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EFFICACY OF A NEW TABLET FORMULATION OF AN ASPOROGENOUS STRAIN OF *BACILLUS THURINGIENSIS ISRAELENSIS* AGAINST LARVAE OF *AEDES AEGYPTI*

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ABSTRACT: A new tablet formulation based on a new asporogenous strain of *Bacillus thuringiensis israelensis* was tested against *Aedes aegypti* larvae in 25 liter containers. The influence of food attractants, stirring of the water, as well as the efficacy of the tablets in plastic containers and earthen-ware jars were determined. A long-term effect of about 30 days with a mortality rate of 86.5-97.6 percent could be achieved. Tablets of *B.t.i.* might be a sensible supplement or even replacement for chemicals such as Abate in *Aedes aegypti* control.

INTRODUCTION

In terms of morbidity and mortality, Dengue is the most serious arbovirus disease confronting mankind. Approximately 1.5 billion people in the tropics, especially Asia, the Western Pacific region, the Caribbean, and Central and South America, live under the risk of dengue infections. It is estimated that more than 600,000 people have been hospitalized and more than 20,000 people have died as a result of dengue and DHF during the past decades (Halstead 1980, 1982). The economic losses caused by this disease are immense.

Epidemiology

The classic form of dengue has been known for more than 100 years. Due to the mild clinical symptoms, it was characterized as a relatively benign disease, transmitted by a few species of mosquitoes. However, the picture of this disease has changed over the last decades. The incidence of dengue and the frequency of epidemics has increased tremendously, thus, changing the clinical picture to that of a severe and fatal haemorrhagic form, known as dengue haemorrhagic fever (DHF).

It is assumed that dengue was originally a zoonosis of monkeys and was predominantly transmitted by *Aedes albopictus*, a species which prefers natural waters as its breeding site and which bites outside of houses (Wellmer 1983).

Wherever human settlements advanced into the forest, people became infected; however very soon acquiring immunity to this relatively mild disease, which then developed into a childhood disease. Simultaneous with the development of large urban settlements, *Aedes albopictus* lost its breeding sites, while at the same time the inhabitants lost their immunity.

When *Aedes aegypti*, originally a tree-hole breeder in Africa, entered Asia at the end of the last century, this mosquito replaced *Aedes albopictus* as vector in urban areas. Due to its excellent adaptation to human habitation, *Aedes aegypti* became the principle vector of dengue and DHF. The breeding sites of this mosquito vary widely, ranging from water containers, which are the most common breeding site, to rock pools, leaf axils, and tree-holes. During and after the Second World War, due to the disruptions caused by the war and the subsequent urbanization in South-East Asia, *Aedes aegypti* encountered excellent developmental conditions. During this period, in the early 1960's, dengue evolved into the haemorrhagic form in South-East Asia.

Dengue haemorrhagic fever was first recognized in the Philippines in 1953, in Bangkok five years later, and in Surabaya, Indonesia in 1968. Today, DHF has spread throughout most of Asia (Bang and Shah, 1987).

The Clinical Picture

Whereas, the classical dengue fever is a disease of older children and adults, the highest risk group for dengue haemorrhagic fever is children over 1 year and under 13 years of age.

Usually, an incubation period of five to eight days follows the infectious bite. In serious cases, the illness starts quickly with fever, stomachache, and vomiting. This phase lasts two to three days. On the third day, while the fever persists, the face becomes swollen and red, and the extremities are damp and cold. At this stage the victim is usually admitted to the hospital. Shortly afterwards, the haemorrhagic symptoms become evident:

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for example, haemorrhaging of the skin and mucous membranes, nose bleeding, vomiting of blood, or passing blood with the stool (Wellmer 1983).

In very serious cases, a drop in temperature is accompanied by a shock of the circulatory system due to the damage of the capillary vessels, allowing protein and liquid to ooze from the bloodstream into the tissue. The blood pressure falls to a point where it can no longer be measured. For the most part, this shock syndrome is associated with a secondary infection of another dengue serotype.

The Virus

The dengue virus belongs to the family of Flaviviridae, of which the yellow fever virus is the type species. This family comprises a large number of arboviruses (Group B), such as the virus of Japanese encephalitis, St. Louis encephalitis, or tick-borne encephalitis. Today, four serotypes are known, called dengue serotype 1, 2, 3, and 4.

