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BIOEFFICACY OF LIQUID FORMULATION OF *BACILLUS THURINGIENSIS* BT_{III} AGAINST *HELICOVERPA ARMIGERA* UNDER FIELD CONDITION IN DIFFERENT FIELDS

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ABSTRACT
Bacillus thuringiensis is a promising agent to control a number of insect pests of order Lepidoptera. A formulation of potential *Bacillus thuringiensis* strain would be beneficial for controlling *Helicoverpa armigera* (a major pest in various crops) larvae. A formulation with high efficacy and low cost would add to the characteristic features of *Bacillus thuringiensis*. In the present study, liquid formulations of *B. thuringiensis* were prepared by using five different types of stabilizers: glycerol, groundnut oil, mustard oil, mineral oil, and sunflower oil. Persistence of Bt cells was monitored at weekly interval. It was found that glycerol supported maximum persistence of Bt cells when formulations were stored at room temperature. Glycerol based formulation showed minimum decline in cell number and could be used to control *H. armigera* larvae for two months.

INTRODUCTION

In recent years, *Bacillus thuringiensis* is receiving increasing attention for its use in integrated pest management programs for agricultural and forest insect pests and insect vectors of human and other mammalian transmissible diseases. Taxonomically, these entomopathogenic bacteria are in the family Bacillaceae and belong to genus *Bacillus*. Typically, they are rod-shaped, form a spore and are motile by flagella. The unique characteristic of this organism is that it produces an insecticidal protein crystal next to the spore at the time of sporulation. *B. thuringiensis* is pathogenic to numerous species of agricultural and forest insect pests and is a component of the soil microbiota worldwide (Martin and Travers, 1989). Many different strains of *B. thuringiensis* (*Bt*) have been isolated from different types of soils; however, most strains used in commercial production of microbial insecticides have been isolated from diseased insects (DeLucca *et al*., 1981).

B. thuringiensis directly causes mortality in insects, and toxins from different strains have similar modes of action. In susceptible insects, the alkaline midgut environment (pH > 8.0) and proteolytic enzymes dissolve ingested crystals and release smaller delta-endotoxins. These proteins, also known as the insecticidal crystal proteins (ICP's), bind to specific receptors on the cellular lining of the midgut. Depending on the Bt strain used, one or several different types of ICP's may be released from the crystal matrix. Once bound to the receptors, ICP's penetrate through the cell membrane and form ion-selective channels. The selective permeability characteristic of the cell membrane is then disrupted, causing the cell to absorb water, swell, and burst. This results in a perforation of the gut and leakage of gut content, including spores, into the hemolymph. At this point, gut paralysis (and in some cases, paralysis of the mouthparts) occurs, the larva stops feeding, and dies in a few hours to a few days (Reardon *et al*., 1994).

B. thuringiensis is commercially produced by liquid fermentation. When cell division is complete, a spore and a diamond-shaped protein inclusion referred as crystal are formed within the vegetative cell (sporangium). At the completion of spore formation, the wall of the sporangium breaks down releasing both the spore and crystal into the growth medium (Dubois and Lewis, 1981). The spores, crystals and other residual fermentation solids are then harvested, stabilized and used for preparation of commercial product. Therefore, commercial formulations of Bt contain both the spore and crystal as their entomopathogenic ingredients.

A microbial pesticide formulation is a physical mixture of Bt cells along with media ingredients which provides effective and economic control of pests. In commercial development of a basic formulation of an entomopathogen, technology concerns maintaining pathogen viability and virulence during the production process and developing a product form which preserves or enhances these properties. Formulations capable of improving persistence would be advantageous because fewer applications would be required to achieve comparable levels of control (Dhingra and Chaudhary, 2005). Different types of formulations like dust or wettable powder, granules, liquid, fumigant, aerosols are in use these days.

The American bollworm, *Helicoverpa armigera* (lepidoptera) is one of the most serious pests of different crops in many parts of the world and is controlled by the use of chemical insecticides. Recently there are a few reports on the development of resistance by *Helicoverpa armigera* to chemical insecticides due to their indiscriminate and excessive

use. Therefore, there is a need to explore potential microbial insecticides so that losses due to attack by *H. armigera* could be minimized. The use of *B. thuringiensis* based insecticides offer various advantages over harmful chemical insecticides being target specific, biodegradable and economical. In view of this, the present investigation was carried out to evaluate the (bio) efficacy of Liquid formulation of *B. thuringiensis* based insecticides against *H. armigera*.

