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Ovlivňují symbiotické bakterie odolnost skladištního roztoče *Acarus siro* vůči biocidním látkám?

Can symbiotic bacteria of storage mite *Acarus siro* alter its response to biocides?

Master's thesis

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Prague, 2022

Declaration:

I hereby declare that I prepared this thesis independently and that I cited all the information and publications used. This work, or substantial part of it, was not previously used to obtain the same or another academic title.

Prague, 4. 1. 2022

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Signature

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Abstrakt

Skladištní roztoč *Acarus siro* (sladokaz moučný) je jeden z nejrozšířenějších skladištních roztočů na světě. Napadá širokou škálu produktů (obiloviny, sušené ovoce, masové výrobky, zvířecí krmiva atd.) a způsobuje alergické reakce u lidí. Z těchto důvodů je nezbytné najít efektivní strategii, která ho dokáže potlačit a ideálně roztoče zcela z nežádoucího prostředí odstranit. V historii u něj byla pozorována rezistence vůči několika pesticidním látkám. V této práci byly čtyři populace *Acarus siro* vystaveny pesticidům v různých koncentracích – nejprve ve formě roztoků a následně jako aditivum v dietě.

Populace vykazovaly odlišnou citlivost na čtyři zvolené pesticidy (pirimifos-metyl, chlorpyrifos-metyl, deltamethrin a deltamethrin v kombinaci s piperonylbutoxidem). Největší rozdíly byly zaznamenány v reakci na roztoky pirimifos-methylu. Ten byl následně přidán v pěti koncentracích do standardní diety, na které roztoči rostli po dobu 3 týdnů. Analýza mikrobiomů z kontrolní diety a z diety ovlivněné pesticidem ukázala, že koncentrace $0,0125 \mu\text{g} \times \text{g}^{-1}$ způsobuje u laboratorního kmene 6L a kmene 6Tu hormoligózu. Stejná koncentrace pak způsobila změnu ve složení mikrobiomu kmene 6Z. Vystavení koncentraci $1,25 \mu\text{g} \times \text{g}^{-1}$ pak způsobilo změnu složení u kmene 6Z a 6Tk. U kmene 6Tk došlo ke snížení zastoupení *Solitalea*-like bakterií na úkor *Sodalis*-like bakterií, které se v kontrolním vzorku prakticky nevyskytovaly. U kmene 6Z došlo ke snížení diverzity mikrobiomu, přičemž při nižší koncentraci měl nejvyšší zastoupení rod *Bacillus*, s vyšší koncentrací pak tvořil téměř polovinu celkového profilu spolu se *Sodalis*-like bakteriemi.

Tato práce ukazuje, že přidáním pesticidů do diety dochází k ovlivnění populačního růstu a ke změně mikrobiomu u vybraných populací *Acarus siro*.

Klíčová slova: roztoči, *Acarus siro*, pesticidy, bakterie, degradace, hormoligóza, mikrobiom, *Solitalea*-like bakterie, *Sodalis*-like bakterie

Abstract

Storage mite *Acarus siro* is one of the most distributed stored product mites in the world. It infests various products (grains, dried fruits, meat products, animal feed etc.) and causes allergic reactions in humans. For these reasons, it is important to find an effective strategy to suppress or even better to eliminate the mite from the storing facilities. Historically, there have been reported cases of the mite being resistant to several pesticides. In this thesis, four populations of *Acarus siro* were exposed to pesticides in different concentrations – first in the form of solutions and next as a diet additive.

The populations showed divergent sensitivity to four selected pesticides (pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin and deltamethrin in combination with piperonyl butoxide). The biggest differences were recorded in response to solutions of pirimiphos-methyl. This pesticide was then added to standard rearing diet in five concentrations. The mite populations were exposed to this diet for 3 weeks. Control and pesticide-treated diet microbiome analyses revealed that $0.0125 \mu\text{g}\times\text{g}^{-1}$ concentration causes hormoligosis in 6L and 6Tu strains. The same concentration was responsible for microbiome change in 6Z strain. Exposure to $1,25 \mu\text{g}\times\text{g}^{-1}$ concentration caused microbial shifts in 6Z and 6Tk strains. In 6Tk strain the amount of *Solitalea*-like bacteria was decreased at the expense of *Sodalis*-like bacteria, that were not present in the control group. In 6Z strain, microbial diversity reduced while exposure to lower concentration of pesticide showed increase of *Bacillus spp.*, when exposed to higher concentration, *Bacillus* and *Sodalis*-like bacteria formed almost half of the profile.

