THE IN VITRO ANTIBACTERIAL ACTIVITY OF "TAZMA MAR" HONEY PRODUCED BY THE STINGLESS BEE *(Apis mellipodae)*

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ABSTRACT: In 1993 the antibacterial effect of "tazma mar" was evaluated on Salmonella typhimurium, Salmonella enteritidis, Escherichia coli, Bacillus cereus and Staphylococcus aureus at concentrations of 10%, 15% and 20% in Brain Heart Infusion Broth. In the absence of "tazma mar", the Gram negative test strains reached counts > 108 cfu/m1 within 12 hours and maintained the count until 48 hours. At 10% concentration, typhimurium, S. enteritidis and E. coli were not inhibited until 12 hours, but thereafter their number declined faster and complete inhibition was observed at 48 hours. Retarded growth and inhibition was noted at 15% and 20% concentrations. A more marked growth retardation and inhibition at all concentrations was noted on B. cereus and Staph. aureus. "Tazma mar" may be effective to treat food-borne infections at low concentrations. [Ethiop. J. Health Dev. 1994;8(2):109-117]

INTRODUCTION

Although honey has been used for dressing wound since ancient times (1), its antibacterial property was recognized only very recently (2). The antibacterial activity was originally believed to be only due to high osmolarity , with its water content rarely exceeding 20% (3). Another antibacterial factor in honey was reported to be its relatively low pH value which is normally around 4 (4). A third factor was believed to be "inhibine" (5), an antibacterial substance, later found to be hydrogen peroxide generated by the action of glucose oxidase in honey (6). White and Subers (7) later observed that some honey samples had antibacterial activity in excess of that which could be accounted for by the action of hydrogen peroxide alone. This antibacterial activity persisted after the removal of hydrogen peroxide by the addition of catalase (8).

Recently, the use of honey as a topical antibacterial agent has been accepted to treat surface infections such as ulcers and bed sores (9, 10), and those resulting from burns, injuries and surgical wounds (11-13).

Many investigators have reported the antibacterial activity of honey against Staphylococcus aureus, Pseudomonas aeruginosa, Citrobacter freundii, Escherichia coli, Proteus mirabilis, Streptococcus faecalis, and Listeria monocytogenes (14-15) .

Most of these studies were made on honey produced by the honey bee. In Ethiopia, honey produced by the stingless bee (commonly known as "tazma mar") is considered to be important in traditional treatment of respiratory ailments, surface infections and various other diseases. Considering the fact that there is a significant association of the potency of honey with the floral type (16), it would be Worthwhile to examine the potency of honey produced by a different species, the stingless bee. The purpose of this work was to evaluate the antibacterial activity of "tazma mar" against some food-borne pathogens, thereby determining the possible role of "tazma mar" in the treatment of food infections.

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METHODS

Preparation of "tazrna mar" "Tazma mar" was purchased from a local market and diluted in Brain Heart Infusion (BHI) Broth (MERCK) in 100 mi amounts in sterile screw capped bottles to give a final concentration of 10%, 15% and 20%. BHlbroth with no "tazma mar" served as a control.

Cultures

The following bacterial cultures were used in this study. Salmonella typhimurium (A 13), Salmonella enteritidis (A 2), Escherichia coli (WS 1323), Staphylococcus aureus (WS 1759) and Bacillus cereus (WS 1537). The cultures were obtained from the culture collection of Bakteriologisches Institute, SVFA, Weihenstephan, former Federal Republic of Germany.

Inoculation with test organisms

The test organisms were separately inoculated in the three dilutions of "tazrna mar" and in the control bottle to get a final inoculum level of around 103 cfu/ml. The mixture was shaken thoroughly and incubated at 32°C for 48 hours. The initial inoculum level was determined by surface plating with appropriate dilutions from the freshly inoculated control bottles on Brain Heart Infusion Agar (MERCK) in duplicates.

