

Studies on the Biological Assay of Insecticides. (XXXIX)*

On the Difference in Susceptibility to the Knockdown Effect of *p,p'*-DDT Powder between Adults of the Common House Fly, *Musca domestica vicina* Macq., reared on the Culture Medium Prepared with the Horse Manure and Those on "Okara," the Residual Product in "Tofu" Making.

Sumio NAGASAWA**

(Takei Laboratory)

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Reports on various culture media for the larval stage of the common house fly which have been studied by previous authors were briefly reviewed. The susceptibility of the flies which were reared on the culture medium prepared with the horse manure and that of the flies those reared on "okara", the residual product in "tofu" making, to the knockdown effect of *p, p'*-DDT powder were compared by the settling dust apparatus method. The utility of the latter culture medium was discussed on the basis of the result of experiment and of the experiences of practical rearing for long years.

INTRODUCTION

As will be reviewed in the next section, studies on the rearing method of the common house fly, *Musca domestica vicina* Macq., especially the studies on the culture medium of the larval stage have already been carried out by many investigators. For the past decade, the present writer has also been studying on the same problem to rear a large number of house flies for the insecticidal tests, and obtained good results using culture media for the larval stage prepared either with the horse manure or with "okara", the residual product in "tofu" making. The former culture medium is a modification of Grady's method,¹⁷⁾ while the latter prepared with "okara" seems to be a new medium devised for the mass culture of the common house fly. In the present paper, the writer wishes to describe on these two methods in detail. And on the basis of the result of experiments designed to compare the flies which were reared

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** 長沢純夫

on the former culture medium and those on the latter culture medium in regard to the susceptibility to the knockdown effect of p , p' - DDT powder when the settling dust apparatus method was resorted to, and also on the basis of the experiences of practical rearing conducted for long years, the writer wishes to discuss especially the utility of the culture medium prepared with "okara".

REVIEW ON THE HISTORY OF STUDIES OF THE CULTURE MEDIUM FOR LARVAL STAGE OF THE COMMON HOUSE FLY

It has been tried for a long time to rear the house fly under the laboratory condition throughout the year by many investigators for use in various researches such as insecticidal test, disease transmission and genetic experiment, etc. However, on account of difficulty in discovering a suitable culture medium for larval stage of the house fly in the winter time, no progress has been made in the study. Formerly, in the temperate zone, the season in which it was possible to rear the house fly continuously on the horse manure medium was approximately from the middle of April to the middle of December. In spite of manifold investigations regarding foods of adult or larval stages, water content of larval culture medium, and the temperature as well as relative humidity in the breeding environment, it has so far been impossible to rear the house fly during the remaining four months of the coldest season.

In 1927, Glaser¹⁶⁾ overcame the difficulty in rearing the house fly during the winter time by supplementing the larval culture medium with yeast cells suspended in water. Namely, he dissolved 453.6 g. (1 lb.) of bakery's cake of commercial yeast in 2000 cc. of water, then autoclaved to kill fungi and stored the mixture on ice. He added this suspension to larval culture medium prepared with the horse manure every two or three days in an amount which was properly determined according to the number of larvae present in a breeding jar. By introducing this procedure he succeeded in overcoming the difficulty in the breeding of the house fly in the winter time. His idea was an important stimulus that has produced a remarkable progress in the studies of this field.

In 1928, Grady¹⁷⁾ slightly modified Glaser's culture medium mentioned above and systematized of mass culture of the house fly. That is, Grady filled the culture jar measuring 15.24 cm. (6 in.) in diameter and 20.32 cm. (8 in.) in height up to the three-fourths of the capacity loosely with fresh horse manure. He found that if about 200 cc. of water was added to the horse manure when the culture was started it was sufficient to keep the medium in a moist condition until the adult emerged. To this, 75 cc. of the supplementary food (yeast cells suspended in water) was added. About 10 cc. or more of supplementary food was poured into the jar every other day until the larvae were about ready to pupate. The amount of yeast to be used somewhat varies with the number of larvae to be reared. He obtained a very good result when 453.6 g. (1 lb.)

of yeast was dissolved in 2500 cc. or 3000 cc. of water, and there was little or no difference in the results even though he used the unautoclaved yeast suspension; he states, however, that it is advisable to sterilize the yeast suspension if an autoclave is available. This culture medium had been widely used for mass culture of the common house flies for various researches until Richardson's medium which will be mentioned below was reported. And the National Association of Insecticide & Disinfectant Manufactures (NAIDM)¹⁾ adopted the use of this culture medium as the official method for rearing the flies for the Peet-Grady fly test in 1932.