This thesis demonstrates that pesticide in diet affects population growth and causes microbial changes in populations of *Acarus siro*.

Key words: mite, *Acarus siro*, pesticides, bacteria, hormoligosis, microbiome, *Solitalea*-like bacteria, *Sodalis*-like bacteria

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1. Introduction

Arthropods represent extremely diverse and rich group of organisms. For many years, the mites have been paid attention to because of their ability to inhabit various niches and ubiquity. It has been recognized that there are mite species colonizing stored products ranging from grains to vegetables, cheese and dried fruits to animal feed and animal products (Campbell et al., 2004, Russell et al., 2013) since these materials provide sufficient nutritional source on which the mites can flourish (Russell et al., 2013). Storage mites represent threat to the stored commodities and thus their presence (or absence) in storages is of great economic importance, because pests can be responsible for losing up to fifth of the entire production of grains (Phillips and Thorne, 2010). The focus of this thesis is on flour mite *Acarus siro* L. (1758) which is a dominant species among stored product mites. It is present all over the world (Griffiths, 1964) with higher densities in temperate regions (Solomon, 1962) which is why its presence is monitored in the Czech Republic and so are the methods of its elimination. Apart from causing economical losses, mites are also responsible for various illnesses, especially allergies. The most common pathway of triggering allergic reactions is contact of the skin with the mite (dead or alive) or its products (Russell et al., 2013). Since this mite is concentrated in storage facilities, illnesses caused by *A. siro* are occupational in most cases (Mullen and Connor, 2019). It was revealed that not only mite bodies but toxins coming from their symbiotic microbiota are responsible for allergic reactions, asthma etc. (Gregory and Lloyd, 2011). Effective control of mites along with recognition of their associated microbiota is hence important to prevent population outbreaks. The mites have, however, developed symbiotic relationships with bacterial community that protect them from the influence of dietary and environmental toxic substances (Van Leeuwen and Dermauw, 2016). This represents a big issue in pests' control as the most common way of controlling mites is the use of pesticides. It has been found that residual or insufficient doses of pesticides may cause hormoligosis in mites, which is a stress response manifesting in greater population growth (Luckey, 1968). Also, after prolonged time of the same pesticide application, the mites were found to develop resistance. There has been several reports of *A. siro* being resistant to various substances (Wilkin, 2003; Thind and Muggleton, 1998; Szlendak et al., 2000). The laboratory culture of *Acarus siro*, also examined in this thesis, was tolerant to chlorpyrifos application (Hubert et al., 2007). The population growth of *A. siro* was greater in the treatments (10, 100, 250 $\mu\text{g}\cdot\text{g}^{-1}$ diet) than in the untreated control (Hubert et al., 2007). Thus, it is likely that application of chlorpyrifos to the diet of *A. siro* caused hormoligosis. In this study, it is tested whether hormoligosis in four cultures of *A. siro* by pirimiphos-methyl addition into diet could be induced. Then the effect of pesticide on mite microbiome composition is observed.

The microbiome was characterized by qPCR with universal primers and barcode sequencing of V4 part of 16S RNA gene. Hence, the aims of this thesis are:

- to compare the response of four populations of *A. siro* to pesticide
- to measure population growth in populations exposed to pesticide-treated and untreated diets
- to analyze populations' microbiome before and after pesticide application and correlate the results with the response to pesticide.