Analysis of samples

Cultures were sampled at 6-hour intervals for 48 hours. Appropriate dilutions of all cultures were separately surface plated on BHI agar and incubated for one hour at 32°C to allow metabolic recovery of injured cells. An overlay of the following agar media was then separately added on to the inoculated plates: XLD for S. typhimurium and S. enteritidis, VRB for E. coli, Mannitol Salt agar for Staph. aureus, and Bacillus cereus agar for B. cereus. Colony counting was done after incubation at 32°C for 24-48 hours.

The pH of the "tazma mar" solutions was measured using a digital pH meter .

RESULTS

S. typhimurium, S. enteritidis and E. coli showed a similar pattern of growth in the control broth and of inhibition at the various concentrations of "tazma mar" (Figures 1-3). They reached a level higher than lOS cfu/rn1 within 12 hours in the control broth and maintained nearly the same level upto 48 hours. At 10% "tazma mar" concentration, growth was not affected until 24 hours, where all reached a count of > 108 cfu/rn1. After 24 hours, however, there was a sharp decline in count resulting in complete inhibition at 36 hours in the case of S. enteritidis and E. coli and 48 hours in the case of S. typhimurium. At 15% concentration, the lag phase for the test organisms was longer, the growth rate was low and the maximum count reached was less than 1& cfu/rn1.

"Tazma mar" concentrations of 20% had a bacteriostatic effect until 24 hours, followed by a sharp decline and then complete inhibition at 36 hours. The Gram positive test organisms (B. cereus and Staph. aureus) showed a different growth pattern from that of the Gram negative ones at the various "tazma mar" concentrations. Growth in the control broth was luxurious, although the count of B. cereus did not reach 108 cfu/rn1 at all times (Figure 4).

At 10% "tazma mar" concentration, the count of B. cereus did not decrease markedly until 5 hours, but a slight decline was observed until 12 hours. Decline in count was sharper after 24 hours but no complete inhibition was observed even at 48 hours (about 10 cfu/rn1). A similar pattern was also observed at 15% "tazma mar" concentration.

A concentration of 20% was effective to reduce the count gradually from 0 hour until complete inhibition at 48 hours. Although Staph. aureus grew to counts > 108 cfu/rn1 in the control broth, its count was maintained under 1Q4 cfu/rn1 at all times at all concentrations of "tazma mar" .A sharp decline in counts started at 30 hours followed by a complete inhibition at 36 hours (Figure 5).

The pH values for "tazma mar" concentrations of 10%, 15% and 20% were 4.0, 3.94 and 3.91 respectively.

Figure 1. Response of *Salmonella typhimurium* to various concentrations of "tazma mar".

