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Review

The Effect of Pesticides on the Microbiome of Animals

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Abstract: In recent decades an increase in the use of pesticides to protect plants from pests, diseases and weeds has been observed. There are many studies on the effects of various pesticides on non-target organisms. This review aims to analyze and summarize published scientific data on the effects of pesticides on the animal microbiome. Pesticides can affect various parameters of the animal microbiome, such as the taxonomic composition of bacteria, bacterial biodiversity, and bacterial ratios and modify the microbiome of various organisms from insects to mammals. Pesticide induced changes in the microbiome reducing the animal's immunity. The negative effects of pesticides could pose a global problem for pollinators. Another possible negative effect of pesticides is the impact of pesticides on the intestinal microbiota of bumblebees and bees that increase the body's sensitivity to pathogenic microflora, which leads to the death of insects. In addition, pesticides can affect vitality, mating success and characteristics of offspring. The review considers methods for correcting of bee microbiome.

Keywords: pesticide; microbiome; animals; taxonomic composition; bacterial biodiversity; bacterial ratios

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1. Introduction

In recent decades, an increase in the use of pesticides to protect plants from pests, diseases, weeds, etc. has been observed [1,2]. Numerous authors have studied various aspects of pesticide toxicity for non-target organisms [3–6]. In recent years, interest in the study of animal microbiome has sharply increased, as well as of its change under the influence of various physicochemical factors [7–14]. The toxicological significance of the interaction of intestinal microbiota with pollutants is a serious concern, since chemicals, interrupting the functions of the intestinal microflora, lead to changes in homeostasis of animals [15]. The number of publications on the effect of various pesticides on the microbiological composition of the intestines of animals, both model and agricultural, has increased. The aim of this work was to systematize data concerning the effect of pesticides on the microbiome of animals, primarily pollinators. Considering that the problem of a sharp decrease in the abundance of pollinators is already a matter of food security. The review considers various aspects of the effect of pesticides on the taxonomic composition and ratios of microorganisms in the intestines of animals, and methods for correcting the microbiome of beneficial insects.

2. The Impact of Pesticides on the Microbiome of Animals from Different Taxonomic Groups

2.1. The Effect of Pesticides on the Microbiome of Model Organisms

There are many studies concerning the effects of various pesticides on certain living organisms. All of them show a negative xenobiotics effect. Majority of studies was conducted on mice and rats, which are the main model objects for toxicological studies on pesticide effects.

Chlorpyrifos is a broad-spectrum insecticide that interfere with signaling from the neurotransmitter acetylcholine [16,17]. It causes microflora dysbiosis and, consequently, leads to a change in the level of microorganisms metabolites [18–20]. Also, it is often found in food, thereby affecting the normal functioning of the endocrine and gastrointestinal systems [21]. It was shown on rats orally fed with special diet with different fat contents [22]. In parallel with the altered nutrition, rats were injected with chlorpyrifos. Chronic introduction of the pesticide caused reduction of luteinizing, follicle-stimulating hormones and testosterone concentrations on a diet with a normal fat content. A high-fat diet and the effect of the pesticide have increased the number of anti-inflammatory cytokines. Pesticide-induced abnormalities in the intestinal microbiome were more apparent in rats fed a high-fat diet. The affected bacteria included short-chain fatty acid-producing bacteria, testosterone-related genus, pathogenic bacteria, and inflammation-related bacteria. The study helped clarify the relationship between disrupted endocrine function and gut microbiota dysbiosis induced by pesticides.

Liang et al. also studied the effect of a similar diet containing chlorpyrifos on mice. Subsequently, antibiotic treatment and microflora transplantation were performed [23]. The results of the experiment showed a violation of the intestinal barrier by chlorpyrifos, which led to increased penetration of lipopolysaccharides into the body and, consequently, the appearance of inflammation in the intestine. In addition, the mice that received the microbiota modified with chlorpyrifos acquired a fat mass and low insulin sensitivity.