Nagasawa and Uruha²⁹⁾, Nagasawa³⁰⁾ further simplified Grady's method when they reared the common house flies for their mosquitocidal incense test. Namely, they filled a glass pot measuring 14 cm. in diameter and 19 cm. in height with the mixture of ca 1500 g. of fresh horse manure and ca 30 g. of waste dried powder of beer yeast and poured 150 cc. of tap water into it. They could rear about 200 very good house flies from a pot of this culture medium throughout the year.

Hockenyos²⁰⁾, at first, adopted Grady's method for rearing the house fly for his experiment. However, as he had noticed during the summer time that larvae of the house fly were more abundant in heaps of the hog manure than in piles of the horse manure, he attempted to adopt this material for laboratory use during the winter months, and he found that a mixture of one part of the fresh hog manure free of straw with three or four parts of the fresh horse manure gave the best result and also that this combination provided the medium with sufficient aeration and at the same time produced more uniform moisture condition. According to his experience, the supplementary food was less required when this method was adopted as this material seemed to provide more nutrition per unit of space than the horse manure alone, and that the larvae usually had a preference for the hog manure.

Ikeda²²⁾ reared the house fly with the mixture of three parts of fermenting wheat bran and two parts of horse manure. Wheat bran containing water 65~70 per cent was left beforehand to ferment for 24 hours in an incubator regulated at 28~30°C. He states that 2 g. of the mixture is suitable for one larva.

Derbeneva-Ukhova¹⁰⁾ and Kusina²⁴⁾ in Russia reared the flies on the horse manure. Lörincz and Makara²⁷⁾ state that the pig manure is most convenient as the larval culture medium in Hungary. In Jerusalem, Feldman-Muhsam¹³⁾ compared the four culture media, viz. Richardson's medium³⁹⁾, coffee grounds which was used by Hase¹⁹⁾, white cheese and the cow dung, and he obtained the best result with the cow dung but he could not good results with the coffee grounds. Formerly, Glaser¹⁴⁾ used fermenting bran, horse manure, hog manure and other materials for larval culture medium and he pointed out that the horse manure was the best and the most practical material for culture of the house fly. These previous workers, however, didn't try to rear the house flies throughout the year by using these materials.

As Basden⁶⁾ stated, the size of the resulting adults is determined in their larval

stage, and the production of vigorous adults with the minimum of variation from the normal size can be accomplished only by ensuring that the larvae have an adequate supply of the suitable food under the optimum condition. To answer this purpose, it is absolutely necessary to supply with food, the quality of which is as nearly constant as possible. It is evident that the horse or pig manure, cow dung, etc. can not be considered suitable for the reason stated above. Besides, as Richardson³⁹⁾ stated, the use of horse manure, or of the mixture of it with the hog manure as a rearing medium has several disadvantages. In the first place, it is not always available for most laboratories. In the second place, it is rather disagreeable to handle. Most important, however, is the fact that frequently a species of red mite, parasites of the house fly, is apt to be brought in with the horse manure. If the mites have once become established and multiplied in great numbers, the mites swarm through the breeding media and infestation of the flies will be nearly 100 per cent. All the jars and cages should be sterilized to get rid of this troublesome mites.

To overcome these disadvantages, Richardson³⁹⁾ devised a culture medium. He found that a wheat bran-alfalfa meal mixture supplemented with small amounts of yeast (453.6 g. of baker's yeast dissolved in 2000 cc. of water) and diamalt (a commercial product of the Fleishmann Yeast Co., containing a large percentage of malt sugar) is a very satisfactory larval medium containing more nutrient than the horse manure medium; and he could rear a large number of flies with this medium. The composition of Richardson's medium is as follows:

Wheat bran	1474.2 g. (3¼ lb.)	} Mixed thoroughly
Alfalfa meal	793.8 g. (1¾ lb.)	
Water	5000 cc.	} Mixed thoroughly
Yeast suspension	300 cc.	
Diamalt	25 cc.	

The procedure is as follows: Add the latter mixture to the former and mix thoroughly. The house fly eggs may be introduced to the medium immediately. The quantity of yeast suspension necessary can probably be lessened considerably by preparing the entire mixture one day before use, so that the yeast cells will have time to multiply.

