

Immunostimulatory effect of disinfectant on bees

Tatiana Romanishina^{1*} Svitlana Gural'ska¹ Tetiana Kot¹ Svitlana Furman¹

Oleh Pinsky¹ Diana Feshchenko¹ Zhanna Rybachuk¹ Olexiy Tkachenko²

Olga Zastulka¹ Svitlana Tushak¹

Abstract

The article presents a modern interpretation of the immunostimulatory effect of the disinfectant "Biocontact Plus" on the bee organism. The minimum threshold concentrations of the disinfectant with bactericidal effect were determined in vitro. The stimulating effect of 0.15 % of the concentration of active substances of the disinfectant has been confirmed in vivo. The immunostimulatory effect of this concentration on the immune status of bee colonies is interpreted (the number of young and mature immunocytes). The immunomodulatory effect of 0.15 % disinfectant "Biocontact Plus" on bee haemolymph allows for the use of alternative therapies for bacterial diseases of bees in the future.

Keywords: *Apis mellifera*, *Klebsiella Aerogenes*, hemolymph, disinfectant, immunostimulant

¹Polissia National University, Saryi Boulevard, 7, Zhytomyr, 10008, Ukraine

²Dnipro State Agrarian and Economic University, Serhii Efremov, 25, Dnipro, 49600, Ukraine

*Correspondence: tveterinar@gmail.com (T. Romanishina)

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Introduction

Modern beekeeping has virtually no specific means of preventing infectious diseases, which contributes to their spread and hinders the development of the industry (Glenny *et al.*, 2017; Galatiuk *et al.*, 2020; Galyautdinova *et al.*, 2020). So, alternative to the use of prophylactic groups of drugs to maintain the immune status of the macroorganism of bee colonies are new tools with polyvector action, which quickly achieve a positive therapeutic effect and increase the body's resources for better and easier recovery after any damaging factors. (Rybachuk *et al.*, 2019). Such drugs are not only standard therapeutic groups, but also small doses of other substances that sensitize the body and can cause activation of the immune system resulting in the synthesis of immunoactive cells or substances (Nasir *et al.*, 2000). There is a known practice of using small doses of substances that are part of some disinfectants or chemotherapeutic agents in oncogenic diseases (Ruggeri *et al.*, 2014), under the action of which the body, receiving small doses of toxic substances, forms a sensitizing immunoactive state. Thus, the introduction of diatomaceous polyunsaturated aldehydes, which are part of some disinfectants, activates the death of human cancer cells (Sansone *et al.*, 2014). Therefore, the use of disinfectants of various compositions in different concentrations and doses for micro-and macro-organisms - the basis of modern disinfection (Paliy *et al.*, 2020) can cause the active uncontrolled introduction of toxic substances into a living organism resulting in active reactions with nucleophilic targets (DNA, lipids, proteins), forming stable and unstable substances that further disrupt cellular functions (Sapkota *et al.*, 2015). Therefore, the study of the action of new drugs in both human and veterinary medicine is a gradual, long-term and time-consuming process that requires informational pathogenetic interpretation and laboratory confirmation.

The purpose of the study was to determine the immunostimulatory concentration of the disinfectant "Biocontact Plus" on the bee organism.

Materials and Methods

A laboratory experiment was performed to determine the nature of the drug's effect on the test culture of bee bacteria *in vitro*. Disinfecting properties of the drug "Biocontact Plus" which includes: glutaraldehyde - 13 %; glyoxylic aldehyde - 4 %; formaldehyde - 11 %; quaternary ammonium salts - 6 %; triamine - 1,5 %; fogging components - not less than 10 % and water (Tushak *et al.*, 2018; Kronos-agro) were studied on a pure test culture, excreted from the bees, of the species of *Klebsiella Aerogenes* (*Enterobacter Aerogenes*) identified by biochemical typing in the State Institution "Zhytomyr Regional Laboratory Center of the Ministry of Health of Ukraine" in 2019 (Galatiuk *et al.*, 2020), *in vitro* by diffusion from discs impregnated with low concentrations (0.05 %; 0.1 %; 0.15 %) of disinfectant solution ("Biocontact Plus") diluted in 50 % sugar syrup. The studies were performed on fifteen Petri dishes for 5 days with a test culture strain of *Klebsiella Aerogenes* (*Enterobacter Aerogenes*). The effect

of the disinfectant was evaluated by the nature of the growth of microorganisms around the discs.