2. Literature overview

2.1 Stored-product mites

Subclass Acari is represented by more than 30,000 species mostly small arthropods (mites and ticks), varying in their body shape. They are distributed worldwide and have been successful at colonizing many different habitats such as soil, deserts, ice fields and oceans. Despite their numbers, relatively few are considered parasites. The subclass is considered to be divided into two superorders – Parasitiformes (or Anactinotrichida) and Acariformes (also known as Actinotrichida). While the Parasitiformes includes bird mites and ticks, the Acariformes includes e.g. plant mites, scabies and free-living stored-product mites that belong to the family Acaridae. Mites have a short life cycle which allows a speedy build-up of the numbers of individuals within populations once they find ephemeral food resources, which they have been very successful in profiting from (Russell et al., 2013). By 2013, 280 mite species have been reported to be associated with 425 stored products, over forty mite species have been reported to be associated with more than ten commodities and more than twenty have been reported to be associated with more than 20 products (Hagstrum et al., 2013). Storage (or grain) mites infest stored food or other materials of plant or animal origin. They can be found on grains, flour, cereals, vegetables, dried fruits, animal feed (Campbell et al., 2004), nuts, cheeses and dried meats, hay and straws (Russell et al., 2013) and dry-cured hams (Zhao et al., 2016). *Acarus siro* and *Tyrophagus putrescentiae* are the most common representatives of the Acaridae family. In Czechia, stored product mites represent serious problem as they infest various food sources with great abundance (Kučerová and Horák, 2004).

2.2 Allergies and mites

Nowadays, more than 250 mite species are associated with health-related issues of both domestic animals and humans. These problems include skin irritation (temporary or persistent), allergies, transmission of pathogenic microbial agents and parasites, invasion of respiratory passages, ear canals or even occasionally internal organs, acarophobia and other psychological conditions (Mullen and OConnor, 2019).

Both “dust” and “storage” mites are responsible for causing allergies. House dust mites like *Dermatophagoides* spp. are sources of a variety of cross-reactive allergens; these are found in not only living mites but also in their body parts, dead skins, excreta and secretions and their presence is associated with atopic eczema and asthma (Russell et al., 2013). Storage mites can cause dermatitis and other allergic reactions through massive infestation of products – people handling heavily contaminated produce may experience dermatitis (Russell et al., 2013). The mites can also climb onto individuals and cause hypersensitivity dermatitis caused by their very presence and their products on the skin, whilst not feeding on skin, blood, or any tissues. Most cases of human dermatitis are the cause of occupational acarine dermatitis and involve workers handling both plant and animal materials, such as flour, hay, dried fruits and vegetables or cheeses and other dairy product in both households and commercial storage facilities (Mullen and OConnor, 2019). Allergens produced by storage mites such as *A. siro* can also be inhaled or ingested, which may result in anaphylactic reactions (Russell et al., 2013). Dermatitis caused by *A. siro* is commonly referred to as grocer’s itch or baker’s itch etc. (Mullen and OConnor, 2019), which clearly illustrates the most affected occupations suffering from said dermatitis. Along with other mite species, *A. siro* has also been observed in sputum of people suffering from pulmonary acariasis, in which mites invade the lungs, albeit it is impossible to say with certainty which of the species are responsible for the more serious problems that involve cough, difficulty breathing, chest pain, expectorated phlegm and blood or restlessness (Mullen and OConnor, 2019). Chemical contaminants associated with stored-product mites include mutagens, carcinogens, and undesirable odors (Hubert et al., 2018). Even though mite-related allergies used to be associated with house dust mites, Wraith et al. (1979) showed that allergy to storage mites might be more widespread and important than it was thought because of their capability to cause strong reactions among patients with house dust allergy. They also stated, that stored-food mites, or their allergens, respectively, might represent risks for those, who live in very damp houses where growth of mould may support the growth of the mites, especially if there are other conditions supporting the development of mite populations, like keeping of pets (Wraith et al., 1979). Dead mite bodies, cast skins, glandular secretion and reproductive products may also be source of allergens themselves or could become ones through the

process of decomposition (Wharton, 1976). Overall, mites are associated with at least 106 groups of allergens (Allergen nomenclature, 2021 [online]). Since storage mites are present in homes as well, they represent danger not only to those with occupational exposure but also to urban populations (Van Hage-Hamsten and Johansson, 1998).