Thomssen and Doner⁴³⁾, Campbell and Sullivan³⁹⁾ used Richardson's culture medium to rear the house flies for their experiments. Campbell and Sullivan⁹⁾ used a battery jar measuring 22.86 cm. (9 in.) in diameter and 25.40 cm. (10 in.) in height as the culture jar. They weighed 1200 g. of bran-alfalfa meal mixture (2 : 1 by weight) into a jar and poured a mixture comprising 40 cc. of a commercial malt syrup, 150 cc. of yeast suspension (proportion of baker's yeast to water is entirely the same as that of Richardson's yeast suspension) and 2500 cc. of water into it and kneaded thoroughly together. They got a very good result with it. McGovran et al²⁸⁾ reared the house flies on Richardson's medium to which powdered dried milk had been added.

A little later, NAIDM²⁾ replaced officially Grady's horse manure culture medium with Richardson's culture medium with a slight modification to rear the house flies for the Peet-Grady test. The prescription of the NAIDM culture medium is as follows : A cylindrical jar measuring approximately 15.24 cm. in diameter by 22.86 cm. in height was preferred as a container. For three jars,

Soft wheat bran (corse)	1200 g.
Alfalfa meal	600 g.
Water (lukewarm)	2700 cc.
Malt extract	50 cc.
Compressed yeast	30 g.

were used. The former two dry materials were thoroughly mixed, and placed in jars to the three-quarters mark, and to each jar 900 cc. of a suspension of the latter mixture were added and kneaded. The proportion of liquid materials to dry materials may be varied slightly to prevent mold growth.

A few years later³⁾, the Ralston Purina Co., St. Louis, Mo., has entered into a special contract with the NAIDM for sale of the former dry larval materials, and has mixed the medium quarterly a year according to the NAIDM specification, while Diamalt specified to the product of the Standard Brand, Inc. Besides, the proportion of combination as well as quantity of the materials of the larval medium were a little changed. Namely, a mixture of 340 g. (12 oz.) standard dry larval media with approximately 750 cc. of an aqueous suspension containing 15 mg. moist cake yeast and 10 cc. non-diatstatic Diamalt was prescribed for a cylindrical battery jar mentioned above. The procedure of preparation is as follows : Mix thoroughly until a loose, flabby media is obtained, transfer it to the battery jar without packing, cover with cloth and set in the insectary. In addition to the above description, it is necessary to note that the amount of suspension required for best rearing results should be determined in each laboratory and that it may be varied to some extent in order to prevent mold growth. It is suggested that the media should be prepared in the late afternoon of the day before the introduction of eggs. At present, this medium is used by many investigators not only for the insecticidal test but also for various other fields of research.

For example, Barbesgaard and Keiding⁵⁾ used this NAIDM culture medium with some modifications for their genetic experiments. Namely, they prepared the medium in the following way. They mixed 400 g. of wheat bran with 200 g. of lucern meal and moistened uniformly with 1000 cc. of milk containing about 15 cc. malt and 15 g. baker's yeast. They found that the yield of pupae considerably increased by using milk instead of water when small rearing jars were used.

Similarly, Lichtwardt et al²⁵⁾ used for their genetic experiments approximately 1800 g. of dry NAIDM materials to which were added 100 g. of Brewer's yeast. This

dry materials was then moistened with a mixture of 6000 cc. of water, 100 cc. of dark Karo syrup and 170.1 g. (6 oz) of live baker's yeast.

Prior to that, Basden⁶⁾ slightly modified Richardson's culture medium using some other materials. Namely, he used the middlings instead of bran which completely passed through a 14 mesh sieve since he could obtain pupae in a clean condition by using this method, and of alfalfa meal he used also the grass meal, which was so fine that nearly all passed through 24 mesh sieve, and that about half of it will pass through a 40 mesh sieve. Moreover, he states that the dry malt extract was more convenient than to use the malt solution which usually contains 75-80 per cent extract. The formula of Basden's medium is as follows:

Middlings	1814.4 g. (4 lb.)
Grass meal	907.2 g. (2 lb.)
Tap water	6000 cc.
Dry malt extract	60 g.
Dried yeast	45 g.

In preparing the medium, the middlings and grass meal are weighed out beforehand, sterilized in a oven, thoroughly mixed and stored dry in a bin. The suspension of yeast and dry malt extract in warm water is prepared when needed and the suspension is mixed thoroughly with the appropriate quantity of the mixture of middlings and grass meal.