The immunostimulating effect of "Biocontact Plus" was studied in bee models *in vivo*. Bees were fed different concentrations of the disinfectant (0.1 %; 0.15 %; 0.2 %) diluted in 50 % sugar syrup for 21 days. The bees of the control group received 50 % sugar syrup. The effect of the action was analysed by observation and microscopy of haemolymph smears. The cytological quantitative and morphological characteristics of haemolymph hemocytes were evaluated according to Emad M. S. Barakat (Barakat *et al.*, 2016).

Our study was approved according to the guidelines and roles of the Animal Researches Committee of Polissia National University dated September 21, 2020 (number of animal use protocol 56).

Results and Discussion

In the first stage of the research, the minimum effective concentration (0.15 %) of "Biocontact Plus" disinfectant with bactericidal (10.4 ± 0.27 mm) and bacteriostatic (26.6 ± 0.45 mm) effects was determined on the test culture *Klebsiella Aerogenes* (*Enterobacter Aerogenes*) species *in vitro* (Figure 1. - I (A, B)). Our studies of the activity of lower concentrations of the drug (0.1 %) characterized a decrease in bactericidal action (9.33 ± 0.42 mm) (Figure 1. - I (A)). The growth of pure culture of *Klebsiella Aerogenes* (*Enterobacter Aerogenes*) species around the discs with a concentration of 0.05 % "Biocontact Plus" did not differ from the biofilm around the control (clean) disc, due to the deficiency of active components of the disinfectant.

The second stage of the study (*in vivo*) found that the highest activity and longevity (21 days) was observed in bees fed a concentration of 0.15 % "Biocontact Plus" diluted in 50 % sugar syrup. The haemolymph of this group of bees differed in the number of prohaemocytes, which are the prototype of animal and human myelocytes (Figure 2). In addition, the intensity of neutrophil phagocyte synthesis by hemolymph prohaemocytes increased (Figure 1 - II (C - 2)). Whereas a concentration of 0.2 % "Biocontact Plus" diluted in 50 % sugar syrup caused mass mortality of bees on days 6-7 with the presence of low differentiated haemocytes (prohemocytes) in haemolymph smears (Figure 1 - II (D)) accordingly, a concentration of 0.2 % "Biocontact Plus" idiluted in 50 % sugar syrup causes a high antigenic load, which provokes bee poisoning. The active ingredients in Biocontact Plus (aldehydes and salts) have a disinfectant effect. Their higher concentrations (0.2 % and higher) cause a damaging effect in the cytoplasm of bee haemocytes on the principle of disinfectant action (Kronos-agro). Reaction of the bee organism to low concentrations (0.15 %) of the disinfectant is similar to the pathogenetic effect of homeopathy in human and veterinary medicine. For example, homeopathic treatment is widely used as an alternative to antibiotic therapy to prevent neonatal diarrhea in calves and to minimize bacterial infection. (Fortuoso *et al.*, 2018). In case reports, the disinfectant in a large dilution has a weak toxicating effect on the bee. The bee's immune system responds to the components of

the drug as a foreign antigen (aldehydes of the drug bind toxins, transforming them into anatoxins that act as antigens for the insect's immune system), which is a cellular target for immunocompetent cells. The effect of increased cell differentiation into young (neutrophil cells) and mature (eosinophil cells) immunocytes might be due to immune activation of the bee organism (Figure 1 - II (C - 1, 2)). In the bee organism there is a non-specific inflammatory reaction, which allows for a long time to avoid immunosuppressive action. The active ingredients of "Biocontact Plus" - glutaraldehyde, glyoxylic aldehyde, formaldehyde - are not end products of cellular metabolism and might possess immunomodulatory effects. As aldehydes are active metabolic substances, they contribute to the activation of the host defence system by forming free chemical bonds in the cells of the bee organism. The drug promotes the activation of the complementary protection system of the host organism, which causes inhibition of the synthesis of surface antigens in the form of sialic acid, which promotes evasion of immunocompetent cells (Vermoote *et al.*, 2011). It is possible that the stimulating effect of the disinfectant "Biocontact Plus" is manifested at the genetic level by

activating genes involved in the colonization of aldehyde molecules of bee hemolymph hemocytes.

