THABIT, T. M. A. M.¹, I. N. NASR¹ AND M. M. RASHED²

1- Central Agric. Pecticides Lab., ARC, Dokki, Giza

2- Biochemistry Dept., Fac. Agric., Cairo University, Giza

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Abstract

Bensultap insecticide (bancol 50% WP) was applied on soybean plant (i.e. genotype Giza-21). Determination of residues was performed on whole plant parts (i.e. foliage, peels and seeds) under the Egyptian normal field conditions. The recommended application rate of 75g active ingredient (a.i.) /100 L water was used. The photodecomposition rate was estimated by exposing bensultap a.i. to some of the effective environmental factors including the direct sunlight, short UV-rays and different temperature degrees. The probable influence on some of the chemical constituents induced by bensultap residues or metabolites remained in seeds were monitored. Recovery rate was done at two different spiking levels 0.1 and 1 mg/kg for all parts and values were greater than 90% for foliage and seeds and 80% for peels. Results indicated that the half-life values (RL₅₀) were 7.03 and 10.45 days for foliage and peels respectively while residue levels were below the limit of detection (0.01ppm) in seeds at all intervals till the harvest time. Direct sunlight was more effective than UVrays in accelerating the degradation rate of bensultap that RL₅₀ values were 5.00 and 5.96 hours for direct sunlight and UV-rays, respectively while it reached 171.88, 54.71, 26.27 and 11.10 hours for 25, 35, 45 and 55°C, respectively. Data indicated that there was not any significant effect on lipid, carbohydrate, ash, moisture, fiber contents and both of the measured elements K and Na, on the other hand crude protein and the organic nitrogen and Ca contents were significantly decreased, while P content was significantly increased.

INTRODUCTION

Bensultap [S,S-2-dimethylaminotrimethylene-di(benzenthiosulfonate], an insecticide developed by Takeda Chemical Industries, Ltd. (now Sumitomo Chemical Takeda Agro Company Ltd), an analogue or propesticide of the natural toxin nereistoxin. Acts as a synaptic blocking agent for the insect central nervous system. Bensultap is one of the recently used second generation insecticides although of the scarcity of data concerning bensultap toxicity unless it is less toxic and show higher affinity to insect than mammalian transmitter-receptors (Matsuda et al., 2001). Bensultap is one of the recommended pesticides for controlling soybean pests. Giza-

21 genotype is one of the new used soybean genotypes used in Egypt which marked by the high yield and resistance to cotton leaf worm which considered the major insect pest attacking crop.

The objective of this work was to estimate the residue levels of bensultap on and in some soybean plant parts (foliage, peels and seeds) of the genotype Giza-21. Photo and thermal decomposition rate under some of the environmental factors (i.e. direct sunlight, UV rays and different temperature degrees) was also investigated. The influence of bensultap residues or metabolites on some of the chemical constituents of seeds was also studied.

MATERIALS AND METHODS

1-Residue analysis:

1.1- Field Experiment, application and sampling procedures:

Plantation was performed at Sakha station for agricultural experiments at Kafr El-Sheikh governorate during the growing season of 2003. The experimental area was comprised to be a representative experimental plot about 70m², one plot applied as control (with no treatment). Sowing date of soybean seeds of the tested genotype Giza-21 was on June 13th, 2003. All plots received the good agricultural practices (GAP) throughout the experimental period. Formulation of bensultap (bancol, 50% WP) was applied on August 31st, 2003 using the recommended rate of 75g a.i. /100 L water. Samples were collected randomly from treated and untreated plots started one hour after treatment as the initial deposit (zero time) for all parts (i.e. foliage, peels and seeds), then 1, 3, 6, 10, 15, 20, 27 days later for foliage samples and 10, 20, 27 and 42 days (harvest time) after treatment for peels and seeds samples. Seeds were removed out from peels to have each separated along. Representative analytical samples have prepared of each foliage, peels and seeds in triplicates, with crushing of dry seeds to be fine flour using seed mill.

