic acid (NAA, 0.25-4.0 x 10^{-3} M; formulation, source, and purity not reported). Inoculated and uninoculated controls, identical in all respects except that NAA was deleted, were also included. Saturated compressed air was passed through a capillary tube into each culture flask throughout the growth period to prevent settling of the Chlorella cells. The cultures were incubated for 8-9 days.

A second experiment was conducted using the same procedures except that the cultures were incubated in a growth chamber (14-hour day at 29 C, with 2,200 footcandles of light and a 10-hour night at 22 C) for 5 days. The algal populations were determined turbidimetrically. The average density for each concentration was converted to percentage reduction of growth in the experimental flasks based on that in the controls.

REPORTED RESULTS:

When percentage inhibition was plotted as a function of the concentration of NAA, the hyperbolic response curve described by the rate equation $v = \frac{V_{max}(H)}{K_m + (H)}$ (where v is the percentage inhibition, H is the molar concentration of NAA, V_{max} is a constant representing the maximum velocity of the growth inhibition, and K_m is the concentration of NAA at 1/2 V_{max}) was produced.

Growth inhibition ranged from 40% at 2.5 x 10^{-4} M (46.5 ppm ; which is the K_m) to 80% at 2 x 10^{-3} M (372 ppm).

A nonlinear dosage-response relationship was obtained by subjecting the data to a probit analysis. This indicated that the inhibition of Chlorella growth by NAA was more efficient at low concentrations than at high concentrations (Figure 1).

DISCUSSION:

1. Aqueous concentrations of NAA ranging from 2.5 x 10^{-4} M (46 ppm) to 4.0 x 10^{-3} M (744 ppm) were used in the study. However, when NAA was applied at 744 ppm it was applied in excess of its solubility (420 ppm in water at 20 C; Herbicide Handbook, Weed Sci. Soc. Am., 1979). It is not known if NAA is soluble at 744 ppm under the conditions of this study.

2. The data for the experiment conducted in the growth chamber were not presented.

3. The double reciprocal plot of the rate equation shows a linear relationship up to the maximum solubility of NAA (2.0 x 10^{-3} M), thus indicating that inhibition of C. pyrenoidosa by NAA follows the laws of first-order kinetics.

4. It was stated that "saturated compressed air" was bubbled through the broth cultures. It is not known if the air was saturated with water. Although the sterility was not stated, it is assumed that this air entered the cultures aseptically.

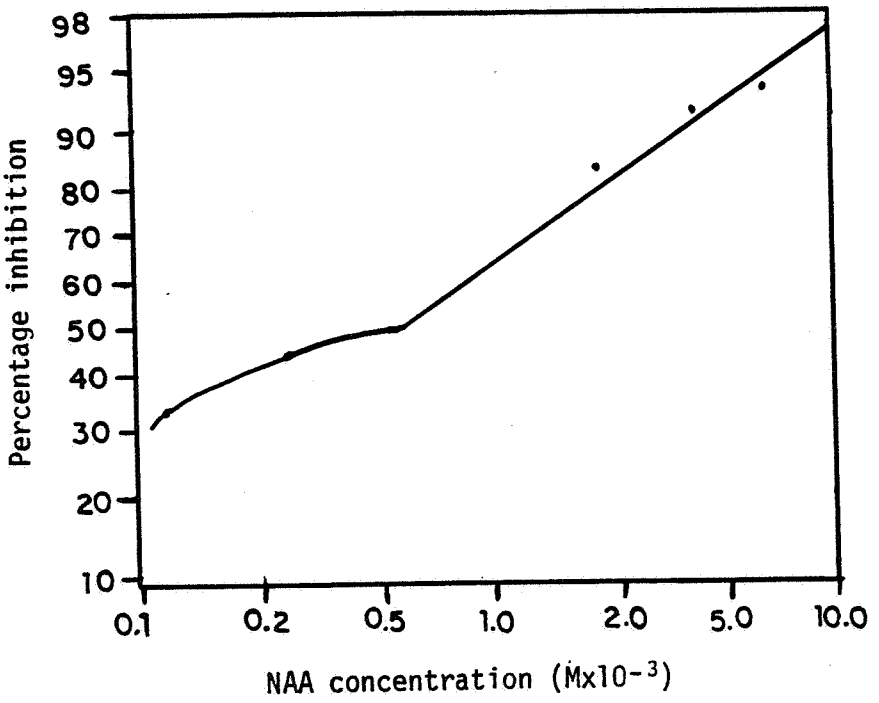


Figure 1. Probit analysis of the percentage inhibition of Chlorella pyrenoidosa as a function of the logarithm of NAA concentration.