Control of Dengue

Although intensive efforts have been made to develop suitable vaccines against dengue, there is no chance for success in the foreseeable future. Within the scope of medical treatment, the only means of reducing the case fatality rate is early diagnosis and proper case management (Bang and Shah 1986).

The only possibility of controlling the disease is by attacking the vector. It is, of course, much cheaper to prevent an outbreak of the disease than it is to diagnose and to treat cases. It is estimated, for example, that each DHF-case costs between 50 and 200 U.S. dollars, depending on the seriousness of the case in question (Sumarmo and Suroso 1987).

The main methods of dengue vector control are:

- Source reduction by means of environmental sanitation. The elimination of all non-essential water containers functioning as breeding water for *Aedes aegypti* is the most effective method in terms of long-term reduction of the mosquito population and of the costs associated with it.

- Protection of water containers, for example, by means of lids to prevent larval breeding.

- Release of larvivorous fish as predators of larvae. School children, for example, can raise fish and sell them for mosquito control.

- Observance of a "weekly dry day," meaning that the containers are to be emptied at least once a week.

- Cleaning the containers before and after the rain season can also help reduce mosquito populations.

- The most commonly used methods are space spraying, for example, with Malathion against adult mosquitoes, and larviciding with Temephos.

Despite all of the control measures mentioned above, sustained "community participation" is still the strongest asset of a successful campaign. An integrated community-based approach, with volunteers from the village, is the most promising method of motivating a community to participate in vector-control programmes. Women can be of vital importance, since their participation enhances the possibility of reaching individual households and of ensuring sustained community support (Yoon 1987). Integration with other community projects, such as water supply, community development, sanitation programmes, child education, and income-generating activities can help to sustain vector control programmes on the basis of community participation. Of course, government coordination, close supervision, monitoring and evaluation, and last but not least, team work with public health personnel, local volunteers, social scientists, and entomologists are necessary to successfully reduce transmission of vector-borne diseases. Entomological, virological, and epidemiological surveillances are the basis for good results.

B.T.I.-TABLETS IN AN INTEGRATED VECTOR CONTROL PROGRAMME IN JAKARTA

Jakarta is one of the most rapidly developing and growing cities in South-East Asia. At the same time, ever since dengue haemorrhagic fever was diagnosed for the first time in 1968, the number of cases has risen quickly and steadily. Whereas, in 1970 only 477 persons suffered from dengue haemorrhagic fever and 90 of these died, in 1986 there were over 16,000 cases of this disease, with a total of 600 deaths. Between 1968 and 1986 a total of 116,567 people contracted the disease and 5,092 of them died (Suroso 1987). Approximately 25 percent of all dengue cases in Indonesia were recorded in Jakarta. Together with the Ministry of Health, the Provincial Government of Jakarta (DKI) is undertaking a tremendous effort to solve the dengue problem (Masyhur 1987). In Jakarta, a full 200 health service employees are involved in DHF control every year. The annual budget amounts to some 200,000 U.S. dollars.

So far, the measures for the control of *Aedes aegypti* focus on the following aspects (Suroso, 1987):

- Health education and environmental sanitation with community participation.
- Mass application of Temephos. Between 1983 and 1985, more than 150 tons of 1%-Abate sand granules were used in Indonesia, with a total value of over 280,000 U.S. dollars.
- Space spraying of Malathion within a radius of 100 meters of the site of a dengue-case occurrence.

In spite of these efforts, it has not been possible to overcome the dengue problem. All that has been achieved is the prevention of an even more drastic increase in the number of cases. Up until now in Indonesia, the organophosphate insecticide Abate (Temephos) has been the most commonly used chemical for mosquito control in water containers, which usually contain 200 liters of water. Abate is also sold to private people; for example, as a 1%-sand granulate. One package contains 10 grams and is sufficient for 100 liters of water. Application at two-month intervals is recommended. However, many people do not like the rotten egg-like odor of this chemical in water used for consumption and the household. Furthermore, it is likely that resistance against Abate will develop in the near future.

For these reasons, there is a need to develop new approaches for controlling dengue haemorrhagic fever. A promising agent is *B.t.i.*, which has been used in Germany for many years with excellent results against floodwater mosquitoes (Becker and Ludwig 1983). Within the scope of a cooperation program between the Health Department of Jakarta, the University of Indonesia in Jakarta, as well as the University of Heidelberg and the German Mosquito Control Organization (KABS), the possibilities of using *B.t.i.* products against *Aedes aegypti* larvae were tested.