MATERIALS AND METHODS

Cultures

The native isolate of *Bacillus thuringiensis* (Bt_{III}) having better insecticidal activity was obtained from Microbial biotechnology lab of the BMB department of CCS HAU, Hisar. The cultures were purified by restreaking on LB agar plates several times and maintained on LB agar slants with 20% glycerol at 4ºC. Agro-industrial based media (1.2% potato extract, 1.0% cotton seed meal in minimal media containing Peptone 2g/L, Dextrose 1.5g/L, Yeast Extract 2g/L, $MgSO_4.7H_2O$ 0.03g/L, $FeSO_4.7H_2O$ 0.02g/L, $ZnSO_4.7H_20$ 0.02g/L, NaCl 5g/L, Tween 60 1mL/L) was used for the biomass production of *B. thuringiensis*.

Preparation of liquid formulation and sterilization

Liquid formulations of Bt_{III} was prepared by using five different stabilizers viz. glycerol, groundnut oil, mustard oil, mineral oil, and sunflower oil. One hundred ml of glycerol, groundnut oil, mustard oil, mineral oil, and sunflower oil were transferred to 250mL autoclavable plastic bottles. These bottles were loosely capped and sterilized at 15 lb/sq inch for 20 min. After cooling, the bottles were removed from the autoclave and capped tightly.

B. thuringiensis Bt_{III} cells were grown in different 2L Erlenmeyer flasks for 48h at 30ºC and allowed to settle at the bottom by adding sterilized mixture of 1% agar and bentonite to it. The supernatant was removed and cells were harvested to prepare Bt suspension of $\sim 10^{12}$ cells/mL (estimated by spread plate counting method). One hundred ml of this Bt suspension was aseptically poured into the bottles carrying different stabilizers. The contents of the bottles were mixed thoroughly and kept at room temperature (~30ºC) under lab conditions.

Table 1: Evaluation of insecticidal activity of *B. thuringiensis* Bt_{III} under lab condition

Isolates	No. of larvae	Larval mortality (%) 0h	24h	48h	72h	96h	120h
Bt_{III}	40	0	50±5	75±2	100±2	100	100
Control	40	0	0	0	0	0	0
S.E.(±)		0.000	2.229	3.502	1.291	1.798	1.586
C.D.(p≤0.05)		0.000	3.381	3.599	4.275	4.955	3.254

Insect bioassay

The second instar larvae of *H. armigera* were collected from the farmers' field (Fetehabad, Haryana), which were infested with *H. armigera*. These larvae were transferred one each to bioassay vial and starved for 24h at 30ºC (so that these larvae feed on the treated leaves vigorously). A cell suspension of *B. thuringiensis* Bt_{III} isolate (@$1X10^8$ cells/mL) was prepared in sterilized distilled water. The fresh leaves were dipped in this suspension and dried. These leaves were fed to the second instar larvae of *H. armigera*. In control sample, untreated leaves were given to the larvae. Percent mortality was monitored at an interval of 24h up to 120h. The non-food grade starch was added to the Bt suspension (@0.1%), which acted as sticker and spreader for Bt formulation on foliage.

Evaluation of persistence of *B. thuringiensis* cells in the liquid formulations

The total number of viable cells in the liquid formulation was determined at an interval of seven days. The contents of the formulation were mixed thoroughly before removing the samples. The sample (1mL) was aseptically removed and transferred into 9mL of water blank. Serial dilutions were made and 50μL of suspension was plated on LB medium plates.