1.2- Extraction and cleaning up procedures:

Extraction procedure mentioned by Johnson and Stansbury (1966) was found to be suitable to extract bensultap residues from all soybean parts which adapted by using dichloromethane instead of chloroform. Samples of 30, 30 and 15g of foliage, peels and seeds, consecutively were macerated with 20g activated sodium sulfate anhydrous (Na_2SO_4) till complete mixing achieved then 150ml dichloromethane (HPLC grade) were added and blended for two minutes on high speed using warring blender. Extracts were dried through sodium sulfate anhydrous, and then a known volume of extract was taken and evaporated till dryness using rotary evaporator at 35°C. The method recorded by MacMahon and Hardin (1994) was used for cleaning up of bensultap residues extracts of soybean foliage, peels and seeds were dissolved in 20 ml ethyl acetate, then mixed with 4, 2 and 1g of the adsorbent mixture that consists of activated charcoal : Celite 545 at the ratio of 1 : 4 w/w, for foliage, peels and seeds extracts, respectively, then were shaken for two minutes and filtrated through activated sodium sulfate anhydrous, then precipitate rinsed with an additional 20ml ethyl acetate, and evaporated till dryness at 35°C.

1.3- Removal of oil from seed extract:

Oil removal from seed extract was carried out as described by MacMahon and Hardin (1994). This process is a very important step to prevent the interference of oils in determination, and considered so effective on sensitivity.

1.4- Determination of bensultap residues:

Knauer High Performance Liquid Chromatograph (HPLC) system equipped with Knauer variable wavelength detector, knauer online degasser and Knauer quaternary Maxistar K-1000 pump provided with Phenomenex Luna C18-5µ, (250 x 4.6 mm i.d.) 100A reversed phase HPLC column was used isocratically with mobile phase of acetonitrile : water at the ratio of 60 : 40 v/v, with flow rate of 1ml/min, was used to determine bensultap insecticide residues, detector wavelength was 210nm. Retention time at these conditions was 3.71min.

1.5- Effect of Environmental Factors studies:

To study the effect of the different environmental factors, aliquots of bensultap a.i. stock solution each representing 200µg a.i. in ethyl acetate were taken and spread as uniformly as possible in uncovered petri dishes (5cm i.d.) and were left to dry at room temperature then were subjected to the different treatments at successive periods. Exposure to direct sunlight for 0, 1, 3, 6, 12, 24, 48, 72 and 96 hours, where the dominant atmospheric temperature was 41±2°C, different temperature degrees at 25, 35, 45 and 55°C for 0, 1, 3, 6, 12, 24, 48, 96 and 144 hours and short UV-rays at 254nm wavelength at a distance of 12cm for 0, 1, 3, 6, 12 and 24 hours. Residues remained in petri dishes were quantitatively transferred into test tubes with known volumes with ethyl acetate, and then were determined with HPLC as mentioned previously.

1.6- Recovery Efficacy Studies:

To examine the efficacy and the limit of quantitation of the used method, untreated samples of foliage, peels and seeds were spiked with bensultap a.i., solution at two levels 0.1 and 1 mg/kg for all matrices, and then procedures of the entire method mentioned were performed. Recovery values were greater than 90%

for foliage and seeds and 80% for peels, for both levels at limit of detection (LOD) 0.01ppm.

1.7- Kinetic studies:

The degradation rate of bensultap insecticide was calculated mathematically according to Timme and Frehse (1980), that degradation behaviour of pesticide residues can be described mathematically as a pseudo-first order reaction, that rate of degradation (K) could be calculate using common logarithms from the following equation:-

 $\log R = \log R_0 - 0.434Kt$ $\log R_0 - \log R$

$$Kt = \frac{\log R_0}{0.434}$$

 R_0 : the residue level at the initial time (zero time).

R : the residue level an interval in days after pesticide application.

Kt: the degradation rate constant at the successive intervals in days.

K: the mean of Kt

-The half-life values (RL₅₀) were calculated mathematically according to Moye *et al.* (1987) from the following equation:-

2- Determination of some biochemical constituents in seeds:

2.1- Proximate analysis:

Moisture, lipid, fiber and ash contents of soybean seeds of tested genotypes were estimated according to A.O.A.C., (2004),

Carbohydrate content was extracted from seeds with aid of acid hydrolysis according to A.O.A.C. (2004), and estimated spectrophotometrically at 490nm, according to Dubois *et al.* (1956).