Mosquito feeding-behaviour plays an important role in the effectiveness of various formulations of *B.t.i.* toxin against different species (Aly et al. 1988). In contrast to Anopheline larvae, which feed at the water surface (surface feeders), and *Culex pipiens*, which filters food from the upper zones of water (suspension feeders), the larvae of *Aedes aegypti* graze at the bottom and the walls of water containers (bottom feeders).

Therefore, a tablet formulation has to fulfill all of the following prerequisites in order to be able to fight *Aedes aegypti* larvae:

1. It must sink to the bottom and be available there as food to the larvae.
2. It must function as a *B.t.i.* depot at the bottom of the container, so that the effect is more or less independent of the water above it. It is advantageous that the efficacy of *B.t.i.* be independent of its concentration in the water; after all, water is regularly taken out of the containers for use and then refilled with fresh water.
3. *B.t.i.* tablets can easily be sold on the market, since this formulation is accepted by the users. If, on the contrary, a powder formulation were put into the water containers, the water would become turbid, giving the impression of contamination.

Due to contamination of the drinking water with live bacteria or spores, the *B.t.i.* material used to control *Aedes aegypti* must be hygienically pure and free of bacteria or spores. In this context, the asporogenous *B.t.i.* strain developed by the BASF is recommended here.

MATERIALS AND METHODS

In the test series thirty-liter containers (e.g. plastic containers or earthen-ware jars) filled with 25 liters of water from a well were treated with different *B.t.i.* tablets. Fifty early fourth-instar larvae of *Aedes aegypti* were then placed into each container. The larvae used in the experiments were obtained from a colony reared in the laboratory. After 24 hours, all of the still living or dead larvae were collected with a pipette and the mortality rate determined. Afterwards either two or four liters of water were taken from the containers and replaced with fresh water in order to simulate normal conditions. Finally, 50 early fourth-instar larvae were added again to each container. The water temperature varied between 25° and 27°C. The tests were carried out in two replicates (exception: series one and two without replicates), and in each experiment two containers remained untreated for control purposes. The mean value and standard deviation were calculated.

It was assumed that a phagostimulant would stimulate the larvae to feed more upon the *B.t.i.* tablet material. Therefore, two different tablet formulations were examined in a first series of tests.

One formulation (tab+) consisted of 500 mg of asporogenous *B.t.i.*-WP, 500 mg of inert material, and 500 mg of wheat flower as a food attractant or phagostimulant; each tablet weighed 1.5 grams.

A second formulation (tab-) consisted of 500 mg of asporogenous *B.t.i.*-WP and 500 mg of inert material, but without a food attractant (weight/tablet: 1 gram).

Plastic containers and earthen-ware jars were used for this test series in order to evaluate whether the material from which the containers is made has any influence on the effectiveness of the tablets.

Both one plastic container and one earthen-ware jar were treated with tab+ (1,5 mg/l) and tab- (1,0 mg/l). The concentration was 0.5 ppm of active B.t.i. material in both test series. One container of each type remained untreated.

In a second series, the influence of turbulence when water was removed from plastic containers and then refilled was tested. In one set of plastic containers, the 4 liters of water were removed carefully each day so that no turbulence occurred, whereas, in a second set of containers, turbulence was caused when the water was removed and added again.

In the third test series, three different tablet formulations (designated as tablet A, B and C) were tested in a concentration of 10 ppm of active *B.t.i.* material. The tablets differed with respect to the composition of their inert material and did not contain additional food attractants. In comparison, Abate was tested in the recommended concentration of 0.1 gram per liter. The tests were carried out in two replicates and two containers were left untreated as controls. In the evaluation, the mortality rates of five-day blocks (pentads) were used. For this purpose, the mean mortality rate for the five successive days of each pentad was determined. Then, the means of the pentads were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The results indicate that as far as long-term effects were concerned, tablets with food attractants were not significantly more active than tablets without attractants (Figure 1 and 2). Whereas, in the earthenware jars the tablets with attractants were slightly more effective than in the plastic containers, the tablets without attractants were effective about one week longer. The reason for this unexpected result might be that tablets with wheat flower dissolve faster than tablets without attractants. Furthermore, it could be possible that the development of microorganisms is stimulated by the wheat flower, thus, causing the *B.t.i.* toxin to decompose more rapidly. It can be assumed that the tablet without attractants already contains a certain amount of organic matter resulting from the fermentation, and that this organic matter is sufficient as an attractant, thus, making it unnecessary to add further attractants to the formulation.