2.3 *Acarus siro*

Acarus siro L., (1758), also referred to as “flour mite”, is a representative of superorder Acariformes, family Acaridae (Hagstrum et al., 2013). It is dominant species within the stored product habitat (Griffiths, 1964). It used to be confused with two other *Acarus* species, namely *A. farris* and *A. gracilis*, but these appear to be more of a field and outdoor species. *A. siro* can feed on whole grains and processed cereals (Griffiths, 1964), but it prefers the latter option, and is one of the most common mites to infest cheese (Mullen and OConnor, 2019). The mites are generally 0.5 mm long with almost colorless oval bodies and brown legs (Griffiths, 1964). Although this mite is distributed basically worldwide (Griffiths, 1964), it is predominantly distributed in temperate regions (Solomon, 1962). When reared in mixed cultures with common house dust mite *Dermatophagoides pteronyssinus*, *A. siro*'s population growth seems to be unaffected by the other mite's presence. The trend shows, however, that *A. siro* exhausts food sources very fast and within a few weeks becomes unable to keep its densities. Nonetheless, *A. siro* is capable of outcompeting dust mite species when in food-rich environment (Wharton, 1976) and is dominant especially in animal feed stores (Wilkin and Thind, 1984).

2.3.1 Development of *A. siro*

A. siro can develop at temperatures of 24–31 °C and a relative humidity greater than 60%. This mite tends to congregate where the relative humidity is 80%–85%, at which its reproductive rate is highest (Mullen and OConnor, 2019), although at 25 °C and 90% relative humidity they still can increase their numbers by seven folds pre week (Solomon, 1962). If the relative humidity drops to 60 % and lower, according to other conditions the population will die out either slowly or quite rapidly (Solomon, 1962). At temperatures from 10–20 °C and relative humidity 60 %, the eggs do not hatch anymore, and adults only survive for a short period of time (Cunnington, 1965). The highest temperature in which *A. siro* can survive and reproduce is slightly above 30 °C (Solomon, 1962). If the temperature reaches 32 °C, the mites fail to develop (Cunnington, 1965). Both temperature and relative humidity limit is noticeably low compared to other insect pests of stored food. The mite can, however, very slowly increase its

numbers at low temperatures (cca 5 °C) and endure at temperatures around 0 °C for a long time (Solomon, 1962). Since *A. siro* relies on moisture, it is no surprise that the individuals can distinguish between humidity levels differing by 5 % on 7 cm long gradients and they are attracted towards the area where relative humidity is 80 % and more. Its higher reproductive rate is also directly associated with higher moisture content (Solomon, 1962). Its reproductive growth is supported especially when the stored material is damp enough to become mouldy, since stored-product mites typically feed on fungi (Mullen and OConnor, 2019).

2.3.2 Fungal associations with mites

It has been previously shown that the presence of fungi is increased by mite presence (Sinha, Van Bronswijk and Wallace, 1970). Commonly found fungi in mites are *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Acremonium*, *Paecilomyces* or yeast (Oh et al., 1986). *Acarus siro*'s relationship with fungi is slightly complicated: it can live upon fungi which enables it to infest even otherwise unsuitable materials if they are just slightly mouldy. By that, it manages to keep down the mould and prevents it from rapid growth (Solomon, 1962). The fact, that mites can use fungi as a food source means that they should have specific enzymes to digest the mycelia (Hubert and Mourek, 2002). The mites carry spores on their bodies and in their digestive tracts, by which they inoculate clean grain or other food sources and they later feed upon these developed fungi. They prefer species requiring higher moisture contents like *Aspergillus* spp. (Solomon, 1962), which suggests that mites are, indeed, selective vectors of fungi (Hubert et al., 2003). Numbers of *Penicillium* spp. colonies and *Aspergillus glaucus* group colonies were less abundant on grain infested by mites (Armitage and George, 1986). It has been reported that *A. siro* can multiply on cheddar cheese contaminated by *Penicillium verrucosum* and on the sterilized mould itself, not as successfully as on the pure cheese though (Peace, 1983).

When the mite culture itself becomes mouldy or doesn't do very well, the mites are very likely to die out (Solomon, 1962). There are cases, when populations of *A. siro* were reduced or died out because fungal contamination emerged on their food source. It was shown that if wheat germ becomes discolored and infested by fungi, the mites leave the germ uneaten. The development turned out to be affected, too – the eggs hatched normally, but only a few of the emerged larvae survived to the first nymphal stage and none progressed further. Approximately half of larvae and first-stage nymphs died, and very small number reached the adult stage. Final-stage nymphs all survived, but females derived from them failed to lay as many eggs as usual and died much earlier. When eggs had been put on infected germ flakes, they had only reached the larval or first nymphal stage, whilst at the same time the eggs that had been put on regular wheat germ had already reached the adult stage (Solomon et al.,

1964). *Wallemia sebi* was proven to be more abundant on grain infested by *A. siro*, which could mean the mites avoid consuming it and also do not suppress the fungal growth through secretion, or the fungus might exploit the absence of competition from other fungi (Armitage and George, 1986).