Table 2: Bioefficacy of liquid formulations of *B. thuringiensis* Bt_{III} against *Helicoverpa armigera* larvae in cotton field

Formulation	Larval population per 10 plants days after spray 0	3	5	7	%Mortality
Glycerol	38.00 ± 1.73	16.00 ± 0.57	12.25 ± 0.00	9.00 ± 0.57	76.3
Mustard oil	37.00 ± 0.57	14.75 ± 0.00	10.50 ± 0.57	9.00 ± 0.00	75.6
Groundnut oil	40.00 ± 1.15	23.25 ± 0.57	21.00 ± 0.57	21.00 ± 0.57	47.5
Mineral oil	47.00 ± 1.15	22.00 ± 0.57	23.50 ± 0.00	21.00 ± 0.00	55.3
Sunflower oil	37.00 ± 1.15	22.75 ± 0.57	19.25 ± 0.57	18.00 ± 0.57	51.4
Control	54.00 ± 0.57	54.00 ± 1.15	54.50 ± 0.00	54.00 ± 1.15	0.00
C.D.(p≤0.05)	3.762	1.542	1.303	1.990	
S.E.(±)	1.179	0.483	0.408	0.624	

Table 3: Bioefficacy of liquid formulations of *B. thuringiensis* Bt_{III} against *Helicoverpa armigera* larvae in gram field

Formulation	Larval population per 10 plants days after spray 0	3	5	7	% Mortality
Mustard oil	58.00 ± 1.15	35.00 ± 1.15	23.50 ± 1.73	17.00 ± 1.15	70.68
Glycerol	95.00 ± 2.88	66.50 ± 3.46	38.00 ± 1.73	15.00 ± 1.73	84.21
Groundnut Oil	75.00 ± 1.15	53.00 ± 1.73	37.50 ± 1.15	26.00 ± 1.15	65.00
Sunflower oil	42.00 ± 1.73	23.00 ± 1.15	18.50 ± 0.57	13.00 ± 0.57	69.00
Mineral oil	83.00 ± 1.73	60.50 ± 2.88	37.00 ± 1.15	20.00 ± 1.15	75.90
Control	63.00 ± 1.15	60.00 ± 1.15	61.00 ± 0.57	60.00 ± 2.88	4.0
C.D.(p≤0.05)	5.640	6.862	3.670	5.620	
S.E.(±)	1.767	2.150	1.150	1.761	

Table 4: Bioefficacy of best liquid formulation of *B. thuringiensis* Bt_{III} against *Helicoverpa armigera* larvae in tomato field

Formulation	Larval population per 10 plants days after spray				% Mortality
	0	3	5	7	
Glycerol based Bt_{III} liquid formulation	64.00 ± 1.73	38.75 ± 1.15	21.50 ± 1.15	16.00 ± 1.15	75.00
Halt (+ve control)	65.00 ± 2.30	35.50 ± 1.73	17.00 ± 1.15	15.00 ± 1.15	76.92
Control	65.00 ± 0.00	65.00 ± 0.57	65.00 ± 0.00	65.00 ± 0.00	0.00
C.D.($p \leq 0.05$)	3.678	4.525	4.402	3.895	
S.E.(±)	1.111	1.366	1.329	1.176	

These plates were incubated at 30ºC for 24h in a B.O.D. incubator. Colonies appearing on the plates were counted and cfu/mL in the liquid formulation was calculated.

Evaluation of bioefficacy of different Liquid formulations of *B. thuringiensis* Bt_{III} under field conditions

The bioefficacy of different liquid formulations of *B. thuringiensis* Bt_{III} was evaluated in cotton, gram and tomato fields which, after survey, were found to be highly infested with *H. armigera*. The experiments were conducted after the emergence of *H. armigera* attack when most of the larvae were of first or second instar. Different liquid formulations of *B. thuringiensis* isolate Bt_{III} were applied in these fields @ 10^8 cells/mL with the help of conventional sprayer. Non-food grade starch (0.1%) and SDS (0.01%) were added in all Bt formulations just before spray. In control plants, sterilized distilled water containing starch and SDS was applied on to the leaves. The larval count was made at 0, 3, 5 and 7 days after application of Bt formulation and percent mortality was calculated.

Experimental results

To monitor the bioefficacy of Bt_{III} culture against *H. armigera* under lab condition, second instar larvae of *H. armigera* were fed with Bt treated leaves (@1X10^8 cells/mL) and decrease in number of larvae was observed over a period of 5 days. It was observed that *B. thuringiensis* isolate Bt_{III} showed 100% larval mortality after 3 days of treatment (Table 1). In control no larval mortality was observed even after 5 days.