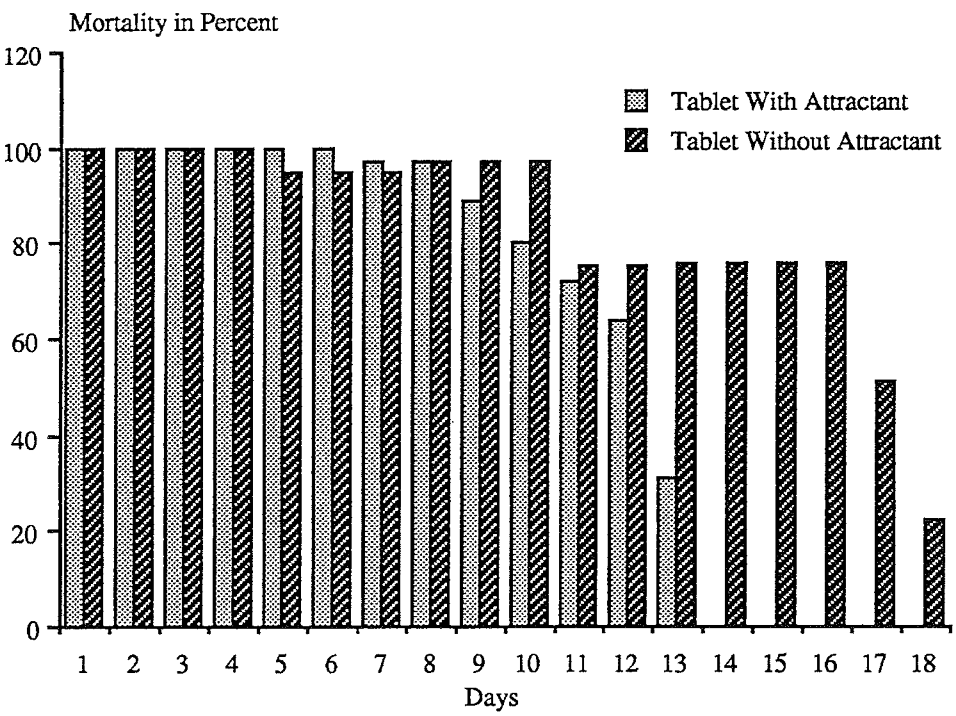

Figure 1. Efficacy of *B.t.i.* tablets with and without attractants in plastic containers.

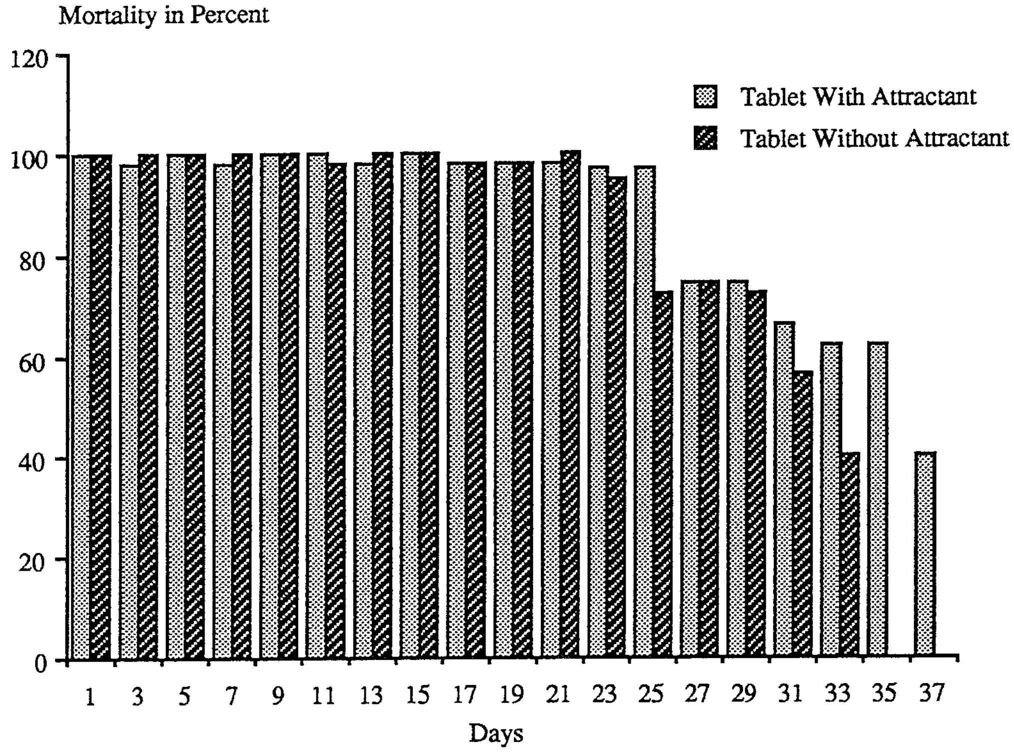

Figure 2. Efficacy of *B.t.i.* tablets with and without attractants in earthen-ware jars.