On the other hand, Eagleson¹¹⁾ used the fermenting mash of oats for the culture of the larval stage of the house fly. He mixed 2000 g. of crimped whole oats with 4000 cc. of water and 200 cubic centimeters of cheap syrup in a No. 2 washtub and permitted to soak two hours; then, he mixed one cake of compressed yeast or 200 cubic centimeters of active yeast culture ("Starter") with the softened oats, and covered the tub with muslin and placed in an incubator at 32°C. After 3 days, when the tub should be strongly odoriferous of fermenting grain, the eggs were transferred on them. In 1941, Eagleson¹²⁾ used this medium for his experiment. Yasuda^{46), 47)} reared the house flies on a nutritive medium which is commonly used in bacteriological technique. It consists of 3% of agar, 0.5 % of NaCl, 1% of pepton and ca 10% of minced meat. Recently Hafez¹⁹⁾ reared the house flies on the cotton that soaked up three parts of milk and one part of water.

Wolf⁴⁵⁾ tried to breed the house flies on sewage sludge. This trial, however, was made from sanitary point of view, and not to find out the method of mass culture

Buei⁴⁾ reared the house flies with fermenting wheat bran alone. Namely, he mixed the wheat bran with the moderate quantity of water and left it to ferment for 24 hours in an incubator regulated at 30°C. When water is used in excess in this procedure, the mixture will be overfermented, and mortality of larvae will increase in the course of development. He states that 1.5~2.0 g. of fermenting wheat bran is suitable for

one larva.

The writer^{31,32,36)} has reared the common house flies on "okara", the residual product in "tofu" making, for some years. Utilization of "okara" for culture of larvae of the common house fly has already been tried by Hori²¹⁾, Kobayashi²³⁾, Tsutsumi⁴⁴⁾, Yasuda^{46,47)} and others. But their trials were rather in a small scale, glass tubes or petri dishes having been used as containers for rearing. The writer has modified their methods and developed a method which is fit for conducting mass culture throughout the year. Following materials are necessary for the preparation of this culture medium. (1) "Okara", residual product in "tofu" making. "Tofu" is one of the commonest subsidiary food-stuff in Japanese diet and is made all over Japan at all seasons. "Okara" is also called "unohana", "yukinohana", etc., and is used partly for the food of man, but a large percentage of it is used for the food of cattle. Thus, it can be obtained at a moderate price. For the culture of the house fly, this must always be obtained in a fresh condition to exclude the possibility of oviposition by other flies. Usually many eggs are found in "okara" when it is not in a fresh state. (2) Straw. Straw is used for absorption of water from "okara" and also for the purpose of maintaining the culture medium in a moderate moist condition. Before using, the straw must be cut into pieces of length of about 3 cm. or so. And, if possible, it is desirable to sterilize by heat to avoid the contamination of mites or undesirable pilzes. (3) Yeast powder. Waste dried powder of beer yeast may well be used for the culture medium. The yeast, however, must be in the state alive. (4) Saw dust. This is used for maintaining the culture medium in the moderately moist and porous state. A better result may be expected if the rice bran is substituted for the saw dust. (5) Rice bran. Wheat bran may also be used for the culture medium instead of rice bran.

For preparing the culture medium, first, fill the pot measuring 14 cm. in diameter and 19 cm. in height with the chipped straw to a depth of about 3 cm. or so. The quantity of chipped straw may be varied according to the temperature and relative humidity of the insectary or to the moisture contents of "okara". Next, mix ca 1000 g. of "okara" with ca 30 g. of yeast powder and ca 30 g. of rice bran uniformly, and place the mixture into a pot. Then transfer about 500~700 eggs on the culture medium in the pot and cover the pot with a gauze to avoid the possibility of oviposition by other flies. Here, if we transfer larvae 1 or 2 days after hatching on the culture medium, better results will be obtained than in the case of using eggs. If there is no possibility of oviposition by other flies, covering with the gauze is not so important in the case where "okara" is used as in the case of the horse manure culture medium where the cover is very useful for maintaining the culture medium moderately moist. To get eggs, a petri dish having an inner diameter of 9 cm. and height of 4.5 cm. with a mixture of 1 part of fish scrap, 2 parts of rice bran and 3 parts of water is placed in the cage containing house flies. In course of one day,

hundreds of eggs are deposited on the medium and the eggs may be transferred to the rearing pot immediately after oviposition or the larvae transferred after hatching. At the temperature of 30°C, the first pupa will be formed in the upper dry layer of the medium five or six days after the transference of eggs. Two days later, all or almost all larvae will have pupated. The writer used to remove the upper part of the culture medium with pupae in it and to pick out the rest of the pupae with a pincette, and put them into the stock cage to obtain the flies that emerge.