2.2-Estimation of organic nitrogen content:

The organic nitrogen content was estimated using micro-Kjeldahl method as mentioned in the A.O.A.C. (2004), then total protein content was calculated by multiplying total N \times 6.25

2.3-Estimation of total phosphorus content:

Total phosphorus content was determined spectrophotometrically at 650nm using Beckman DU-7400 spectrophotometer in presence of blank and potassium dihydrogen phosphate as a standard, according to the A.O.A.C. (2004).

2.4-Estimation of Elemental composition:

Na, Ca and K contents were determined in the digested solution using Thermo Jarrell Ash (TJA) AA- Scan1 atomic absorption system, as mentioned in the A.O.A.C. (2004).

2.5-Statistical analysis:

Student's *t*-test was used for analyzing the obtained data statistically to define the significance levels with the procedure outlined by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1- Bensultap residues:

Table (1) show residues and loss rates of bensultap insecticide on and in soybean foliage of the tested genotype Giza-21. The initial deposit which determined on foliage one hour after treatment was 37.48ppm, this amount dropped to 32.66ppm one day after application indicating a loss rate of 12.86%. Residues gradually decreased to 0.51ppm with loss rate of 98.63% after 27 days from application. RL_{50} value was 7.03 days from application with degradation rate of 0.0985.

Table 1. Residues and loss rates of bensultap insecticide on and in soybean foliag	ge of
the tested genotype Giza-21 at rate of 75g a.i./100 L water.	

	foliage of Giz	a-21 Soybean genotype			
Time after application in days	Residue*	Loss			
	(ppm)	(%)			
0**	37.48±1.18	00.00			
1	32.66±1.22	12.86			
3	24.52±0.83	34.57			
6	18.80±0.98	49.84			
10	14.83±1.05	60.43			
15	9.06±0.66	75.82			
.20	5.95±0.36	84.12			
27	0.51±0.32 98.63				
K	0.0985				
RL ₅₀ (days) 7.03					

* : Each value represents an average of three replicates.

** : Samples were taken one hour after application (zero time).

LOD : 0.01 ppm.

RL₅₀ : Half-life value.

K : Constant of degradation rate.

Mean ± Standard Deviation (SD).

Residues and loss rates of bensultap insecticide on and in soybean peels and seeds of the tested genotype Giza-21 were demonstrated in Table (2). The initial deposit of bensultap residues on and in peels were 15.90ppm, decrease to 8.88ppm indicating loss rate of 44.15% at 10 days after treatment. Residue levels decreased to 0.16ppm indicating loss rate of 98.99% at the harvest time (42 days after application). The calculated RL₅₀ value was 10.45 days after treatment with degradation rate (K) of 0.0663. Seeds at all intervals were free of any bensultap detectable residues till the mature seeds at harvest time which were free from any detectable residues of bensultap at detection limit of 0.01ppm too. The obtained data are in agreement with the findings reported by Mojasevic et al. (1996) and Rajkovic et al. (2002) which declared that all plant commodities treated with bensultap were in the safety limit at the harvest time. Concerning health aspects, the maximum residue, limit (MRL) of bensultap residues on soybean recorded by the Japan Food Chemical Research Foundation (2006), is (3.0ppm), so the pre-harvest interval value (PHI) for bensultap on soybean should be 22 days from application on the vegetative parts to be safe for human consumption.

	Giza-21 soybean genotype						
Time after application in days	Pe	Seeds					
	Residue**	Loss (%)	Residue	Loss (%)			
0*	15.90±1.18	00.00	ND				
10	8.88±0.35	44.15	ND				
20	4.14±0.25	73.96	ND				
27	1.18±0.18	92.57	ND				
42	0.16±0.12	98.99	ND				
K	0.00						
RL ₅₀ (days)	10.						

Table 2. Residues and loss rates of bensultap insecticide on and in soybean peels and seeds of the tested genotype Giza-21 at rate of 75g a.i./100 L water.

: Samples were taken one hour after application (zero time).

** : Each value represents an average of three replicates.

LOD : 0.01 ppm.

ND : Not detected.

42 days: Harvest time.

RL₅₀ : Half-life value,

K : Constant of degradation rate.

Mean±SD.