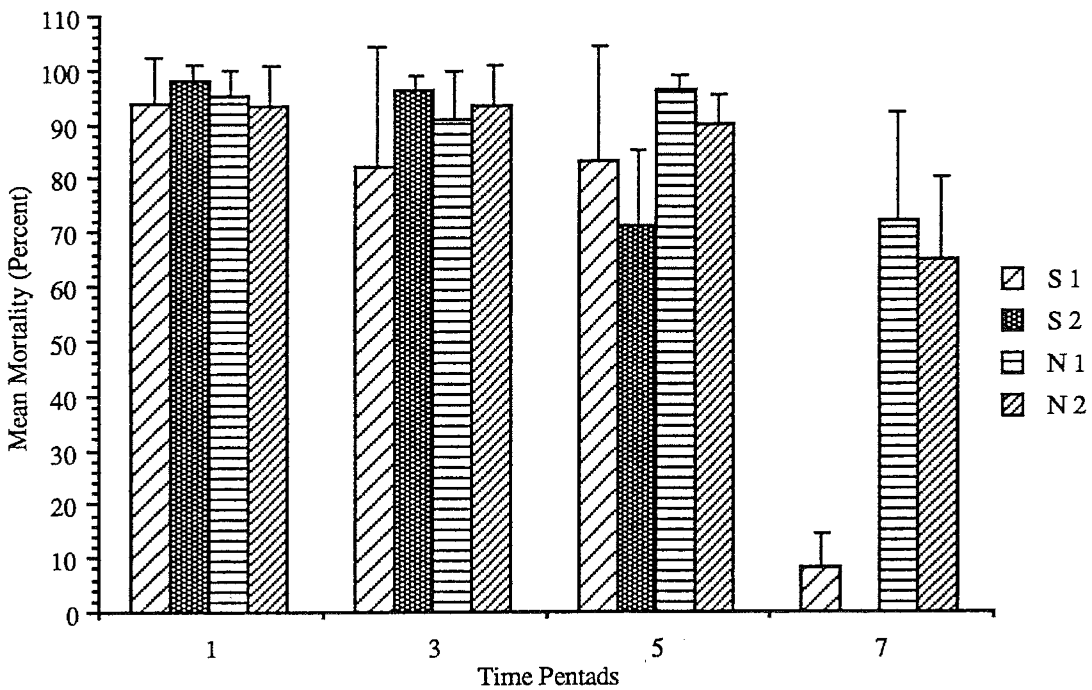

Figure 3. Impact of water turbulence on the long-term effect of *B.t.i.* tablets.
S = stirred, N = not stirred (1 and 2 = replicates), ⊢—⊣ = standard deviation.

As is also shown in Figure 1 and 2, the material of the containers does have some influence on the long-term effect of the tablets. In plastic containers the tablets are less active than in earthen-ware jars. This is perhaps due to the longer persistence of *B.t.i.* toxins in the pores of the clay material.

The results presented in Figure 3 show that the tablets remain effective considerably longer if no turbulence occurs. This indicates that the tablet formulation can have a lasting long-term effectiveness due to its greater retention time.

In Figure 4 and TABLE 1, it is shown that the mortality rates caused by tablet B and C were already down to less than 70 percent after the fourth week, whereas, tablet A still had a mortality rate of about 90 percent. In the fifth week, tablet A had mortality rates of 70 percent, while tablet B and C were significantly less effective. In comparison, Abate showed mortality rates of 99.2 to 100 percent for at least five weeks (7 pentads). In the control the mortality rates were between 0 and 1.2 percent.

CONCLUSION

From these results the conclusion can be drawn that *B.t.i.* might be a promising agent in the fight against dangerous diseases such as DHF in the tropics. *B.t.i.* preparations in suitable formulations are a sensible supplement or even replacement for chemicals such as Abate. It can also be assumed that, by applying *B.t.i.* in integrated programmes, the onset of resistance can be prevented at least for the time being.