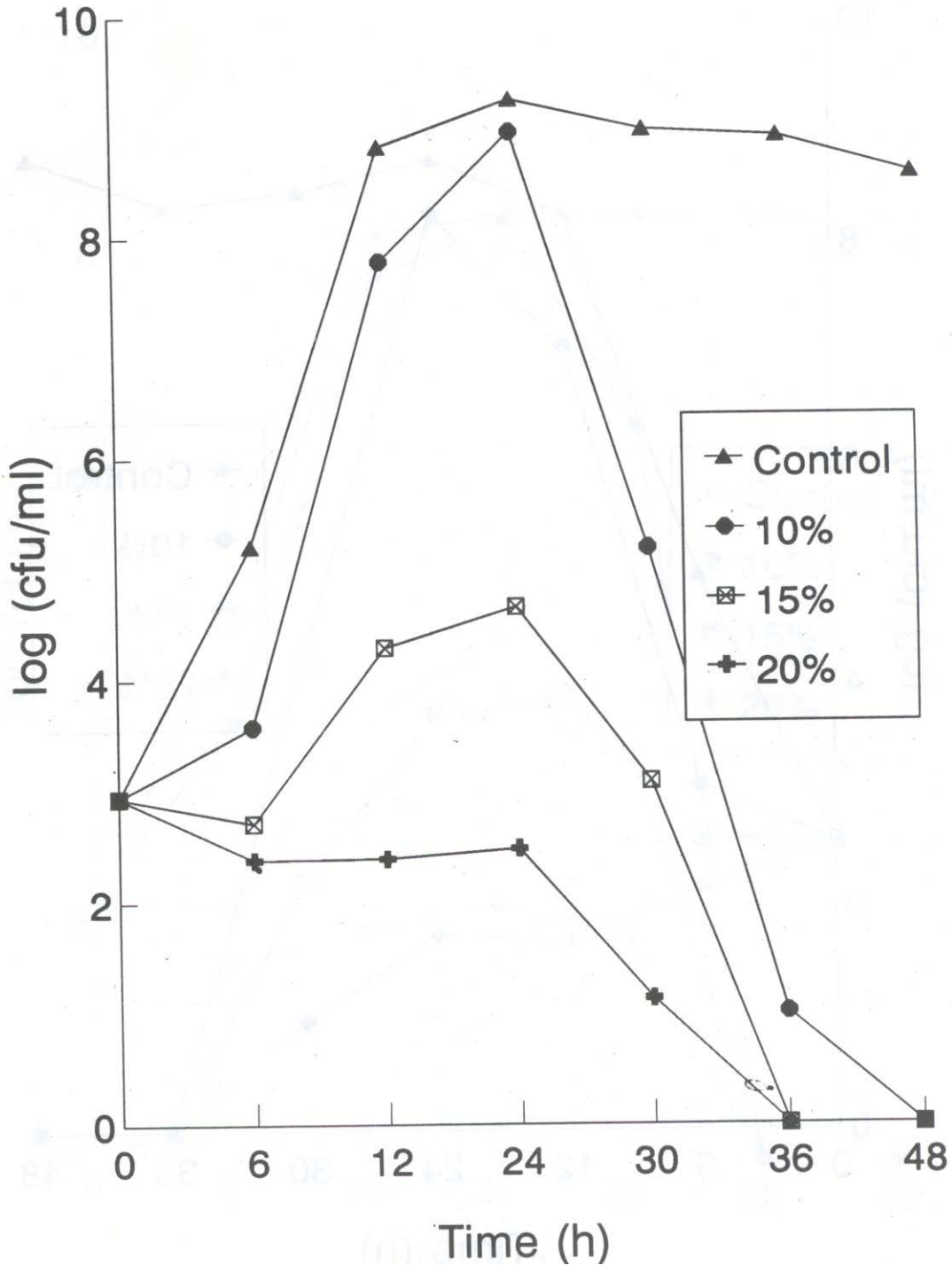


Figure 2. Inhibition of *Salmonella enteritidis* at various concentrations of "tazma mar".