TOXICOLOGY DATA EVALUATION RECORD PAGE 1 OF

CASE 050125 NAPHTHALENEACETIC ACID STUDY 5 DP BK 11/20/79

CHEM 055502 1-naphthaleneacetic acid

BRANCH EFB DISC 20 TOPIC 1.99 GUIDELINE 40 CFR 163.62-6.6

FORMULATION 00 = ACTIVE INGREDIENT

FICHE/MASTER ID 05010980 CONTENT CAT 01

Klein, D.T. (1962) Effect of growth-regulators on mutants of "Neurospora crassa". Physiologia Plantarum 15:237-245.

SUBST. CLASS = S.

DIRECT RVW TIME = 9 (MH) START-DATE END DATE

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CONCLUSIONS:

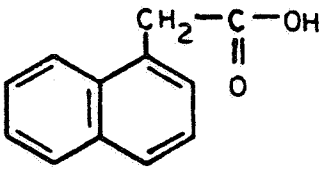
Metabolism - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Naphthaleneacetic acid (NAA) at 10-50 ppm severely inhibits (28-68%) a lysineless mutant (225) of Neurospora crassa. At less than 10 ppm, NAA had almost no inhibitory effect (<10%) on a p-aminobenzoic acidless mutant (1633A) of N. crassa, but at 30-50 ppm had a slight inhibitory effect (12-25%).

MATERIALS AND METHODS:

Naphthaleneacetic acid, NAA 800,
Fruitone N, Rootone, Transplantone,
Tre-Hold



1-Naphthaleneacetic acid

Naphthaleneacetic acid (NAA, Calbiochem; formulation and purity not reported) and p-aminobenzoic acid were added to 10 ml Fries' synthetic medium before autoclaving and inoculated with a p-aminobenzoic acidless (PABAless) auxotroph 1633A mutant of Neurospora crassa. The fungi were incubated in shake culture for 3-4 days at 26 C.

NAA was added to 10 ml Fries' synthetic medium and autoclaved, and then sterile lysine was added to the medium. The medium was inoculated with a lysineless colonial mutant 225 of N. crassa and incubated in stand culture at 26 C for 7 days.

The mycelia of both mutants were harvested, dried at 60 C in a vacuum oven, and weighed.

Linear growth rates were determined by growing the fungi at 26 C in growth tubes containing 10 ml of agar medium. The growth tubes were marked every 24 hours for 4 days for the PABAless mutant and every 48 hours for 14 days for the lysineless mutant to indicate the advance of the mycelia.

REPORTED RESULTS:

The growth (as measured by dry weight) of the lysineless mutant was reduced by 9% at 1 ppm NAA and 68% at 50 ppm NAA. The growth of the PABAless mutant grown with PABA at 0.5 µg/ml was reduced by 7 and 19% at 1 and 50 ppm NAA, respectively. When the mutant was grown in the presence of PABA at 0.002 µg/ml, NAA stimulated growth 7% at 1 ppm, had no effect at 10 ppm, and inhibited growth 12 and 25% at 30 and 50 ppm, respectively (Table 1).

The linear growth of controls for both mutants remained constant during the incubation period. NAA at 1, 10, and 50 ppm reduced the growth of the lysineless mutant by approximately 10, 25, and 20%, respectively. At 1 ppm, NAA had no effect on the growth of the PABAless mutant, but at 10 and 50 ppm it reduced growth by about 5 and 20%, respectively.

DISCUSSION:

The environmental importance of the sensitivity of N. crassa mutants to NAA is questionable.

Table 1. Effect of NAA on the dry weight of Neurospora crassa lysineless colonial mutant (225) and PABAless [a] mutant (1633A).