Another study revealed the effect of propamocarb fungicide on the microflora and intestinal metabolism of mice [24]. Analysis of the operational taxonomic unit (OTU) showed that 32.2% of the cecum OTUs was changed after exposure to propamocarb. In a similar experiment, the fungicide imazalil was used [25]. The microbiota of the contents of the cecum and feces changed at phylum and genus levels after application of the fungicide, this showed the sequencing of 16S rRNA. Operational taxonomic unit (OTU) analysis showed that 14.0% of fecal OTUs and 31.1% of cecal OTUs changed after imazalil exposure. The results showed that high doses of fungicide disrupt the metabolism of mice by altering the intestinal microbiota. In addition, imazalil significantly increased the number of bacterial infections in the mucous membrane of the colon of mice, affecting the intestinal barrier function.

A decrease in abundance and bacterial diversity was also shown after consumption of systemic triazole fungicide penconazole [26]. In mice treated with penconazole and its enantiomers, the relative content of the microbiota in the intestine, in particular, the cecum, changed. The relative abundances of seven gut microflora (at the genus level) were altered following exposure to penconazole. Penconazole caused significant changes in the relative abundances of five gut microflora. Metabolism analysis showed metabolic profile disturbance after exposure to this substance. This indicates the harmful effects of this pesticide on the animals.

In addition to studying the effect of pesticides on the intestinal microbiota, studies have been conducted on the combined use of antibiotics and pesticides. The use of antibiotics directly affects the intestinal microbiota, reducing the number and diversity of bacteria [27,28]. In turn, the intestinal microbiota changed by antibiotics can affect the chemical transformation of xenobiotics in the body [29,30]. Finding the relationship between the effects of antibiotics and pesticides have been conducted where rats with an antibiotic-modified microbiota were exposed to triazine herbicides [31]. The results showed that antibiotic administration reduces the number of bacteria in rats: The relative abundance of *Ruminococcaceae* species decreased, and the *Bacteroides* species increased. It was also found that antibiotics suppress the gene expression of hepatic metabolic enzymes and increased the expressionof proteins associated with intestinal adsorption. All this together increases the risk of exposure to triazine herbicides (atrazine, simazine, ametrine, terbuthylazine, and metribuzin) on the body, leading to an increase in their bioavailability.

2.2. The Influence of Pesticides on Microbiomes of Soil Animals

In modern studies [32–34], the bacterial composition of soils is used as one of the important indicators of the negative consequences of pesticides usage. Pesticides have been shown to act on bacteria that fix nitrogen [35], cause changes in plant probiotic soil microflora [36] and total soil microbial diversity [37]. It is little-known about the effect of fungicides on the bacterial community of the soil. The effect of different concentrations of azoxystrobin fungicide on the soil and intestinal microbiota of soil animals was studied using the example of *Enchytraeus crypticus* annelid worm [38], as well as the effect of the insecticide monocrotophos on earthworms, which are actively used in studies as a model object for assessing environmental risks from the usage of chemicals [39]. In general, the results are similar: A decrease in the number of beneficial bacteria in the intestine and a decrease in microbiota were shown. *Enchytraeus crypticus* showed an increase in the spread of proteobacteria in response to an increase in the concentration of azoxystrobin. A significant change in the structure of the soil microbial community was observed under the effect of penconazole, carbendazim, pencicuron, and fludioxonil [40,41]. On adding 10 mg/kg carbendazim, the change in soil microbial composition was greater than 40%. The relative abundance of bacteria also significantly changed under the influence of penconazole, pencicuron and fludioxonil.

2.3. The Effect of Pesticides on the Microbiome of Aquatic Organisms

Trichlorfon insecticide is often used in agriculture and horticulture [42]. This compound is actively used to combat various parasitic infections in aquaculture. It is well soluble in water, and, accordingly, the excessive use of such a phosphorus-containing pesticide leads to environmental pollution [43]. The effect of various concentrations of trichlorfon was identified on the intestinal microbiome of common carp *Cyprinus carpio* [44]. Exposure to the pesticide significantly reduced the height of intestinal villi, and also reduced the level of gene expression for claudin-2, occludin, ZO-1. Exposure to trichlorfon influenced the composition of the microbiota community and reduced the diversity of bacteria in the intestines of carp. The proportions of probiotic bacteria, namely, *Bifidobacterium*, *Akkermansia*, and *Lactobacillus*, were observed to be reduced after trichlorfon exposure. Together, this proves the negative effect of trichlorfon: It can damage the intestinal barrier, cause oxidative damage to the intestine, cause an inflammatory reaction and change the structure of the intestinal microbiota in carp.