The presence of aldehydes as organic compounds is known in the bee body. Also, these organic components are part of honey - formic, acetic, propionic, isobutyric, isovaleric. (Homutov, 2017). In Oxford, propolis from around the world (Austria, Ecuador, Germany, Israel, Great Britain and the United States) was analyzed, where the presence of more than 129 components, including aldehydes, was determined by chromatography. Similarly, aldehydes can be secreted by metabolites of bacteria contained in the intestines of bees. Therefore, these aromatic organic chemicals do not act as signaling agents for bee hemolymph hematocytes. They may be used by insect cells to synthesize compounds important for their own metabolism until the level of molecules of organic and synthetic compounds in the bee organism reaches a toxic threshold (Homutov, 2017). Therefore, when evaluating the action of an immunostimulant for bees, it is important to determine its threshold concentration *in vitro* (Figure 1 - I), followed by analysis of haemolymph components *in vivo* (Figure 2).

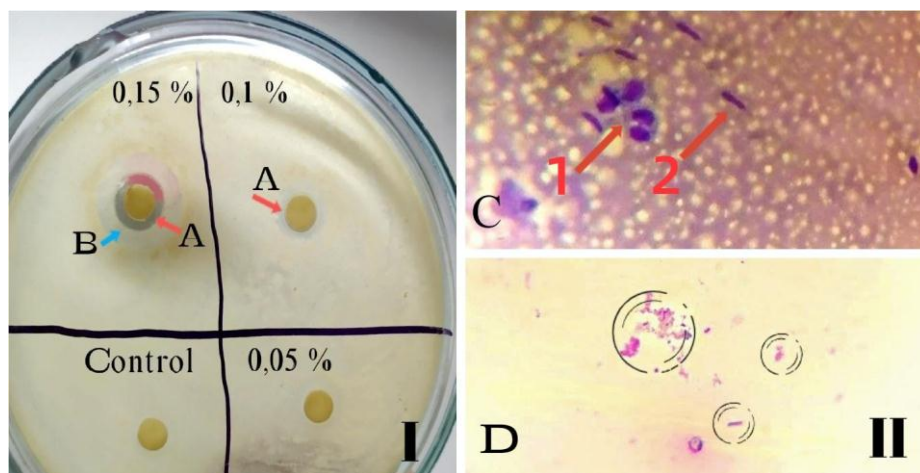


Figure 1 I - Disinfectant activity "Biocontact Plus" of different concentrations against test culture of *Enterobacter Aerogenes* (*Klebsiella Aerogenes*) on meat-peptone agar medium (A - bactericidal effect; B - bacteriostatic effect); II - Light microscopy of haemocyte morphology of test groups of bees - C (0.15 % "Biocontact Plus"): 1 - neutrophil cells, 2 - eosinophil cells; D (0.2 % "Biocontact Plus") - prohemocytes (x400)

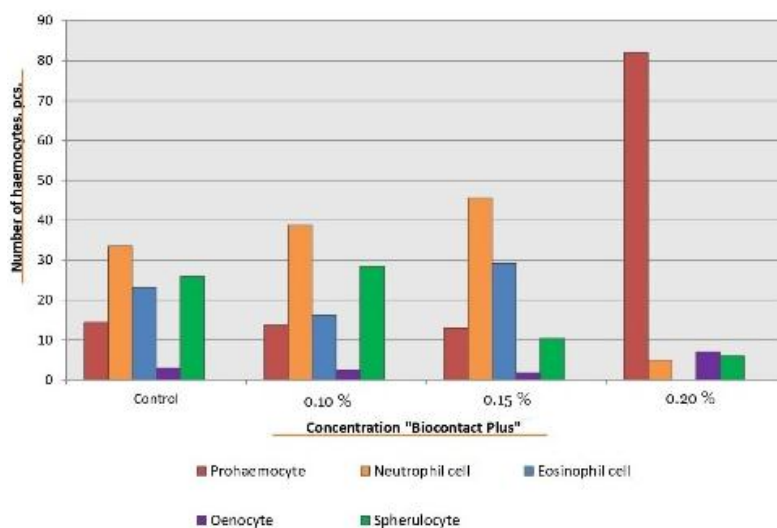


Figure 2 Parameters of haemolymph of bees when exposed to different concentrations of "Biocontact Plus"

In conclusion, the obtained data indicates the possibility of introducing into the development of prevention of bacterial diseases of bees low-dose disinfectants with pronounced bactericidal and bacteriostatic action against *Klebsiella Aerogenes* (*Enterobacter Aerogenes*). The disinfectant "Biocontact Plus" 0.15 % concentration diluted in 50 % sugar syrup is a potential alternative to aggressive immunostimulants to increase resistance in intestinal bacterial diseases of bee colonies.

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