Conditions Which Determine the Efficiency of Ammonium Sulphate in the Control of *Prymnesium Parvum* in Fish Breeding Ponds

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The phytoflagellate *Prymnesium parvum* has been reported in Europe (Otterstroem *et al.*, 1939, Liebert *et al.*, 1920), where it occurs sporadically and causes mass mortality among fishes. In 1947 *Prymnesium parvum* first appeared in Israel fish ponds, rapidly spreading through regions where water was brackish. Since then it has caused extensive damage to the fish breeding industry and is now the most serious natural obstacle to fish breeding in Israel. *Prymnesium* kills fish by means of an extracellular protein-like toxin. The control of *Prymnesium parvum* can be based upon methods which destroy the organism, or upon methods which destroy only the toxin (Shilo and Aschner, 1953; Shilo *et al.*, 1953).

After the first appearance of *Prymnesium parvum* in Israel it was found by Reich and Aschner (1947) that ammonium sulphate exerted a lytic effect on *Prymnesium parvum*. Low concentrations of the substance lysed *Prymnesium parvum*. Its effect on other forms of life present in the fish ponds was negligible; in addition, the relatively low cost, high solubility, ease of dispersion and concomitant fertilizer effect of ammonium sulphate made it a most desirable means of controlling *Prymnesium*. Although the killing effect of ammonium sulphate was found to be of short duration, constant vigilance entailing immediate application of ammonium sulphate upon appearance of *Prymnesium* in ponds has enabled fish breeding to be continued in the infected regions comprising over one-fourth of the country's total fish-breeding area.

Of late, it has been observed repeatedly that ammonium sulphate may in some cases prove ineffective as a control measure, even when used in 8-fold, the usual concentration. Failure of control by ammonium sulphate has occurred largely in a particular region (Beisan Valley) and has been found to be especially marked in winter. In order to determine the factors governing the lytic action and the most effective use of ammonium sulphate, a program of investigation, both in the laboratory and in the field, was instituted.

The first step was to determine whether the ineffectiveness of ammonium sulphate was connected with the random appearance of sensitive strains of the phytoflagellate, or with a natural selection of a resistant mutant in the course of the years of continued use of ammonium sulphate. Strains of *Prymnesium* were collected from scores of ponds where resistance to ammonium sulphate was observed, and these cultures were tested in the laboratory for sensitivity to ammonium sulphate. All of the strains, when tested under controlled conditions of temperature, pH, and illumination, were found to be equally and highly sensitive to ammonium sulphate. From the foregoing, the explanation of reduced effectiveness of ammonium sulphate by development of resistant strains appeared unlikely and it became evident that external conditions must play a major role in the lytic action of the ammonium salt on the microorganisms. Hence, it was decided to determine the relative importance of these various conditions for ammonium sulphate activity.

Investigation of the temperature dependence of lytic activity of ammonium sulphate

In the range 2 to 30 C the lytic activity of ammonium sulphate was found to be a function of temperature; the activity increased as temperature increased. In addition, lysis was completed more rapidly at higher temperatures. Experimental data illustrating this effect are presented in figure 1. The decreased activity of ammonium sulphate observed in ponds during the winter season when water temperatures are usually below 10 C may be accounted for, in part, by this effect.

Investigation of the pH dependence of the lytic activity of ammonium sulphate

In the range pH 6.5 to pH 9.5 (representing the range which is found in local fish ponds and one which is not intrinsically harmful to *Prymnesium*) the lytic activity of ammonium sulphate was found to be a function of pH; the activity increased as pH increased. The results of a typical experiment are plotted in figure 1. This effect will be discussed in detail elsewhere. The findings are consistent with the interpretation that free ammonia and not the ammonium ion is the agent responsible for lysis. It is of interest to point out that a similar correlation has previously been noted in relation to effect of pH on the toxicity of ammonium sulphate to fish (Wuhrmann *et al.*, 1947, 1948) and that a similar explanation of the phenomenon has been advanced in that connection.