2-Bensultap photo and thermal-decomposition:

Data in Table (3) show the photodecomposition of bensultap insecticide a.i. when exposed to the direct sunlight and short wave UV-rays. Data indicated that generally exposure to direct sunlight was more effective than exposure to UV-rays in accelerating the rate of photodecomposition, that loss rate values after one hour of exposure were 24.85 and 19.19% reached to 90.38 and 91.07% after 24 hours of the exposure to direct sunlight and short UV-rays, respectively. The estimated RL₅₀ values were 5.00 and 5.96 hours, for sunlight and UV-rays exposure, respectively and the estimated degradation rate (K) was 0.1387 and 0.1161, respectively.

Table 3. Influence of direct sunlight (atmosphere temp. about 41°C±2) and short UV-

Time of	Direct su	unlight	UV-rays		
exposure in hours	µg**	Loss (%)	hđ	Loss (%)	
0* .	200.00±0.02	00.00	200.00±0.14	00.00	
. 1	147.30±2.13	24.85	161.61±1.12	19.19	
3	103.15±1.88	44.92	136.87±1.33	31.56	
6	74.36±0.86	62.82	96.11±1.98	51.94	
12	40.34±1.05	79.83	40.14±2.07	79.93	
24	14.23±0.22	90.38	17.85±2.18	91.07	
48	4.14±0.09	95.93			
72	0.25±0.16	99.87			
96	BDL		÷		
К	0.13	87	0.11	61	
RL ₅₀ (hours)	5.0	0	5.9	6	

rays on the dissipation rate of bensultap insecticide a.i.

: Samples were taken directly after the quantitative transfer (zero time).

** : Each value represents an average of three replicates.

BDL : Below detection limit.

: Constant of degradation rate. K

: Half-life value. **RL**50

LOD : 0.01ppm.

Mean±SD.

Table (4) shows the effect of exposure to different temperature degrees on bensultap a.i.. Data indicated that bensultap influenced by increasing temperature degree and period of exposure. The loss percentage values of bensultap residues were 3.85, 13.23, 19.09 and 46.76% after the first 6 hours from exposure at 25, 35, 45 and 55°C, respectively. While values were 24.78, 62.43, 94.87 and 99.58% after 144 hours from exposure at the same previous temperature degrees, respectively. The calculated RL_{50} values of bensultap residues were 171.88, 54.71, 26.27 and 11.10 hours after exposure, indicating degradation rates (K) of 0.0040, 0.0127, 0.0264 and 0.0624 at 25, 35, 45 and 55°C, respectively.

The obtained data agreed with the findings of Barakat *et al.* (2006) and Eissa *et al.* (2006) who mentioned that bensultap showed a high degradation when exposed to the direct sunlight and high temperature degrees (55°C), so it is recommended to use bensultap in areas with dominant temperature not exceed 30°C. Degradation rate is also positively correlated with the period of exposure and chemical structure. UV-rays produce many chemical changes such as hydrolysis, oxidation and isomerization in large number of pesticides. The radiation energy produced from UV-rays and direct sunlight might be absorbed by a pesticide molecule so that it may increase the transitional, rotational or electronic energy of the molecule.

Generally sunlight proved to bemore effective than UV-rays in accelerating photodecomposition rate and this may due to thermal, evaporational and light intensity considerations.

2-Effect of bensultap insecticide residues on some biochemical constituents in seeds:

Proximate analysis of soybean seeds of the tested genotype Giza-21 and the influence of bensultap on the biochemical constituents and macro elements in seeds during the successive intervals 0, 10, 20, 27 and 42 days (harvest time) were illustrated in Tables (5) and (6). Data indicated that there was not any significant effect of bensultap application on lipid, carbohydrate, ash, moisture, fiber contents and both of the measured elements K and Na. On the other hand crude protein, organic nitrogen and Ca contents were significantly decreased, while P content was significantly increased, depending upon statistical analysis using *t*-test at $P \le 0.05$. The obtained results are in agreement with the reaveled findings reported by Thabit (2002) and Hegazy *et al.* (2006) for all measured component except protein results which was in the same direction of those found by Ismail *et al.* (1993).