2.4 Food competition and economic relevance

Stored food pests have been recognized as a serious threat since the first half of 20th century. Postharvest losses caused by stored-product insects, including mites, are estimated to range from 9 % in developed countries all the way up to 20 % and more in developing countries (Phillips and Throne, 2010). In farms or other commercial facilities, high relative humidity, storing damp wheat, poor air circulation, and scarification of the wheat during storage manipulations, or even water seepage and accumulation of vegetable debris are factors contributing to massive mite and insect infestations (Krantz, 1955). Even though the mites do not attack the grain when the coat is intact (Solomon, 1946), during storing and threshing, more than 90 % of the grain get scarified and therefore destroyed. High numbers of mites also lead to increase of temperature and moisture content of the grain, which simplifies the growth of microorganisms (Krantz, 1955), not to mention their presence usually does not go unnoticed by their predators, which as a result contaminate stored food also, so they create another group of possibly dangerous and risky animals for humans (Hubert et al., 2018). Moisture content is also critical when it comes to attacking the germ – *A. siro* only infests such material, where the water content is 14 % and higher (Mullen and OConnor, 2019). On the other hand, it does not typically eat out endosperm unless it is mouldy (Solomon, 1962).

By infesting stored food, mites can affect food safety through the change of humidity and temperature of such food. These factors may contribute to higher growth rate of pathogenic or toxigenic fungi and bacteria. Mites probably act as vectors for both as they are likely to host these organisms in their digestive tract and therefore contribute to the spread of pathogenic organisms (Hubert et al., 2018).

2.5 Control

As stated before, the flour mite is widely distributed in temperate regions (Solomon, 1962) and doesn't represent such threat in subtropical or tropical parts of the world (Griffiths, 1964). Since the development of *A. siro* is dependent on temperature and humidity, the easiest way to reduce its numbers would be to keep the storage as cool and dry as possible to lower the risk of outbreaks (Büchi, 1991). In warmer areas of the world, *A. siro* is present, but in accordance with listed information, its

populations and therefore infestation of stored food is not as excessive as in temperate regions (Emmanouel et al., 1994). Even though controlling pests involves prevention as described (controlling temperature and humidity), an important part of prevention are inspections, because it is not uncommon that commercial producers start to eliminate mite populations only once they are visually observed (Zhao et al., 2016). For monitoring and detection, traps can be used as they attract numerous species and provide early warning (Thind, 2005) but their effectivity depends on the temperature and relative humidity of the environment (Wakefield and Dunn, 2005).

2.5.1 Biological control

As a biological control, predator species *Cheyletus eruditus*, the most common cheyletid in stored products, has been commercially used in warehouses and granaries (Mullen and O'Connor, 2019). It is, however, often limited by temperature or humidity, and so *A. siro* can maintain its population, even if very small, but manages to emerge next season again, whilst *Cheyletus* fails to survive (Solomon, 1962). As stated by Solomon et al. (1964), some fungi might be poisonous for mites. It is very likely that mites get poisoned by digesting food infested by *Wallemia sebi* (syn. *Sporendonema sebi*) or *Aspergillus restrictus* – either the fungi are poisonous, or they make the food material poisonous, or both. It is safe to say that growing fungi on stored products as mite control is highly unlikely, but if the chemical compounds produced by these fungal species that restrict mite populations are identified, it could lead to the development of new acaricide (Solomon et al., 1964). If selective fungal transport via mites is considered, the importance of effective control is even enhanced (Hubert et al., 2003).