Mutant	Concentration of PABA (µg/ml)	Growth (% of controls) at different NAA concentrations (µg/ml)				
		0	1	10	30	50
PABAless	0.5	100	93	94	83	81
	0.002	100	107	100	88	75
Lysineless	0[b]	100	91	72	48	32

[a] PABA = p-aminobenzoic acid.

[b] Contains lysine at 50 µg/ml.

CASE 650023 NAPHTHALENEACETIC ACID STUDY 6 DATE 11/25/74

CHEM 050012 1-naphthaleneacetic acid

BRANCH EFB DISC 2 TOPIC 19 GUIDELINE 9-CFR 163.62-23

FORMULATION 00 = ACTIVE INGREDIENT

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Loveless, L.E.; Speert, E.; Weisman, I.M. (1954) A survey of effects of chemicals on division and growth of yeast and "Escherichia coli". Journal of Bacteriology 68(1):1.

SUBCL. CLASS = B.

DIRECT RVW TIME = 10 (MN) START-DATE END DATE

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CONCLUSIONS:

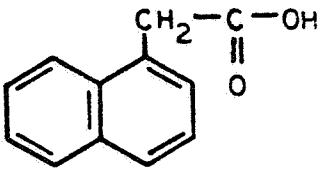
Metabolism - Effects of Pesticides on Microbes

1. This study is scientifically valid.

2. Naphthaleneacetic acid (NAA) at 300 and 500 ppm inhibits the growth of Escherichia coli and Saccharomyces cerevisiae, respectively, by about 50%. In addition, NAA inhibits the ability of S. cerevisiae cells to divide.

MATERIALS AND METHODS:

Naphthaleneacetic acid, NAA 800,
Fruitone N, Rootone, Transplantone,
Tre-Hold



1-Naphthaleneacetic acid

Yeast cells (Saccharomyces cerevisiae; isolated from Fleischmann's baker's yeast cake) were inoculated into sterile (sterilization technique not reported) liquid medium containing glucose, ammonium tartrate, asparagine, vitamins, and basal salts and incubated for 6-7 hours at 30 C. After centrifugation and washing, the cells were suspended in fresh medium and grown to a concentration of 1 x 10^7 cells/ml. Aliquots of the culture were transferred to smaller flasks containing different chemicals, including naphthaleneacetic acid (NAA; source, formulation, and purity not reported). The solvent used to dissolve NAA was not specified; however, the solvent reportedly was nontoxic. After incubation with shaking for 4 hours, aliquots of the culture were removed for cell mass and cell count determinations. Cell mass was determined by dry weight measurements. Cell counts were made with a hemacytometer.

Bacterial cells (Escherichia coli, strain B) from agar slants were suspended in sterile salts-glucose medium enriched with yeast extract and peptone, and incubated for 6-8 hours at 37 C. Cells were resuspended in sterile salts-glucose medium and allowed to grow with shaking to a concentration of about 4.5 x 10^8 cells/ml. Aliquots of the culture were then transferred into smaller flasks containing NAA or other chemicals to be tested and grown for 1.5 hours. Cultures were maintained at 0-4 C after the growth phase to prevent further growth and division. Cell counts were made with a Petroff-Hauser counter, and cell size was determined by dry weight measurements.

REPORTED RESULTS:

NAA at 500 µg/ml (500 ppm) inhibited the growth of S. cerevisiae by 58% and inhibited cell division (treated cells were 190% the size of control cells). At 300 µg/ml (300 ppm) NAA inhibited the growth of E. coli by approximately 50% but did not inhibit cell division.

DISCUSSION:

1. The study was performed using acceptable procedural protocols. However, data were not presented for all concentrations of NAA used. Only data for the concentrations at which growth of the organisms was inhibited by approximately 50% were presented.

2. Due to the large number of chemicals tested, the NAA data were limited. The data for nontoxic chemicals did not state the concentrations used for each chemical, but there was an implication that they were 300 and 500 ppm for E. coli and S. cerevisiae, respectively.

3. If ethanol or benzene were used as the NAA solvent, as was suggested in the study, then the sterility of the NAA could be reasonably assured. The brief incubation period (1.5 or 4 hours) of the logarithmically growing cultures, in any event, would be expected to render the sterility inconsequential, as noted in the study.