Time of	25°C		35°C		45°C		55°C	
exposure in hours	, µg**	Loss (%)	þð	Loss (%)	þð	Loss (%)	hð	Loss (%)
0*	200.00±0.04	00.00	200.00±0.09	00.00	200.00±0.05	00.00	200.00±0.07	00.00
1	198.42±0.60	0.79	196.09±1.28	1.95	191.66±1.11	4.17	178.90±0.16	10.55
3	196.27±0.38	1.86	187.88±0.33	6.06	177.58±1.83	11.21	140.45±1.90	29.77
6	192.29±0.98	3.85	173.54±1.41	13.23	161.81±1.70	19.09	106.47±1.44	46.76
12	188.94±2.12	5.53	165.05±0.20	17.47	132.51±0.70	33.74	85.57±1.51	57.21
24	183.19±0.97	8.40	155.39±1.11	22.30	117.28±1.01	41.36	55.81±1.00	72.09
48	175.05±0.63	12.47	135.04±1.35	32.48	66.12±1.49	66.94	37.54±1.38	81.23
96	159.46±0.58	20.27	90.92±1.38	54.54	30.64±0.69	84.68	10.94±1.41	94.53
144	150.43±2.19	24.78	75.14±1.47	62.43	10.25±0.04	94.87	0.84±0.23	99.58
к	0.0040		0.0127	0.0127			0.0624	
RL ₅₀ (hours)	171.88		171.88 54.71 26.27			11.10		

- I. 1.00

Table 4. Influence of different temperature degrees (i.e. 25, 35, 45 and 55°C) on the dissipation rate of bensultap insecticide a.i.

: Samples were taken directly after the quantitative transfer (zero time). : Each value represents an average of three replicates. *

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Constant of degradation rate. Half-life value. Κ

1 2

RL₅₀

LOD : 0.01ppm.

Mean±SD.

2333

Table 5. Influence of bensultap insecticide on protein, lipid, carbohydrate, ash, fiber and moisture content of soybean seeds of tested genotype Giza-21 at the successive intervals, on dry weight basis.

	Constituents											
Time after	Prote	ins	Lin	Lipids		Lipids Carbobydrates Ach		Fiber		Moisture		
treatment	(0/	\			(0/)				(0())		(94)	
(davs)	(%	· · ·	(9	(%)		<u>(0)</u>	(9	(o)	(%)		(%)	
(00)0)	C** `	т	С	т	С	т	С	т	С	т	С	т
		1						-				
0*	43.65±0.80	40.20±1.83	23.80±0.96	23.21±0.30	15.38±0.20	15.33±0.15	6.19±0.35	6.11±0.09	5.14±0.08	5.11±0.09	69.71±0.86	69.55±0.81
10	42.90±1.76	39.85±0.67	23.36±1.95	23.20±1.10	15.34±0.15	15.11±0.18	6.11±0.21	6.08±0.02	5.17±0.24	5.16±0.22	65.51±0.86	65.48±0.84
20	42.89±1.15	42.65±1.55	22.92±1.37	22.29±0.18	15.24±0.27	14.58±0.35	6.15±0.29	6.08±0.13	5.12±0.10	5.13±0.04	56.40±1.09	56.98±1.15
27	42.98±0.53	42.86±2.69	23.26±1.81	23.20±1.05	15.23±0.16	15.20±0.09	6.15±0.21	6.12±0.14	5.18±0.14	5.19±0.26	7.81±0.43	7.58±0.34
42	43.99±0.17	42.94±3.13	23.36±1.81	23.15±1.10	15.61±0.26	15.52±0.23	6.14±0.33	6.14±0.22	5.18±0.28	5.18±.22	6.13±0.31	6.16±0.35

* : Samples were taken one hour after application.

** : Each value represents an average of three replicates.

C : Control

: Treatment

42 days : Harvest time Mean±Standard Deviation

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Table 6. Influence of bensultap insecticide on N, P, K, Ca and Na elements content of soybean seeds of tested genotype Giza-21 at the successive intervals, on dry weight basis.