Persistence of *B. thuringiensis* isolates in liquid formulation

Liquid formulation is generally composed of culture broth. Sometimes additives such as vegetable oils are also used which act as stabilizer of cells. Glycerol is being used as preservative of microbial cultures. Liquid formulation was prepared by using five different stabilizers (glycerol, groundnut oil, mustard oil, mineral oil, and sunflower oil). It was observed that *B. thuringiensis* cells were equally present in all types of liquid formulations up to 21 days. Thereafter, the viable cell number starts declining rapidly. The persistence of *B. thuringiensis* isolates Bt_{III} was maximum in glycerol based formulation (Fig.1 to 2). There was a sharp decline in viable cell number of *B. thuringiensis* isolates Bt_{III} and S_6 in LB medium (control) when no stabilizer was added in the formulation (Fig. 3).

This indicated that some stabilizers did not affect the survival of *B. thuringiensis* and cell number remained constant upto 30 days. In glycerol based formulation, cells of *B. thuringiensis* isolates showed maximum persistence. The viable cell number of Bt cells was reduced from 3.0 X 10^{12} cfu/ml to 1.4 X 10^{10} cfu/mL after 90 days in glycerol based formulation of *B. thuringiensis* isolate Bt_{III} (Fig. 1); whereas other formulations showed significant reduction in the cell number of Bt cells.

To determine the bioefficacy of liquid formulation of *B. thuringiensis* Bt_{III} against *H. armigera* in cotton (Table 2) and gram (Table 3) fields, the liquid formulations of *B. thuringiensis* @ 10^8 cells/mL were sprayed in the farmer's field with the help of a sprayer. The bioefficacy of each formulation was determined by estimating the reduction in number of larvae in 10 plants at different days. In control, the plants were sprayed with sterilized distilled water. Halt (commercial synthetic insecticide used against *H. armigera* was used as positive control. The best liquid formulation was again rechecked against *H. armigera* in tomato field (Table 4).

To determine the LC_{50} dose of *B. thuringiensis* isolate Bt_{III}, the second instar larvae of *H. armigera* were fed with different concentrations of isolated insecticidal crystal proteins (ICP) of *B. thuringiensis* isolates. The LC_{50} value of *B. thuringiensis* isolates was calculated by determining the ICP concentration at which 50% larval mortality was observed. It was observed that when 8mg/mL of ICP of *B. thuringiensis* isolate Bt_{III} was given to the *H. armigera* larvae, 50% of larvae died after 48h (Table 5). The commercial formulation Halt @ 5mg/mL showed 50% larval mortality after 24h. This indicated that the LC_{50} dose of *B. thuringiensis* isolate Bt_{III} was 8mg/mL at 48h.

RESULTS AND DISCUSSION

Designing a cheap, industrial fermentation medium is one of the most important aspects of process development. *B. thuringiensis* strains are known to utilize a large number of different carbon and nitrogen sources for growth and delta-endotoxin production (Schisler *et al.,* 2004). Use of molasses for Bt based insecticide production has been reported (Icgen *et al.* 2002). Various agricultural nitrogen sources like deoiled and expeller cakes of mustard, cottonseed, groundnut, corn steep liquor have been used to design low cost fermentation media in batch culture process (Johnson *et al.,* 1994; Dhingra and Chaudhary, 2007). In the present study, agricultural products and by-products in the form of 1.2% potato extract and 1% cotton seed meal added in the basal medium was used as sources of nutrients for large scale production of *B. thuringiensis* biomass.

Tablet formulation (Zhang *et al.,*1997; de Melo-Santos *et al.,* 2001, Medeiros *et al.,* 2005), microgel formulation (King *et al.,* 1997), granular matrix formulation (Ridgway *et al.,* 1996), ice granule formulation (Becker, 2003), oil and water based formulation (Dennett *et al.,* 2000) and wettable powder formulation (Arunsiri *et al.* 2003) of *B. thuringiensis* have been evaluated by number of investigators. The shelf life of these formulations of *B. thuringiensis* was reported to be one month.