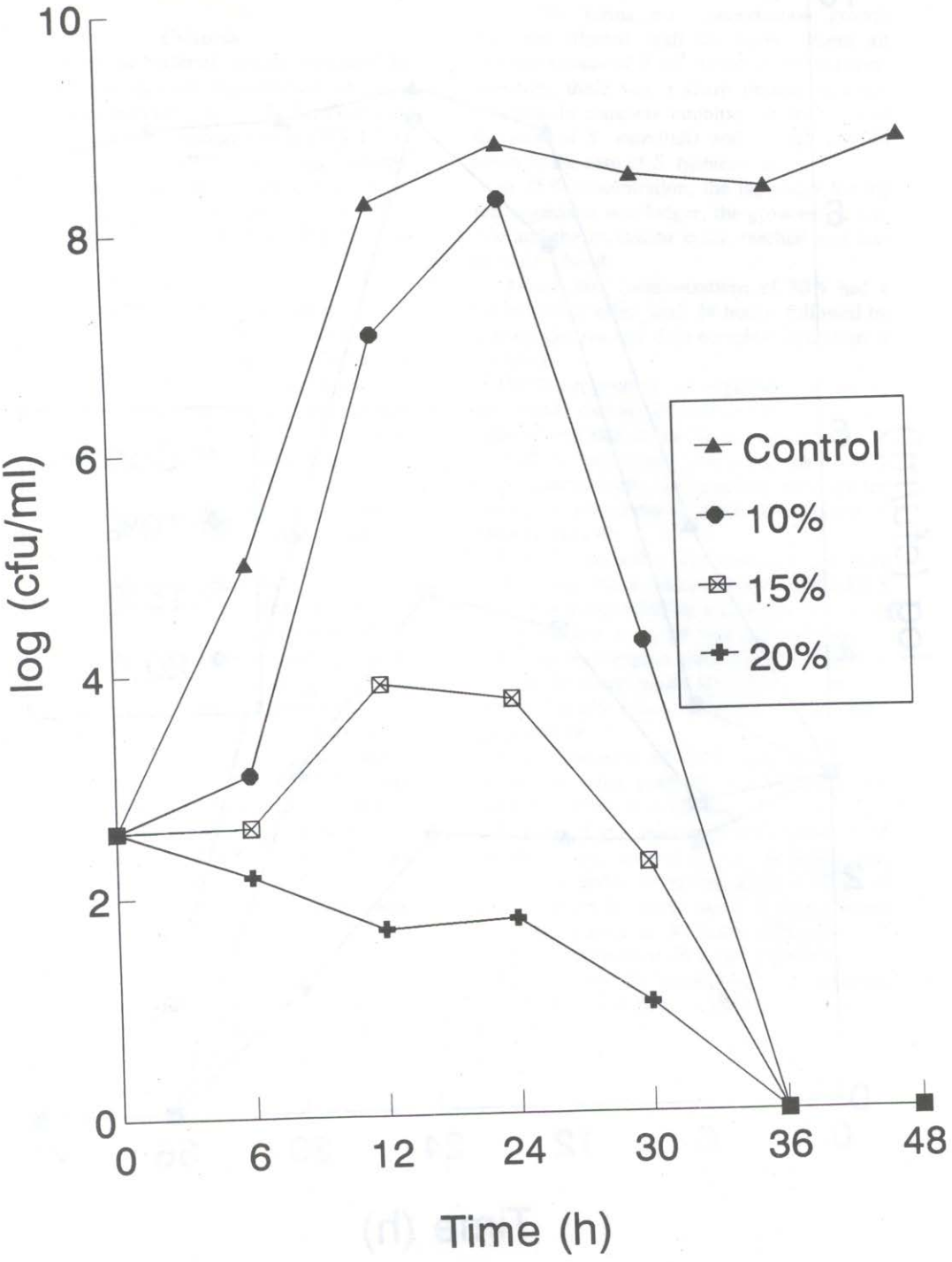


Figure 3. Fate of *Escherichia coli* at various concentrations of "tazma mar".