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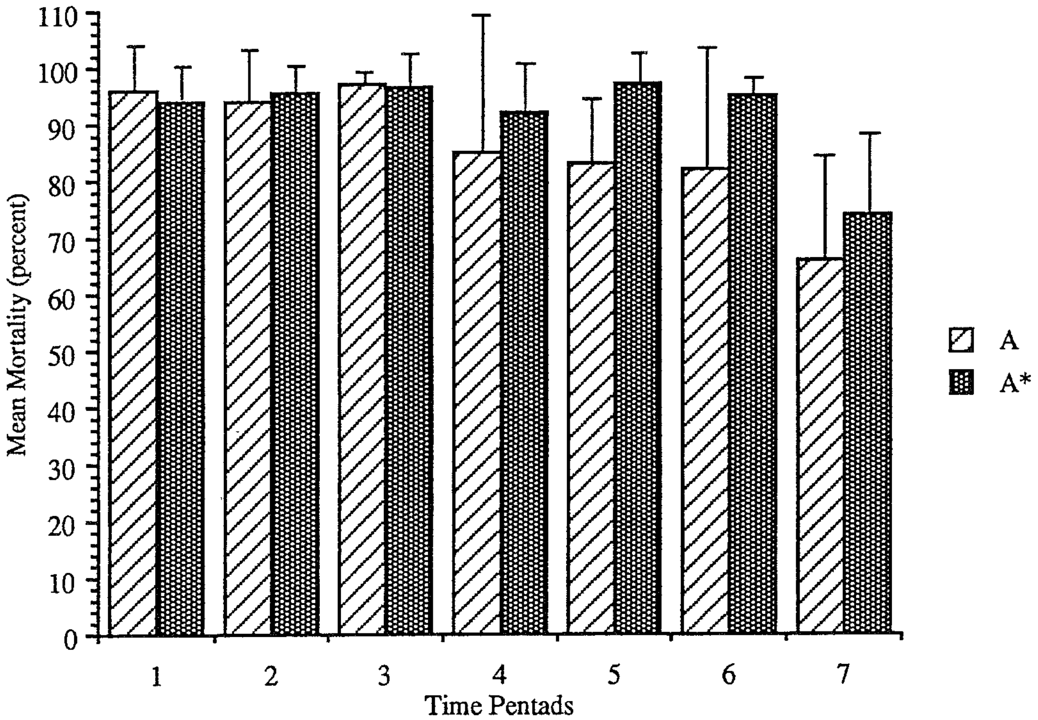

Figure 4. Efficacy of tablet A and replicate A*. The means of pentads 1 to 6 are not significantly different (P> 0.05).

TABLE 1. Efficacy of various tablet formulations (Tablet A, B, and C) of *Bacillus thuringiensis israelensis*. The means of pentads 1 to 6 of Tablet A and A* and of pentads 1 to 4 of Tablets B and B* as well as C and C* are not significantly different (P> 0.05). SD = standard deviation.

Formulation	Mean Mortality in Percent and SD						
Pentads	1	2	3	4	5	6	7
Tablet A	96.4±7.13	93.2±9.01	98.0±1.41	85.6±23.55	83.2±9.45	82.4±20.17	65.6±18.02
Tablet A*	93.6±6.23	95.2±4.82	97.2±4.38	90.8±9.01	98.0±3.74	95.2±3.35	74.4±13.64
Tablet B	96.8±3.03	92.8±9.96	94.4±4.78	82.4±12.20	69.2±15.07	52.4±18.19	0.8±1.10
Tablet B*	95.2±4.60	90.0±11.92	94.4±4.34	92.4±6.39	75.2±13.54	65.6±13.67	2.0±2.00
Tablet C	98.0±2.45	92.0±12.33	96.4±2.61	84.4±14.24	70.8±13.54	44.8±20.52	0.0±0.00
Tablet C*	92.8±8.08	92.4±13.81	93.2±7.56	96.0±3.16	89.6±4.34	89.2±6.72	64.8±16.10
Control	0.8±1.79	1.2±2.68	0.8±1.10	0.8±1.79	0.4±0.89	0.8±1.79	0.8±1.79
Control*	0.8±1.10	1.2±1.79	0.4±0.89	1.2±1.79	0.0±0.00	0.0±0.00	0.0±0.00

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