Investigation of daily fluctuations of pH in ponds in different regions

In order to determine whether the pH dependence of the activity of ammonium sulphate was a factor operative under natural conditions, measurements of the pH of ponds in different regions were undertaken. pH readings made at various times throughout the day in ponds located in different regions are graphically presented in figure 2. Examination of the data shows a well-defined diurnal rhythm of pH with the maximum at noon and minimum at dawn. This periodicity is most probably due to heightened photosynthetic activity of phytoplankton during hours of sunlight (Cerni, 1948). This conclusion is substantiated by the observation that newly-filled ponds poor in plankton show markedly reduced pH fluctutation. Data for such ponds are included in figure 2. A comparison of pH curves of ponds in Ein Hamifratz (Zebulun Valley) with pH curves of ponds in Messiloth or Tel Amal (Beisan Valley) shows that there is a difference in the heights of the pH peaks, the latter being appreciably higher in the Zebulun Valley. These differences are undoubtedly due to qualitative differences in soil composition. The higher lytic activity of ammonium sulphate in the Zebulun Valley over that found in the Beisan Valley is in good accordance with the higher pH levels found in the former region. In addition to the marked difference of pH in ponds of different regions there are even pH differences between individual ponds of the same region. Hence the pH of any particular pond is an important index of the suitability of ammonium sulphate as a *Prymnesium* control measure for that pond.

It was predicted from the pH-activity and pH-time curves (figure 2) that control by ammonium sulphate would be most effective when the ammonium sulphate is added a few hours before a pH peak. The validity of this prediction has now been confirmed by field trials.

Persistence of ammonia-nitrogen in the ponds

Since a number of periodically changing factors had been found to influence the lytic activity of ammonium sulphate, the time of persistence of ammonia-nitrogen in the ponds was investigated. A number of typical experiments are given in figure 3. It may be seen that the concentration of ammonia-nitrogen decreases very rapidly after ammonium sulphate is added and falls to one half the maximum value in approximately 24 hours.

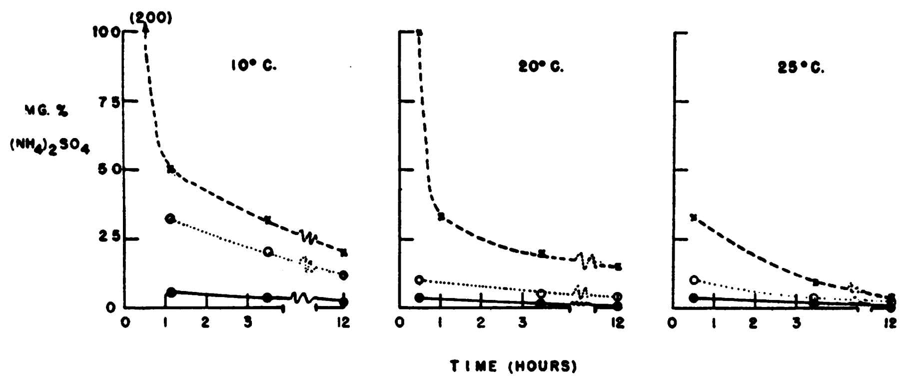

FIG. 1. Lytic activity of ammonium sulphate on *Prymnesium* under different conditions of pH and temperature. The minimum lytic concentration of ammonium sulphate in mg % is plotted as a function of time. Each group of curves gives data for an experiment at a different temperature. The full line gives results of experiments at pH 8.8, the dashed line at pH 7.7, and the dotted line at pH 7.2. Ten per cent of a M/5 phosphate buffer was used to adjust the pH.