The massive usage of pesticides leads to a change in fresh algal biocenoses, causing water blooming. Azoxystrobin is a widely used broad-spectrum strobilurin fungicide. It has been shown that azoxystrobin stimulates the growth of cyanobacteria by inhibiting the growth of competitive organisms, for example, Chlorophyta, and also inhibits the growth of parasitic cyanobacteria, fungi, pathogenic bacteria, and viruses [45].

2.4. The Effect of Pesticides on Insect Microbiome

Insects live in a world full of toxic compounds (plant toxins, artificial pesticides). Insects evolutionarily adapted to the effects of these factors (neutralization of toxins, changes in the target site, the mechanism of detoxification). It is believed that all these mechanisms of resistance are encoded by their own insect genomes, but analyzes with omic technologies have shown that a number of organisms possess specific intestinal microorganisms, some of which contribute to the insect's resistance to phytotoxins and pesticides [46]. These are evolutionary symbiotic associations of microbes that help the body survive and adapt to its environment.

There is evidence of the resistance of some mosquitoes *Culex nigripalpus* to pesticides [47], but the sources of this resistance are still unclear. In addition, cockroach species *Blattella germanica* is considered as one of the most resistant organisms to insecticides. Therefore, these organisms are the most interesting for the analysis of various pesticides. In the studies on mosquitoes [48], the results showed that microbiota may be responsible for pesticide resistance. A gammaproteobacterial genus *Salinisphaera* that produce detoxification enzymes involved in the degradation of pesticides was detected at >10% relative abundance in pesticide resistant mosquitoes. In studies on *Blattella germanica*, its bacteria were involved in the detoxification of xenobiotics, including synthetic pesticides [49,50]. Increase in insect sensitivity to insecticides under the effect of antibiotics has occurred. Also, transplantation of antibiotic-affected microflora of cockroaches to resistant lines was carried out, which led to a significant loss of resistance and death of cockroaches.

An organophosphate insecticide, phoxim, was studied on the body of *Bombyx mori*, a silkworm. This species has been found to be much more susceptible to pesticide [51]. An analysis of the intestinal microbiota showed that the quantity of bacteria *Methylobacterium* and *Aurantimonadaceae* in the intestine was reduced, while the number of non-dominant bacteria, such as *Staphylococcus*, increased significantly. In addition, phoxim inhibited the expression of antimicrobial peptides and enhanced the pathogenesis of *Enterobacter cloacae*.

2.5. The Effect of Glyphosate on Animal Microbiome

One of the most popular herbicides used worldwide for weed control is glyphosate [52–54]. It is actively used on islands near the coastline, that effects on surrounding marine and coastal species [55,56]. Various studies have been conducted to study the effect of this pesticide on the intestinal microflora of animals. For example, in Hawaiian green turtles, under different concentrations of the pesticide, a decrease in density and inhibition of bacterial growth were observed [57]. This fact indicates the adverse effect of glyphosate on the general condition of the animal. In other species (Chinese mitten crab), the antioxidant ability of the intestine decreased with the effect of the pesticide and the content of malondialdehyde increased. As a result of sequencing, an analysis was performed that showed that glyphosate reduced the diversity of the intestinal microbiota of the Chinese mitten crab, and the taxonomic richness of bacteroids and proteobacteria increased significantly [58]. Glyphosate, absorbed with food, directly contacted with the intestinal microbiota of pollinating insects. Quantitative PCR revealed a significant change in the composition of the intestinal microflora [59], the appearance of an unbalanced microbiota, which reduces the bee's resistance to pathogens. Glyphosate caused a strong decrease in the bacteria *Snodgrassella alvi*, a partial decrease in *Gilliamella apicola* and an increase in *Lactobacillus* spp.