MATERIAL AND INSECT

***p, p'*-DDT powder** : For the present investigation, four grades of *p, p'*-DDT (mp 107~108°C) powder of the concentration of 1, 2, 4 and 8% were prepared by the solvent application method³⁶⁾. The carrier used for preparation of the powder was the Kampaku kaolin which was quarried at Kampaku, Haguro-mura, Kouchi-gun, Tochigi-ken, Japan., and manufactured to the powder to be used for carrier by the Hōmei Shōji Co., Ltd. It was screened through the 325 mesh screen of Tyler's standard sieve. The distribution of particle size was not determined. The result of chemical analysis by the Government Research Institute for Ceramics, Agency of Industrial Science and Technology is as follows: Si₂ 57.80, TiO 0.51, Al₂O₂ 30.5, Fe₂O₃ 0.30, CaO 0.43, MgO 0.25, K₂O 0.2, Na₂O 0.22, Ignition loss 10.44, Total 100.22.

The test insects : The insects used were the common house flies, *Musca domestica vicina* Macq., which were reared under the condition of ca 30°C and ca 50% relative humidity. Paste of wheat powder was given to the adults as the food. The larvae were cultured using either Nagasawa and Uruha's culture medium prepared with the horse manure or Nagasawa's culture medium prepared with "okara" as described above in detail. Healthy flies of uniform size 4~5 days old after emergence were used for experiment. On the strain of this common house fly the writer has already reported in a previous paper^{34,36)}. Though no determination of the sexes was made before experiments, the results of determination made at the end of the experiments showed that the sex ratio was 1 : 1 in almost all the cases.

APPARATUS AND METHOD

For the present experiment, the settling dust apparatus was used. The construction of the apparatus and the method of its operation was already described in a previous paper^{34,36)}. Experiments were so designed as to compare the susceptibilities of two populations which were simultaneously on two different culture media.

RESULT AND DISCUSSION

The result of the experiment presented as the relation between time *T* (minutes)

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Table 1. Time T (min.)-per cent knockdown Y_K of adults of the common house fly, *Musca domestica vicina* Macq., reared with culture medium prepared with horse manure and those with "okara", residual product in "tofu" making, for p, p' - DDT powder in the range of concentration C from 1 to 8%. Average of ten tests. (12-20/XII, 1949. 20°C).

Culture medium		Horse manure				"Okara"			
Concentration		1	2	4	8	1	2	4	8
Number of individuals		214	229	237	252	238	251	249	243
Time	6	0.94	5.68	11.81	16.67	0.84	3.19	10.04	17.70
	8	2.80	13.54	27.00	46.83	4.62	15.94	30.12	47.74
	12	18.69	46.29	67.09	81.75	25.21	49.80	70.68	84.77
	16	39.71	68.56	85.23	94.84	46.22	76.10	88.76	97.12
	24	76.64	93.01	98.73	99.60	80.67	96.81	99.60	100.00
	32	90.65	99.13	100.00	100.00	89.08	100.00	100.00	—
	48	98.13	100.00	—	—	99.16	—	—	—
	64	99.07	—	—	—	100.00	—	—	—

and the cumulated knockdown percentage Y_K for each concentration C (%) is shown in Table 1. The experiments were carried out in the period from December 12th to 20th, 1949 under the room temperature of 20°C. By transforming the cumulated knockdown percentage Y_K to probit y_k and the time T to logarithm t , the parameters of the equation of the time-knockdown regression isodoses

$$y_k = 5 + b_c(t - \bar{t}_c)$$

were calculated. The results are shown in Table 2 and Fig. 1.

Table 2. Characteristics of time-knockdown regression isodoses of adults of the common house fly, *Musca domestica vicina* Macq., which were reared with culture medium prepared with horse manure and those "okara", residual product in "tofu" making, for p, p' - DDT powder in the range of concentration C from 1 to 8%.