Enhancement of the lytic effect of ammonium sulphate by unslaked lime

As was shown previously, the lytic activity of ammonium sulphate is greater at elevated pH values. In an attempt to utilize this property to increase the effectivity of ammonium sulphate in the ponds, the application of ammonium sulphate was supplemented with powdered, unslaked lime. Laboratory tests showed that the concentration of ammonium sulphate necessary for complete lysis could be reduced at least 3-fold by the addition of 2.5 mg. per cent of calcium oxide. Field conditions were simulated on a moderate scale by partially embedding rectangular bottomless sheet-iron frames in the bottom soil of the pond, thus forming an enclosure in which tests of the different treatments could be performed. A typical experiment is summarized in table 1. It can be seen from the data that the calcium oxide markedly enhanced the effectivity of ammonium sulphate.

The partitioning of a small area of an ordinary fish pond into test cells by embedding bottomless containers

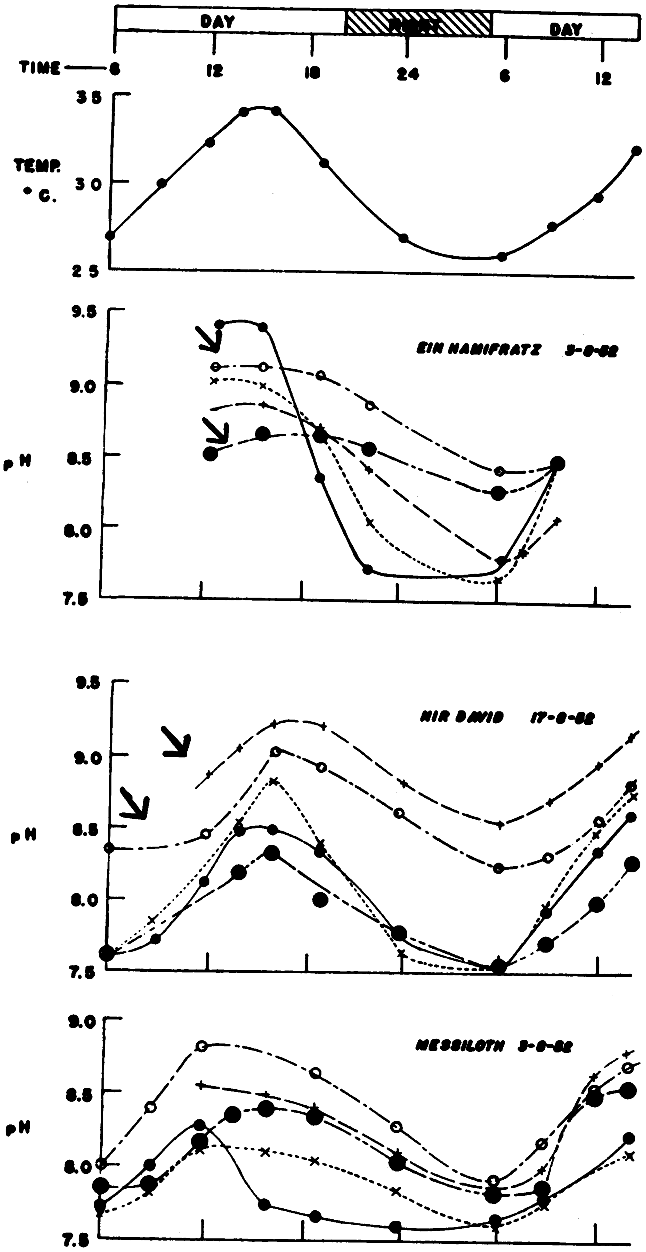

FIG. 2. Daily variations of pH in fish ponds in different regions. The values represent readings of the pH of water samples taken at 10 cm below the surface from a constant selected point in the pond. Measurements were made with a Beckman model 2 H pH meter no more than 10 minutes after collection of samples. All measurements were made during the month of August.

Each curve of the lower three groups of curves is a plot of pH variations with time in a different pond. Curves for ponds which had been filled a short time before testing and which were therefore especially low in phytoplankton are indicated by an arrow (↓). The average diurnal temperature variation of the top layer of water in the ponds tested is plotted at the top of the figure.

into the pond soil may be of more general interest as providing a means of performing a parallel series of controlled field experiments conveniently on a moderate scale. Test compartments of this type duplicate the conditions of the original pond and preserve the biological equilibrium between water and pond bottom. Their convenient size permits great ease of manipulation and observation.