T 0		2			Elem	ients	(a)			
treatment	N (%)		P (g/kg)		K (mg/kg)		Ca (g/kg)		Na (mg/kg)	
(days)	, C**	Τ	C	Т	Ċ	Т	С	Т	С	Т
0*	6.98±0.12	6.43±0.29	5.81±0.11	6.61±0.07	7.50±0.18	7.25±0.33	1.71±0.01	1.52 ± 0.01	6.88±0.28	6.18±0.18
10	6.86±0.28	6.37±0.10	6.62±0.10	7.42±0.03	7.35±0.20	7.89±0.21	1.65±0.02	1.55 ± 0.01	7.18±0.32	6.92±0.13
20	6.86±0.18	6.82±0.24	6.89±0.07	6.89±0.07	7.36±0.21	7.18±0.27	1.51±0.03	1.28±0.01	6.69±0.33	6.94±0.22
27	6.87±0.08	6.85±0.43	7.75±0.06	7.45±0.03	6.83±0.31	7.01±0.42	1.67±0.02	1.69 ± 0.01	6.70±0.30	6.55±0.42
42	7.03±0.02	6.87±0.50	7.92±0.07	7.98±0.05	7.98±0.14	7.80±0.31	1.79±0.01	1.73±0.01	7.22±0.21	7.40±0.40

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* : Samples were taken one hour after application.

** : Each value represents an average of three replicates.

C : Control

T : Treatment

42 days : Harvest time

Mean±Standard Deviation

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سلوك متبقيات المبيد الحشري بنسولتاب علي نبات فول الصويا مع القاء الضوء على معدل تحلله الضوئي و الحراري كذلك تأثيره علي بعض المكونات الكيميائية للبذرة

تامر محمد احمد محمد ثابت ، إسلام نعمان نصر ، محمد مجدي راشد

١- المعمل المركزي للمبيدات – مركز البحوث الزراعية – دقى – جيزة
 ٢- قسم الكيمياء الحيوية – كلية الزراعة – جامعة القاهرة

تم اجراء هذه الدراسة بهدف تقدير ثبات المبيد الحشري بنسولتاب على نبات فول الصويا صنف جيزة-٢١ على أجزاء النبات المختلفة (أوراق-قشور-بذور) على فترات متعاقبة بعد المعاملة بالمعدل الموصى به تحت الظروف المصرية. كذلك تم رصد معدل تحلل هذا المركب بيئيا عن طريق التعريض لأشعة الشمس المباشرة و اشعة UV و درجات الحرارة المختلفة كذلك تــأثير هــذه المتبقيات الموجودة في البذور او نواتج تمثيلها على بعض المكونات الكيميائية بها. أوضحت الدراسة أن فترة نصف العمر لمبيد البنسولتاب كانت ٧,٠٣ و ١٠,٤٥ يـوم لكـل مـن الأوراق و القشور على التوالي بينما لم تكن هناك أي متبقيات موجودة في البذور التي جمعت على مدار الفترات المختلفة ابتداء من بعد المعاملة مباشرة و حتى الحصاد جيث كانت اقل من جدود القياس للمبيد و هي ٠.٠١ جزء في المليون. كذلك أوضحت النتائج أن تعريض المادة الفعالة للبنـسولتاب لضوء الشمس المباشر كان له تأثيرا اكبر على سرعة تحلل المركب ضوئيا أكثر من التعريض للأشعة فوق البنفسجية حيث كانت فترة نصف العمر هي ٩,٠٠ و 5.96 ساعة على التـوالي بينمــا التعريض لدرجات الحرارة المختلفة اوضح انه كلما زادت درجة الحرارة و زمن التعبريض زاد معدل التحلل اى ان اشعة الشمس المباشرة كان لها التاثير الأكبر في زيادة معدل التكسير متناسبا طرديا مع زمن التعريض. اظهرت النتائج ايضا انه لم يكن هناك اى تاتير معنوى للمعاملة بهذا المبيد على كل من محتوي البذور من الليبيدات، الكربو هيدرات، الرماد، الألياف الخام و الرطوبة على مدار الفترات المختلفة من بداية المعاملة وحتى الحصاد بينما ادت المعاملة للنقص المعنوي في محتوي كل من البروتين الخام و النيتروجين العضوي و عنصر الكالسيوم. على الجانب الاخر ادت المعاملة للزيادة المعنوية في محتوي عنصر الفسفور بينما عنصري البوتاسيوم و الصوديوم لم يحدث لها اي تاثير.

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