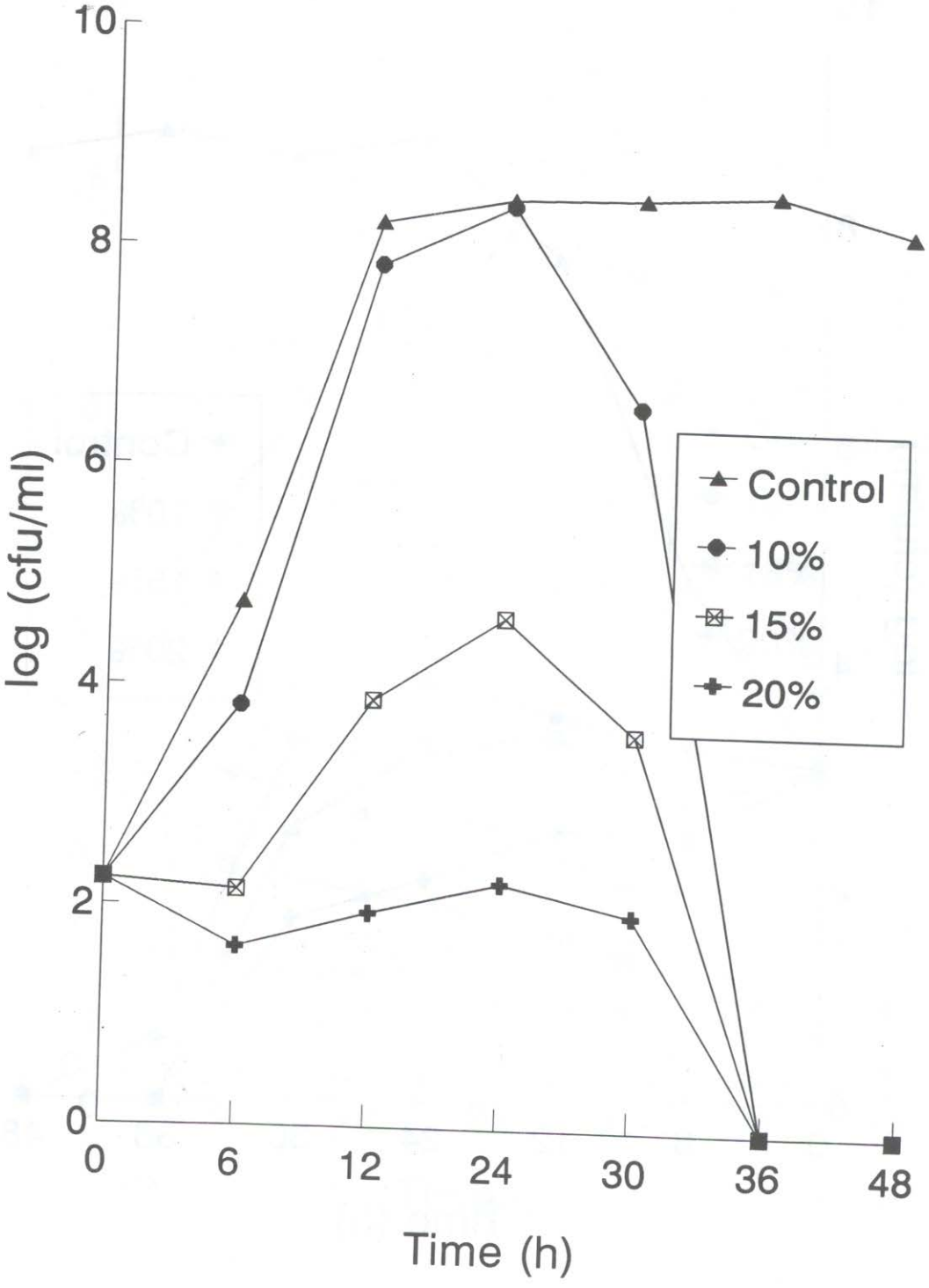


Figure 4. Response of *Bacillus cereus* to various concentrations of "tazma mar".