In addition to the glyphosate effect on the growth of intestinal bacteria, this pesticide has an effect on the synthesis of amino acids (shikimate pathway), in particular, it inhibits the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS). The gene encoding the shikimat pathway enzyme is present in all sequenced genomes of intestinal bacteria of the honeybee [60]. This fact indicates a high potential sensitivity of bacteria (and, consequently, bees) to glyphosate. A decrease in the dominant intestinal microflora of insects effected by glyphosate has been demonstrated. Moreover, the effect of this herbicide increased the mortality rate of bees due to increased susceptibility to the influence of the pathogen *Serratia marcescens*. Thus, glyphosate perturbes the beneficial intestinal microflora of honey bees, potentially affecting their health and pollination efficiency.

Studies have shown that glyphosate affects both adult insects and larvae. The experimental data showed a decrease in survival rate, development rate, mass of larvae and bacterial diversity of the middle intestine *Apis mellifera* [61]. Xenobiotics in high concentrations have the most negative effect on colonies of immature bees, while adults do not experience stress due to its effect.

Interrelationship between pesticide and intestinal bacteria that synthesize vitamins was shown. Human MAIT cells (invariant T-cells associated with the mucosa) can respond to vitamin metabolites: Riboflavin and folate. *Escherichia coli* cells were shown to activate MAIT cells, while *Bifidobacterium adolescentis* and *Lactobacillus reuteri* inhibited MAIT cell activation [62]. Exposure to chlorpyrifos significantly increased *E. coli* colonies mediated by the activation of MAIT cells, and the effect of chlorpyrifos together with glyphosate inhibited colonies growth. In this case, proteomic analysis showed that glyphosate had an effect on the biosynthesis of riboflavin and folate. Thus, chlorpyrifos and glyphosate increase the anti-inflammatory immune response.

A long-term effect of the *Roundup* herbicide (made from glyphosate) on the microbiota of rat intestines demonstrated the growth of *Bacteroidetes* bacteria and a decrease in the number of *Lactobacillaceae* [63]. The culture method showed that *Roundup* has a direct effect on the intestinal microbiota: Bacteria showed different sensitivity to the herbicide, including the identification of a resistant strain of *Escherichia coli*, which is associated with the absence of the EPSPS gene. Thus, *Roundup* accumulations in the environment have a significant negative effect on rat health.

3. The Effect of Pesticides on the Microbiome of Bees and Bumblebees

3.1. Problem for Pollinators

At this time there is a global trend of pollinators decreasing [64–68]. Possible reason for this is the toxic pesticides effects [69,70]. The active usage of pesticides in agriculture and the decline in the growth of colonies of pollinating insects can be interrelated processes. Recent studies have shown that pesticides can affect on bumblebee colonies—An important and annually decreasing group of pollinators [71]. It has been shown that the effect of thiamethoxam, a neonicotinoid insecticide, on bumblebees reduces the proportion of incubating queens in the hive that lay eggs, and also increases the vulnerability of individuals to pathogenic microorganisms [72]. Thus, neonicotinoids have a significant effect on the dynamics of the pollinators population. Current studies examine neonicotinoid target sites, as well as the metabolism of neonicotinoids, not only in pests, but also in beneficial insects such as bees [73]. The obtained data contribute to the development of a new generation of pesticides targeted at nicotinic acetylcholine receptors of insects.

It is known that a honeybee has a special reproductive behavior that can change under the influence of stress factors. Widely used insecticide fipronil, as shown by studies, leads to a decrease in sperm concentration and their viability in combination with an increased rate of spermatogenesis, which has led to a deterioration in the fertility of male bees [74]. The results of this study indicate the detrimental consequences of pesticides usage on the reproductive potential of bees. Pesticides can contribute to honeybee population reduce as a result of distorted fertility, an example of which is fipronil. Similarly, an experiment was conducted with another high-potent insecticide. Chronic consumption of neonicotinoid thiacloprid by bees influenced the behavior of insects in the field. The behavior, navigation, and social communication of bees were disrupted. It has been shown that thiacloprid applicable at low-dose at the feeding sites accumulates over time in the food [75]. Thus, pesticides pose a significant risk to honey bees, disrupting learning and memory functions, and also lead to the losses of insects.