In the present study, liquid formulations of *B. thuringiensis*

Table 5: The LC_{50} dose of the *B. thuringiensis* isolate Bt_{III} crystal protein against *H. armigera* larvae

Bt_{III} protein (ICP) conc.	Larval population(%) alive days after treatment					
	0	1	2	3	4	5
1mg/mL	100.00	100.00	86.00	86.00	71.43	71.43
2mg/mL	100.00	71.43	71.43	71.43	57.00	57.00
4mg/mL	100.00	71.43	71.43	57.00	50.00	50.00
8mg/mL	100.00	57.00	50.00	43.00	43.00	43.00
10mg/mL	100.00	43.00	43.00	43.00	29.00	29.00
Control	100.00	100.00	100.00	100.00	100.00	100.00
Halt@5mg/mL (positive control)	100.00	50.00	40.00	40.00	30.00	20.00

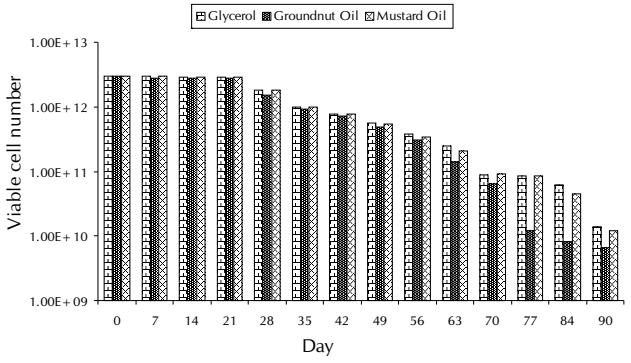

Figure 1: Persistence of *B. thuringiensis* Bt_{III} cells in stabilizer based liquid formulations (part1)

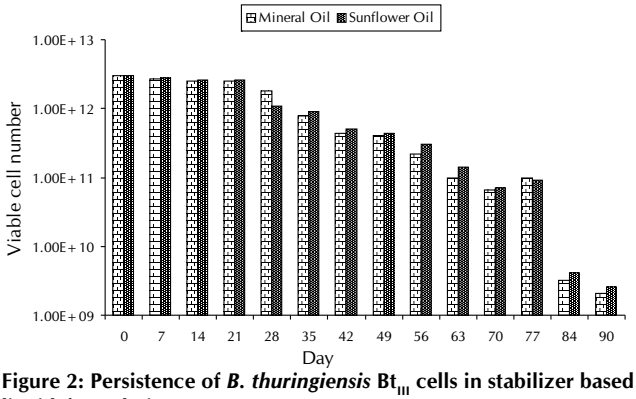

Figure 2: Persistence of *B. thuringiensis* Bt_{III} cells in stabilizer based liquid formulations (part2)

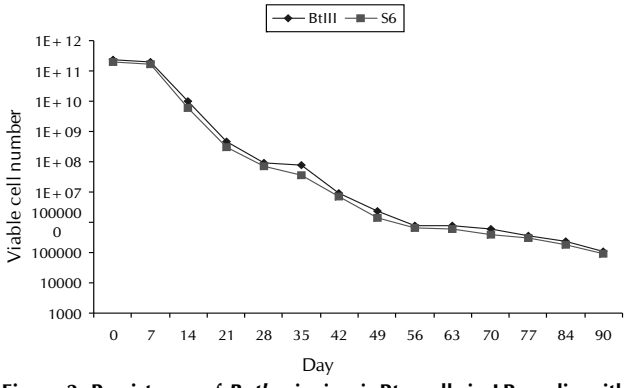

Figure 3: Persistence of *B. thuringiensis* Bt_{III} cells in LB media without any carrier material or stabilizer

were prepared by using five different types of stabilizers: glycerol, groundnut oil, mustard oil, mineral oil, and sunflower oil. Persistence of Bt cells was monitored at weekly interval. As clear from Fig. 1, 2 and 3, glycerol supported maximum persistence of Bt cells when formulations were stored at room temperature. Glycerol based formulation showed minimum decline in cell number and could be used to control *H. armigera* larvae for two months. Groundnut oil based Bt formulation also gave high level of persistence of Bt cells when stored at room temperature. The persistence of *B. thuringiensis* in mineral oil and sunflower oil was very low and declined by a factor of 1000 times within three months (Dhingra, 2006). The results in the present study indicated that bioefficacy of glycerol based Bt formulation was found to be 76.3% with *B. thuringiensis* isolates Bt_{III} after 7 days of application. The results in the present study indicated that the LC_{50} dose of *B. thuringiensis* isolates Bt_{III} was 8mg /mL at 48h. At this dose, 50% of larvae died after 48h of application (Table 5).