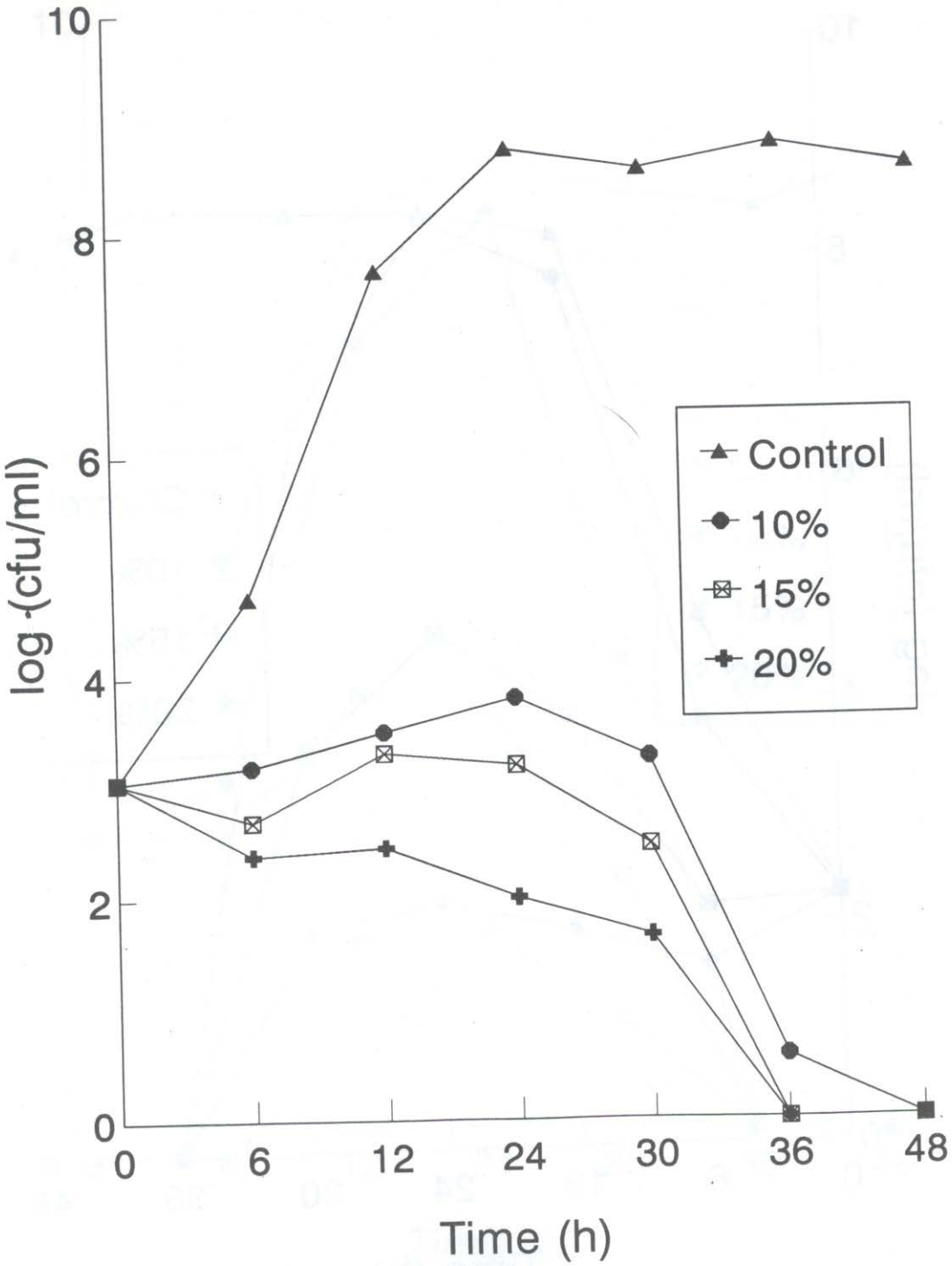
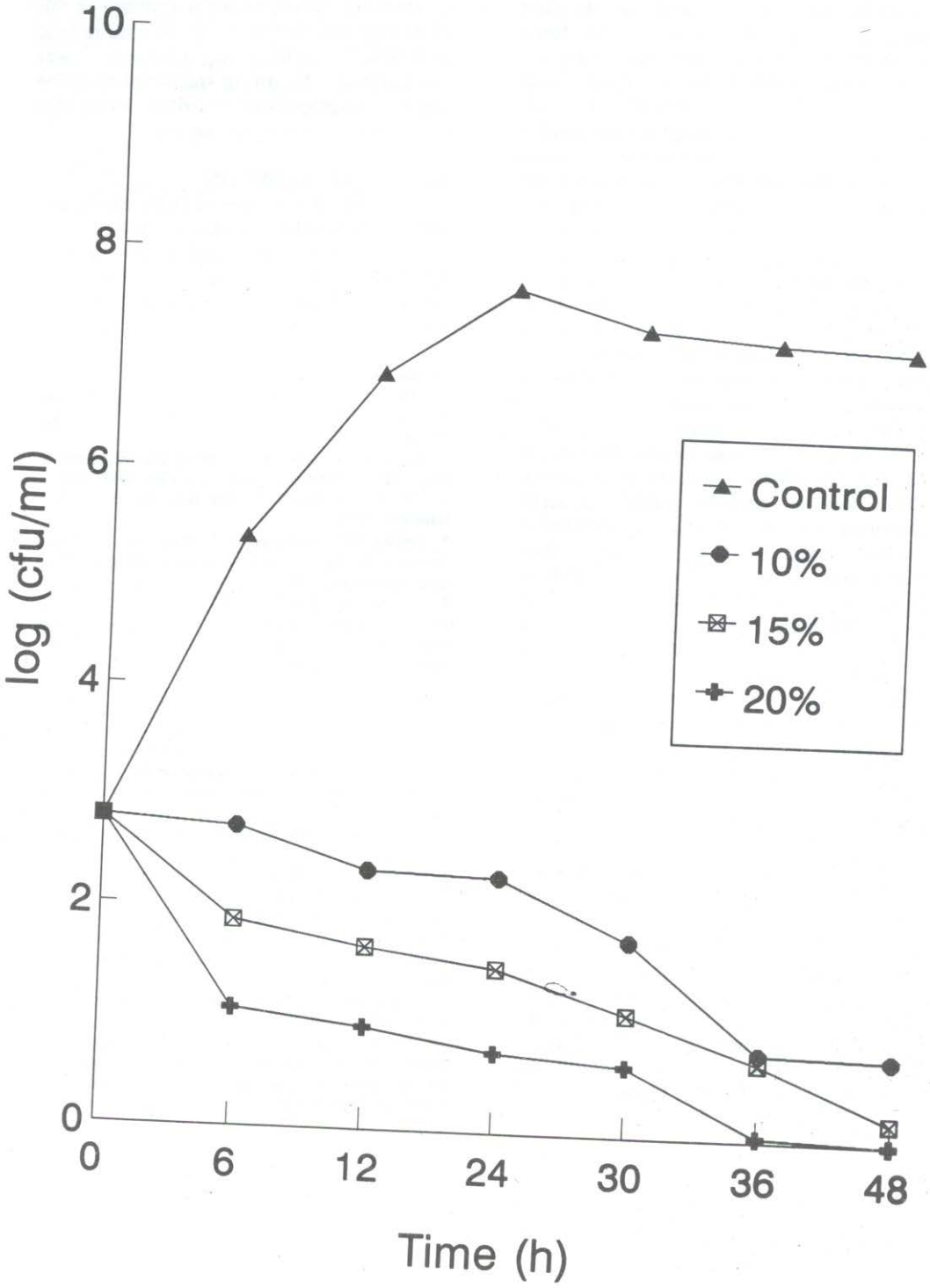