Currently, in addition to stress, bees are constantly exposed to a mixture of agrochemicals and at the same time suffer by parasites. Stressors do not act separately, the effect of pesticides can disrupt both detoxification mechanisms and immune responses, making bees more susceptible to parasites [76,77]. Chronic exposure to several interacting stressors leads to the loss of honey bees colonies and a decrease in the abundance of wild pollinators. Analyzing the history of pollinators extinction, an assessment was made for extinction rate of bees in Britain from the middle of the 19th century to the present [78]. The fastest extinction phase is associated with changes in agricultural policy that began in the 1920s and ends with the ill-considered application of farming methods.

One of the mechanisms of the toxic effect of small doses of pesticides may be their effect on the microbiological composition of insects. Next, data on the effect of pesticides on the microbiome of animals of various taxonomic groups will be considered.

3.2. Microbiome of Bumblebees and Honey Bees and the Impact of Pesticides

The study of intestinal microbiota is an important area of the research of the ecological and functional dynamics of the intestinal environment. Especially the study of the intestinal microbiota of honey bees and bumblebees, which are important pollinators. Due to the specific intestinal habitat, *Apis mellifera* is a valuable model system. All these data together helps to research the microbiota and develop experiments in this field [79].

Intestinal microbial communities play a crucial role in maintaining the health of many insect species. However, these bacteria are very sensitive and it is still unknown how the host and pathogen interact at anthropogenic changes in the environment. The bacterial composition of the microbiota of bumblebees *Bombus terrestris* taken from forest and urban habitats was analyzed [80]. The results showed a significant predominance of bee-specific main bacteria, such as *Snodgrassella* and *Gilliamella* in urban areas, and their small abundance in the forest zone. Conversely, the abundance of pathogens *Crithidia bombi* and *Nosema bombi* was greater in the forest zone compared to the urban one. This confirms the role of beneficial bacteria in protecting against pathogens in the body.

In addition, similar studies support this hypothesis. When analyzing the effect of the parasite *Crithidia bombi* on the intestinal microbiota of *Bombus terrestris*, differences were found: After microbiota transplantation, bumblebees with different resistance caused a different immune response [81]. In studies related to the infection of bumblebees with *Apicystis bombi*, a eukaryotic parasite, insects had a higher abundance of *Arsenophonus* sp. and *Phyllobacterium* sp., which are symbionts. These organisms are mainly present in the fat body, also a correlation has been found between bacteria in the intestine and the fat body [82]. Studies conducted by Näpflin and Schmid-Hempel in Switzerland were aimed at researching the body's properties that provide protection and resistance against pathogens, in particular *Crithidia bombi*, since these mechanisms are still unknown [83,84]. It turned out that the structure of the microbial community does not differ between infected and uninfected individuals. Although the microbial communities of the intestines and their functions in adult bumblebees are widely studied, data on the microbiota of the intestines of the larvae are still limited. It has been shown that the microbiota of adults and larvae differs significantly, in particular, typical main intestinal bacteria in adult bumblebees are absent in larval bumblebees, where Enterobacteriaceae and Lactobacillaceae predominate [85]. The functions of this microbiota have yet to be determined.

As known, intestinal symbionts of insects inhibit the action of pathogens. The main symbiont bacteria, *Snodgrassella alvi* [86] and *Gilliamella* [87], were studied in the body of *Apis mellifera* and *Bombus* sp. It turned out that bees contain a greater number of bacterial strains than bumblebees, which confirms the hypothesis that the foundation of colonies by swarms of workers allows to keep a greater diversity than the foundation of colonies by one queen. Analysis of the intestinal isolates of the bumblebees *Bombus pascuorum*, *Bombus terrestris*, *Bombus lucorum,* and *Bombus lapidarius* showed the presence of several new bacterial taxa and species [88], in particular, 4 new *Gilliamella* bacteria species [89]. The presence of accurately identified microbial isolates will contribute to a future assessment of the functional potential of the bumblebee intestinal microbiota.

As described above, it has been suggested that the use of pesticides has a negative effect on the microbiome of the honey bee. However, the general mechanisms of the toxic effect of insecticides on microbiomes are poorly understood and require detailed study to minimize this problem. *Apis mellifera* honey bees were exposed to nitenpyram [90] and imidacloprid [91]. Decrease in the general condition, behavioral characteristics (food intake), and overall insect survival were observed. The result of sequencing of the 16S rRNA gene showed a change in the intestinal microbiota of bees, i.e., the reduction of microbial density that contribute to metabolic homeostasis and insect immunity in general.