Culture medium	Concentration	Regression coefficient	Standard deviation	Log median knock down time	Median knock down time
	C	b_c	σ_c	$\bar{t}_c$	$\bar{T}_C$ (min.)
Horse manure	1	5.045	0.198	1.25325	17.92
	2	5.259	0.190	1.10060	12.61
	4	5.688	0.170	1.00804	10.19
	8	6.052	0.165	0.92892	8.49
"Okara"	1	4.975	0.201	1.22551	16.81
	2	5.925	0.169	1.07859	11.98
	4	6.006	0.167	0.99108	9.80
	8	6.512	0.154	0.91684	8.26

Then, the concentration C transformed to logarithm c , and the relation of c to t was plotted on the graph. Thereupon, as shown in Fig. 2, nearly parallel two lines which are concave upward are obtained. It is apparent that Ostwald's formula

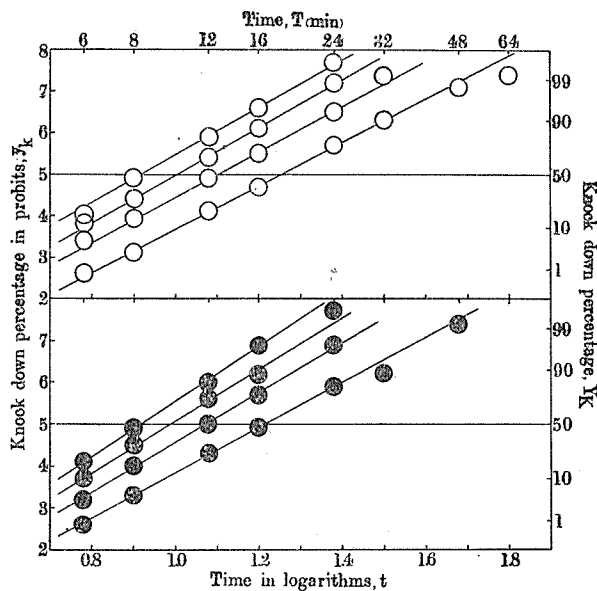


Fig. 1. Time-knockdown regression isodoses of adults of the common house fly, *Musca domestica vicina* Macq., which were reared with culture medium prepared with horse manure (upper figure) and those with "okara", residual product in "tofu" making, (lower figure) for p, p' -DDT powder in the range of concentration C from 1 to 8%.

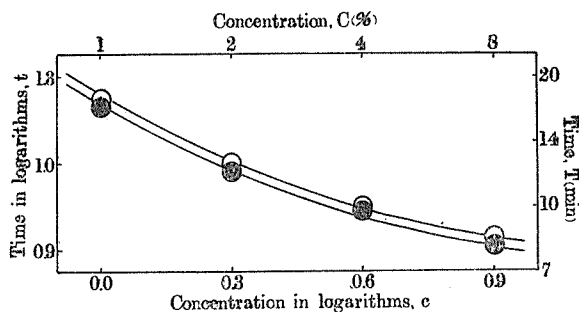


Fig. 2. Relation between log time t and log concentration c at the 50 per cent knock down of adults of the common house fly, *Musca domestica vicina* Macq., which were reared with culture medium prepared with horse manure (upper line with hollow circles) and those with "okara", residual product in "tofu" making, (lower line with solid circles) for p, p' -DDT powder in the range of concentration C from 1 to 8%. The equation of the two lines in original units are $(C - 0.743)^{0.223}t = 13.243$ and $(C - 0.743)^{0.213}t = 12.585$ respectively.

$(C - C_0)^n t = k$ can be applied to the relation of these two variables. In this formula C is the concentration of chemical used, C_0 is the threshold of concentration, t is the length of exposure time in logarithms and n and k are two constants, of which the former represents the slope and the latter the position respectively.* As the result of estimation by the method described by Bliss³⁷⁾, the approximate value of C_0 seems to be 0.743. The equation calculated for the two series are shown in Table 3. As the

Table 3. Relation between log time t and log concentration c at the 50 per cent knockdown of adults of the common house fly, *Musca domestica vicina* Macq., which were reared with culture medium prepared with horse manure and those with "okara", residual product in "tofu" making, for p, p' -DDT powder in the range of concentration C from 1 to 8%.

Culture medium	Regression equation $\log T + b_2 \log (C - C_0) = a_2$ or in original units $(C - C_0)^n t = k$	Precision of the equation			
		S^2	$V(a_2)$	$V(b_2)$	$V(C_0)$
Horse manure	$t + 0.223 \log (C - 0.743) = 1.12200$ or $(C - 0.743)^{0.223} t = 13.243$	0.0000178	0.0000045	0.0000195	0.0000829
			at $\bar{c} = 0.22071$		
"Okara"	$t + 0.213 \log (C - 0.743) = 1.10999$ or $(C - 0.743)^{0.213} t = 12.585$	0.0000059	0.0000015	0.0000064	0.0000276
			at $\bar{c} = 0.22071$		