Figure 5. Inhibition of *Staphylococcus aureus* at various concentrations of "tazma mar".



DISCUSSION

There is no information available in the scientific literature on "tazrna mar" to make comparisons. But similar studies on honey produced by the honey bee have shown that honey could inhibit S. aureus, Pseudomonas aeruginosa, Citrobacter freundii. E. coli, Proteus mirabilis, and Streptococcus faecalis (15). Other workers have reported that honey has an antibacterial effect on Salmonella spp . and E. coli, but the inhibitory effect was much more pronounced at 75-80% of honey concentration (14). The complete inhibition of organisms causing surgical infection or wound contamination was also effected by honey concentration of 100% and partial inhibition at 50% (17). In contrast to these reports, "taima mar" , in this study, could inhibit most of the test organisms at very low concentrations (10-20%). The antibacterial property of "tazma mar" could be due to various factors. Its low pH (around 4) could be inhibitory to B. cereus, which does not normally multiply in acidic conditions. The other organisms are reported to tolerate a lower pH (18,19). In addition, since there was no marked difference in pH values of the various "tazma mar" concentrations, the high growth rate of the Salmonella spp. and E. coli at 10% concentration indicated that pH alone is not an important inhibitory property; it is worth noting that highly osmotolerant strains like S. aureus were markedly retarded at a concentration as low as 10% .The inhibitory property of hydrogen peroxide in honey may not
be so significant since all the test organisms were catalase producers which can break down hydrogen peroxide. "Tazma mar" may, in addition, have other antibacterial substances which are effective at lower concentrations. Bogdanov (20) characterized a flavonoid compound as the antibacterial substance in honey
produced by the honey bee and very recently Russel et. al. (21) identified trimethoxybenzoic acid, methyl syringate and syringic acid as the antibacterial constituent of honey. Further studies are, therefore, required to identify the important antibacterial constituents of "tazma mar".

The effectiveness of "tazma mar" in retarding or inhibiting growth of the test strains in this study may indicate that it may be used to treat food-borne infections at relatively lower concentrations. Its use in traditional medicine may thus be properly evaluated and it may also serve the food preserving industry .

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