Another study related to the effects of neonicotinoids on the body in general and intestinal microbiomes in particular, was an experiment with *Bombus terrestris* [92]. A relationship was found between the effects of clothianidin on the microbial composition of the intestine, both at the individual level and at the colony level. A xenobiotic has been shown to reduce the size and weight of bumblebees, and reduce reproductive ability, but clotianidin does not cause a decrease in resistance to pathogenic microorganisms.

Intestinal microorganisms play a dominant role in the nutrition and other vital processes of the bee. There is a dependence of the intestinal microflora of the honeybee on the general health of the insect [93]. This composition of the microbial community can be used as an indicator of the biological health of the insect. Diaz et al. found a negative effect of imidacloprid insecticide on the composition of the microbial community of the intestines of bees [94]. Gram-positive and Gram-negative biomarkers were affected, whereas fungal biomarkers were unaffected. Both insecticides and generally accepted safe herbicides aimed at controlling weeds cause changes in the intestinal microbiome and increase the body's sensitivity to pathogenic microflora, which leads to the death of insects [95].

In this regard, research directions are currently being actively developed related to the study of microflora changes under the influence of various pollutants, including pesticides. Majority of chemical compounds, used as pesticides, has serious threat to the intestinal microflora [96], and they also negatively affect to the endocrine and digestive systems. These substances play a significant role in the development of metabolic disorders, which leads to intestinal dysbiosis.

The most widely-used pesticides, such as amitraz, chlorpyrifos, dimethoate, were studied for acute or chronic toxicity in *Apis mellifera* and *Apis cerana* [97]. It was found that dimethoate most strongly affects the survival rate and diet consumptionof bees. Data on the highest susceptibility of *A. mellifera* to chlorpyrophos and dimethoate were obtained, while *A. cerana* was more sensitive to amitraz. All major intestinal bacterial types of honey bees were found, including Proteobacteria, Firmicutes, and Bacteroidetes. However, during the experiment, a significant difference in the species diversity of the intestinal microflora of insects appeared.

3.3. Pollinators Microbiome Healing

Today, the honey bee is under stress from a number of biotic and abiotic factors such as pesticides that reduce pollination processes and the overall productivity of the hive. Researches of intestinal microbiota are very important for understanding the general ecological mechanisms [79,98]. The knowledge concerning the effect of the composition of the intestinal microbiota on the health of bees allows us to develop optimal concepts for increasing the productivity of apiaries and general natural populations of honey bees. Thus, the "probiotic concept" is actively transferred to beekeeping. A significant correlation was found between the increase in honey yield and the use of probiotics (*Lactobacillus salivarius*) comparing with the control group which did not receive probiotic [99]. Adding a probiotic to honeybee feed reduces yeast colonies [100], which subsequently affects the overall immunity of insects [101].

The main probiotic strains of the intestines of bees are *Lactobacillus*, *Bifidobacterium*, *Bacillus*. The beneficial effects of these microorganisms on the health and productivity of bee hives were indicate, especially if strains of bee origin are used [102]. Lactic acid bacteria are widespread in nature due to their beneficial effects on the body and are actively used as probiotics. They are used in animal breeding, beekeeping, poultry farming to improve health and increase reproductive functions. Lactic acid bacteria isolated from the hive showed inhibition of twenty human and animal pathogens, some were resistant to antibiotics [103,104]. The intestinal microflora of the honeybee has been classified as heteroenzymatic lactic acid bacteria [105]. They also inhibited the growth of the main pathogen of the honey bee—*Paenibacillus larvae*, which means that the studied lactobacilli have healthy probiotic properties. Lactobacilli are able to survive at acidic pH values, bile salts, and show vitality in the gastric juice [106], which makes them a potential candidate for use in the nutraceutical and pharmaceutical industries. Tests with *Lactobacillus* bacteria also revealed their probiotic effect [107]. One of the results of their application was an increase in egg laying, an increase in the number of bees, and therefore a high yield of honey. Probiotics also reduced the incidence of honey bees by nosematosis and warrosis. The addition of a prebiotic preparation containing *Lactobacillus johnsonii* CRL1647 to the hives, in general, favorably affected the activity of honey bees [108,109]. The commercial probiotic *Lactobacillus rhamnosus* and inulin as a prebiotic caused the survival of honey bees that were infected with the pathogen *Nosema ceranae* [110]. However, the study pays great attention to the correct choice of doses of pro- and prebiotics used, since excessive saturation of the diet of honey bees with these food additives could not prevent the development of the disease from the pathogen, but rather disrupt the regulation of the immune system of bees and increase insect mortality.