value of n in the two equations of the time-concentration line at the 50 per cent knockdown, $(C - C_0)^n t = k$, are almost equal for two series, a combined regression coefficient has been computed from the sum of the numerators and the denominators for two curves to obtain an improved value applying to both relations of two series. The combined regression coefficient is $(0.22337 + 0.21267)/2 = 0.21802$. Revised equation for the horse manure culture medium series is $t + 0.218 \log (C - 0.743) = 1.12082$ or $(C - 0.743)^{0.218} t = 13.203$ and for the "okara" culture medium series is $t + 0.218 \log (C - 0.743) = 1.10113$ or $(C - 0.743)^{0.218} t = 12.622$. The difference of susceptibilities of the house flies in the two series which is expressed as the ratio of the median knockdown concentration for a certain exposure time is $(1.12082 - 1.10113)/0.218 = 0.09032$. Thus, the flies reared on the "okara" culture medium is $\log^{-1} 0.09032 = 1.2312$, or 1.23 times as susceptible as the individuals reared on horse manure culture medium.

Many previous authors have already reported that the susceptibilities of insects to insecticides are influenced by the quantity or quality of food which the test insects have taken in the larval or adult stage. For example, Quayle³⁸⁾ reported that the citrus scale insects attacking the fruits were more resistable to fumigants than that attacking the leaves and twigs. Richardson and Casages⁴⁰⁾ stated that three strains of the green peach aphid, *Myzus persicae* (Sulz.), attacking the different plants showed

* It should not be confused with a subsequent use of the same symbol n for degree of freedom in statistical discussion and k for knockdown which is used in this paper.

three grades of susceptibilities to the fumigation of nicotine. Sun⁴²⁾ reared larvae of the confused flour beetle, *Tribolium confusum* Duv., on foods of eight different combinations of various materials and tested their susceptibilities to carbon disulphide. According to his result, the rate of growth was greatly influenced by the difference of food combination, especially the body weight was markedly influenced, and their susceptibilities to carbon disulphide were conspicuously different. And also he got a similar result with the adult stage of the confused flour beetle and the granary weevil, *Sitophilus granarius* (L.), which were bred with various combinations of different materials and he discussed on the relation between body weight and susceptibility. Saito⁴¹⁾ investigated on the relation between the nutrition of the rice weevil, *Sitophilus oryza* (L.), and the small rice weevil, *Sitophilus sasakii* (Takahashi), and their susceptibilities to carbon disulphide and orthodichlorobenzene, and tried to find explanation in the differences found in body weight, water content and crude fat content, etc. Phillips and Swingle³⁷⁾ reared the larvae of the southern house mosquito, *Culex quinquefasciatus* Say, with various food materials and found that their susceptibilities to rotenone and nicotine were influenced by the kinds of food, and especially largely influenced by its quantity. Lord²⁶⁾ states that the susceptibilities of the pomace fly, *Drosophila melanogaster* Meigen, to nicotine varied with the proportion of yeast in medium on which the flies were reared. Nagasawa³⁹⁾ observed that the common house flies which were given various foods in the adult stage showed different susceptibilities to the knockdown effect of *p*, *p'*-DDT powder.

The trend that the house flies reared on the "okara" culture medium are more susceptible to the toxic action of *p*, *p'*-DDT powder than those reared on the horse manure culture medium was also observed in the experiments using the BHC powder, pyrethrum mosquitocidal incenses and other various insecticides. Therefore, it may be stated that the house flies reared on the former culture medium are somewhat unsuitable for testing the effectiveness of highly toxic insecticides which are not diluted before use, such as liquid household insecticides. In such cases, some devices on the testing method will be required.

The culture medium prepared with "okara" has not such disadvantages as pointed out by Richardson and Basden; it has still several other disadvantages. Namely, putrefaction quickly sets in and a foul smell is very strong. And when the moisture contents of "okara" is very great, all the medium become creamy. As the result, pupation of larvae is impaired to some extent. This tendency is especially marked under the condition of high temperature of summer. This tendency, however, can be easily overcome by regulating the temperature in insectary at ca 25°C and the relative humidity at 50% or so and adding the chipped straw or wheat bran.