REFERENCES

Arunsiri, T.A., Suphantharika, M., Ketunuti, U. and Uthai, K. 2003. Preparation of spray dried wettable powder formulation of *B. thuringiensis* based biopesticides. *J. Econ. Entomol.* **96(2):** 292-299.

Becker, N. 2003. Ice granules containing endotoxins of microbial agents for the control of mosquito larvae - a new application technique. *J. Am. Mosq. Control Assoc.* **19(1):** 63-66.

De Melo-Santos, M. A., Sanches, E.G., de Jesus, F.J. and Regis, L. 2001. Evaluation of a new tablet formulation based on *B. thuringiensis israelensis* against *Aedes aegypti*. *Mem. Inst. Oswaldo. Cruz*. **96(6):** 859-860.

DeLucca, A. J., Simonson, J. G. and Larson, A. D. 1981. *Bacillus thuringiensis* distribution in soils of the United States. *Can. J. Microbiol.* **27:** 865-870.

Dennett, J. A., Lampman, R. L. and Novak, R. J. 2000. Evaluation of methylated soy oil and water based formulation of *B. thuringiensis* var. *israelensis* and Golden Bear Oil. *J. Am. Mosq. Control Assoc.* **16(4):** 342-345.

Dubois, N. R. and Lewis, F. B. 1981. What is *Bacillus thuringiensis*. *J. Arboric.* 7 : 233-240.

Dhingra, H. and Chaudhary, K. 2005. Development of *Bacillus thuringiensis* var. *kurstaki* formulation in carrier based cultures packets. *Annals of Agri Bio Research*. **21(1):** 83-86.

Dhingra, H. 2006. Development and evaluation of different formulations of *Bacillus thuringiensis* for management of *Helicoverpa armigera*. Ph.D. Thesis, Department of Biotechnology and Molecular Biology, CCS HAU, Hisar.

Dhingra, H. and Chaudhary, K. 2007. Effect of different Nitrogen sources on Biomass production of *Bacillus thuringiensis* biomass. *Annals of Biology*. **23(1):** 1-4.

Icgen, Y., Icgen, B. and Ozcengiz, G. 2002. Regulation of crystal protein biosynthesis by *B. thuringiensis* : II. Effects of carbon and nitrogen sources. *Res. Microbiol.* **153(9):** 605-609.

Johnson, V., Shah, R. N., Shah, D. N., Patel, K. A. and Mehta, M. H. 1994. Production of *Bacillus thuringiensis* based bioinsecticide:

Influence of various nitrogen sources on process economics. In: *Microbes for better living*. 161-167.

King, A. M. L., Gunasekaran, K., Sriram, A. N., and Narayanan, R. J. 1997. Efficacy of a microgel formulation of *B. thuringiensis* var. *israelensis* in controlling *Culex quinquefasciatus*. *Indian J. Experi. Biol.* **35(1):** 62-66.

Martin, P. A. and Travers, R. S. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Appl. Envir. Microbiol.* **55:** 2437-2442.

Medeiros, F. P., Santos, M. A., Regis, L. and Rios, E. M. 2005. Development of a *B. sphaericus* tablet formulation and its evaluation as a larvicide in the biological control of *Culex quinquefasciatus*. *Mem. Inst. Oswaldo. Cruz*. **100(4):** 431-434.

Reardon, R., Dubois, N. and McLane, W. 1994. *Bacillus thuringiensis* for managing gypsy moth: A review. *USDA Forest Service*. FMH-NC-01. **94:** 3-30.

Ridgway, R. L., Illum, V. L., Farrar, R. R. and Calvin, D. D. 1996. Granular matrix formulation of *B. thuringiensis* for control of the European corn borer. *J. Econ. Entomol.* **89(5):** 1088-1094.

Schisler, D. A., Slininger, P. J., Behle, R. W. and Jackson, M. A. 2004. Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathol.* **94(11):** 1267- 1271.

Zhang, J. B., Ming, G. Z. and Yuan, F. Y. 1997. Efficacy of fizzy tablets of Bti against *Aedes albopictus* larvae. *Chinese J. Vector Biology Control.* **8(3):** 169-171.

.....From P. 196

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