In addition to lactobacilli, *Brevibacillus laterosporus* was isolated from the digestive tract of the honey bee, which can stimulate the growth of bee colonies [111]. This bacterium has a unique shape and morphological structure. The cyclic peptide (Leu-Pro) of the bacterium has biological activity against several pathogenic microorganisms, in addition, the total number of broods, production of bee pollen, honey is better with bees treated with a suspension of *B. laterosporus*. Similar results were obtained in the analysis of *Bacillus subtilis* subsp. subtilis Mori2, which increases the productivity of bees, stimulates egg laying, the growth of bee generations, therefore, causes an increase in honey, and also reduces the spread of bee diseases [112].

Modulation of the intestinal microbiota of the honeybee is recognized as a successful practical approach. In European studies, the preparation of sugar syrup containing bifidobacteria and lactobacilli isolated from the intestines of the bees was sprayed onto a part of an apiary located in an open field [113]. Analysis of the hives showed a significant increase in the brood population, as well as of pollen and collected honey. Analysis of intestinal microbiota showed an increase in the number of species of *Acetobacteraceae*, *Bifidobacterium*, which contribute to the normal metabolism of the insect.

The probiotic effects of seven isolated intestinal bacteria species from *Apis mellifera jemenitica* to larvae of a bee infected with *Paenibacillus* were studied. The following intestinal bacteria were used in the experiment: *Fructobacillus fructosus*, *Proteus mirabilis*, *Bacillus licheniformis*, *Lactobacillus kunkeei*, *Bacillus subtilis*, and *Enterobacter kobei*, *Morganella morganii* [114]. It turned out that adding these probiotic microorganisms to the diet significantly reduces the mortality rate of honey bee larvae. The use of *Bacillus licheniformis* and *Lactobacillus kunkeei* showed the largest positive effect.

American foulbrood is a disease of honey bees associated with the bacterial pathogen *Paenibacillus larvae*. This disease can be treated with antibiotics (e.g., tylosin), which is not desirable. There is interest to the search for an alternative medicine in the form of a probiotic [115]. The antagonistic effect of lactic acid bacteria against the pathogen was most effective. During the experiment, it was shown that this probiotic under laboratory conditions affects the decrease in the number of pathogen, but in practice this experiment did not show a positive effect [115,116]. The mechanisms governing the dynamics of bee microbiota are complex, and potential drugs identified in laboratory studies must be thoroughly tested in situ.

European foulbrood is a bacterial disease of honey bee larvae. Studies have shown that the intestinal bacteria of bees act as probiotic microorganisms and reduce the risk of developing this disease [117]. The analysis showed that most isolates of intestinal bacteria belong to *Bacillus*, *Staphylococcus*, *Pantoea*. Under the influence of pathogenic bacteria, the inhibitory effect of *Bacillus* on these pathogens is shown.

4. Conclusions

Intestinal microbial communities play a crucial role in maintaining the health of animals. We have shown that pesticides can affect the microbiome of animals of various taxonomic groups. It is noteworthy that pesticides of various classes (insecticides, fungicides and herbicides) can affect the intestinal microbiota of animals. Glyphosate has a significant negative effect on the intestinal microbiota, both mammals and pollinators. Changes in the microbiome induced by pesticides ultimately affect the immunity of animals, reproductive ability and even their behavioral characteristics. Pesticides have a significant effect on the taxonomic composition and ratio of bacteria in the gut of bumblebees and bees. Solutions for the correction of pollinator microbiome are needed.

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