The optimum condition in rearing the common house fly using "okara" seems to be 25°C and 50% relative humidity. Under this condition, as shown in Fig. 3, the upper layer of the culture medium will dry up and break into small pieces by the

time when almost all larvae pupate. But even under the optimum condition, as shown in Fig. 4, the upper layer of the culture medium will dry up and break into very fine pieces when too many larvae were placed into the jar and the pupae will become

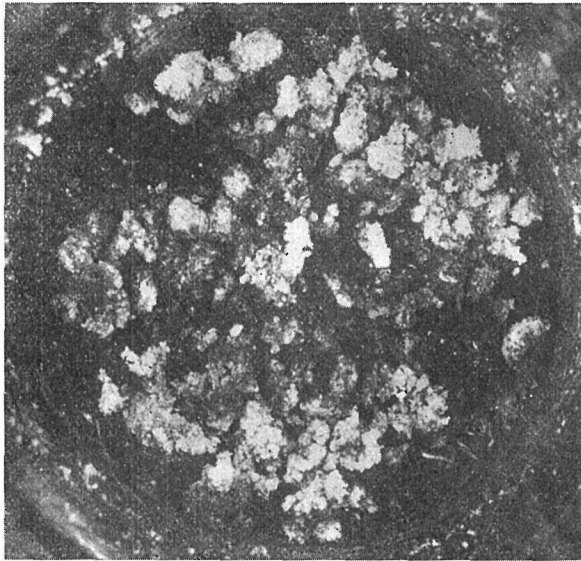


Fig. 3. Upper layer of the "okara" culture medium, under the optimum condition, dried up and broken into small pieces by the time when almost all larvae pupate.

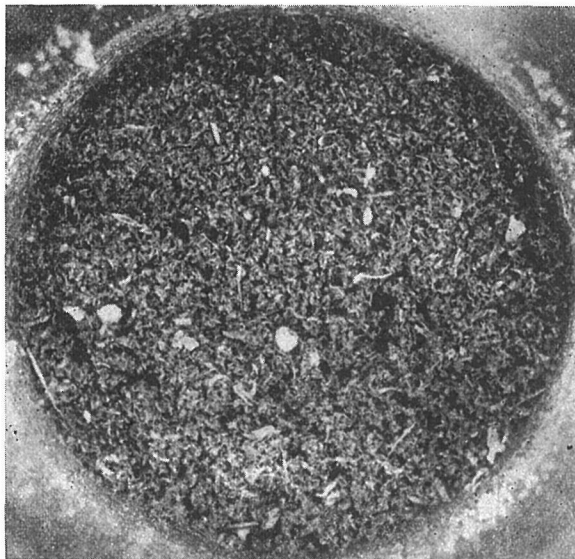


Fig. 4. Upper layer of the "okara" culture medium dried up and broken into very fine pieces when too many larvae were transferred into the jar.

very dwarf. On the contrary, when the number of larvae is too small, the culture medium will dry up making a large block and the mold will grow out it as shown in Fig. 5. As mentioned above, though the culture medium prepared with "okara"

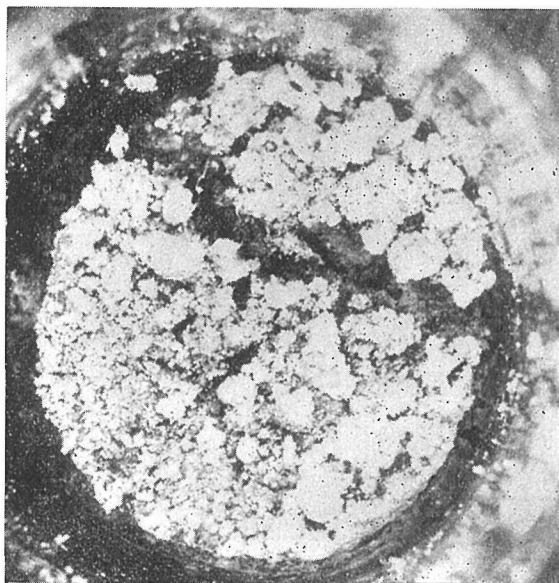


Fig. 5. Upper layer of the "okara" culture medium making a large block and the mold grown out it when the number of larvae was too small.

has some disadvantages, they can be overcome perfectly by regulating the environmental condition and by increasing the additional materials. Therefore, it may be concluded that this culture medium well answers the purpose of the mass production of the common house fly for use in various researches.

SUMMARY

Studies on various culture media for the larval stage of the common house fly which have been studied by previous authors were briefly reviewed. In the review, the writer's culture medium prepared with horse manure and with "okara", residual product in "tofu" making, has been described especially in detail. As the result of studies using the settling dust apparatus, it is realized that the flies reared on the latter culture medium is ca 1.23 times as susceptible as those reared on the former culture medium. Under the condition of 25°C and 50% relative humidity, the mass production of the common house fly using "okara" is very easy and efficient; and it may be concluded this culture medium will well answers the purpose of mass production of the house fly for use in various researches.

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