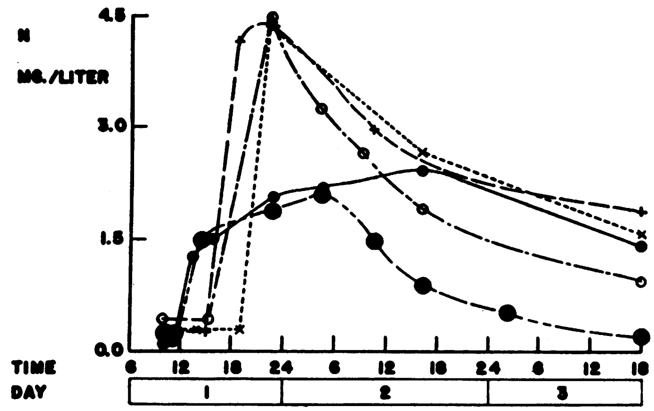

FIG. 3. Persistence of ammonia in pond water after addition of ammonium sulphate to fish ponds. Ammonia was determined as described in the Standard Methods for the Examination of Water and Sewage (1936). Results are expressed as milligrams of ammonia-nitrogen per liter. Ponds to which 15 to 20 kg/dunam (Webster, dönüm—.22 acre) of ammonium sulphate had been added were analyzed. (Ammonium sulphate was added between 12°° and 15°° on the first day.) Each curve gives values for a separate pond. Values are averages of two samples taken from different parts of the pond. The data given are from the ponds of Nir David and Messiloth.

TABLE 1. *Effect of unslaked lime on lytic activity of ammonium sulphate on* Prymnesium

EXPERIMENT NO.	TREATMENT g/100 l		*Prymnesium*/mm³ HOURS AFTER TREATMENT			pH HOURS AFTER TREATMENT		
	$(NH_4)_2SO_4$	CaO	0	7	24	0	7	24
1	0	0	140	170	250	8.1	8.5	8.6
2	10	25	100	20	0	8.4	9.1	8.6
3	15	25	120	0	0	8.4	9.1	8.8
4	20	25	120	0	0	8.1	9.0	8.7
5	10	0	170	110	130	8.0	8.1	8.1
6	15	0	130	170	120	7.8	7.8	7.7
7	20	0	170	120	100	8.2	8.2	8.4
1 a	0	0	120	80	50	8.7	8.9	8.8
2 a	0	25	50	30	40	9.5	9.2	9.3
3 a	0	50	70	20	10	9.7	9.6	9.4

The experiments were conducted in bottomless enclosures located side by side in the same pond. Prymnesia were counted in a blood-counting chamber.

The pH of water, collected at a depth of 10 cm from the surface, was measured with a Beckman 2 H pH meter within 10 minutes after collection.

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Summary

Lytic activity of ammonium sulphate on *Prymnesium* was found to vary with temperature, being higher at elevated temperatures. The lower activity of ammonium sulphate in fish ponds during the winter may be due to this fact.

Lytic activity of ammonium sulphate on *Prymnesium* varies with pH, activity being greater at higher pH.

The close correlation between the pH dependence of the lytic activity of ammonium sulphate and the increased concentration of free ammonia of high pH values, due to dissociation of the ammonium sulphate, strongly suggests that free ammonia is the active lytic agent.

The pH of fish ponds has been found to vary rhythmically during the course of the day, the degree of variation differing in different regions. This provides a plausible explanation for the regional localization of relative inactivity of ammonium sulfate.

The efficiency of ammonium sulphate can be improved by adding it to the ponds during the daily pH rise. Supplementing the application of ammonium sulphate with unslaked lime further increases the pH and enhances the lytic effect on *Prymnesium parvum*.

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