2.5.2 Physical control

Acarus siro has rather low tolerance limit for unfavorable temperature and humidity conditions as was described by Cunnington (1965) and Solomon (1962). Compared to other foodstuff insect pests, *A. siro*'s highest temperature limit for survival and reproduction is only above 30 °C (Solomon, 1962). Since its cuticle is permeable to gases, thus also to water vapour, the mite loses water rapidly in dry atmosphere; on the other hand, it can obtain moisture from the atmosphere once these levels are above 70 % (Solomon, 1962). It has also been reported that *A. siro* can be killed by being exposed to –15 °C for 60 minutes or 55 °C for 30 minutes (Žďárková and Voráček, 1993). The importance of temperature and relative humidity, thus the grain moisture content, was also demonstrated by Armitage, Cogan and Wilkin (1994) as they reported increased numbers of *A. siro* once these levels increased to 17% m.c. and 80% r.h. Cooling the stored material at 15% m.c., 70% r.h. and 5 °C can prevent the development

of mites in the grain mass, it does not, however protect it from substantial infestations on the surface (Arimtage, Cogan and Wilkin, 1994). Alternative option might be the high temperature/short time (HTST) technique, which uses heated air in combination with infra-red radiation to rid the wheat germ of mites. Almost complete reduction can be obtained by exposing the wheat to 200–250 °C for only 6 seconds, thorough eradication was achieved with exposition to 400–450 °C for 6 seconds. Moreover, none of these options affects the germination properties or the quality of the grain (Mourier and Poulsen, 2000). *A. siro* also cannot survive when exposed to low pressure – even after 24 hours of exposure to the pressure of 190 or 95 mm Hg, 100 % of mites were not able to survive. Exposing packing of materials such as dried fruits, cheese or spices to vacuum of 190 mm Hg or less can therefore protect the products from mites, even if the material would have been previously infested. *Acarus siro* also seems to fail to reproduce and develop properly when kept under constant light for 3 weeks (Žďárková and Voráček, 1993). Quite interesting method of controlling the mites is the use of inert dusts. It involves diatomaceous earth, which is a natural product formed from fossilized diatoms and the dust is mostly made of amorphous silica. The dusts have both abrasive and sorptive effect so as the mites pick up the dust as they walk over the treated surface, their cuticle gets disrupted. The mites end up losing water and eventually die from desiccation (Ebeling, 1971). Even at a quite low dose (0.5 g/m²), the dusts are capable of up to 100% control of *A. siro* (Collins and Cook, 2006)

2.5.3 Chemical control

There have been attempts to control mites with fumigation. Sinha, Berck and Wallace (1967) reported that *A. siro* could be removed by fumigation with phosphine (PH₃), however the effect only lasted about five months. After this time, the mite reoccurred and started multiplying as usual (Sinha, Berck and Wallace, 1967). Similarly, phosphine and methyl bromide were tested to control other two (*Tyrophagus putrescentiae* (Schrank) and *Caroglyphus berlesei* (Mich.)) storage mite species effectively, because both were able to eliminate adults and eggs. However, the fumigants were more effective in higher temperatures, especially in eliminating the eggs as those are more resistant than the adults. Both stages were more susceptible to methyl bromide (Jalil, Ross and Rodriguez, 1970). *A. siro* was found susceptible to fumigation with methyl chloroform, carbon tetrachloride, ethylene dichloride, methyl formate, ethylene dibromide, ethylene dichloride, ethylene oxide, acrylonitrile, ethyl bromide, ethyl formate, methallyl chloride, methyl bromide and phosphine. The eggs were able to survive fumigation with ethylene oxide and phosphine, so, with phosphine, the procedure was repeated to kill individuals that had emerged from the surviving eggs. The interval between the treatments was dependent on temperature – at 20 °C it was 10–14 days, at 15 °C about 21 days and at 10 °C, intervals of from 5 to 9 weeks to achieve complete kill of the mite. Since the adults and mobile stages are more

susceptible than the eggs, it only required low dosages, thus the concentrations for both treatments were much lower than that required in a single fumigation (Bowley and Bell, 1980). Methyl bromide, though successful in trials, has been found to be an ozone depleting substance and listed as such under the Montreal Protocol, which ordered to lower the production to eventually discontinue the use of it by 2005, with specific exceptional applications (UNEP, [online]) such as in dry-cured ham industry as there is no effective alternative for controlling mites infesting dry-cured ham. Phosphine was considered an option because it was very successful at the laboratory trials, but under commercial conditions the gas had only limited success. It is also highly corrosive so its use under commercial conditions would be quite difficult, complicated (Zhao et al., 2015) and also expensive because of severe electrical damage and the harm the fumigation did to the copper fittings (Zhao et al., 2016).

Apart from fumigation, there have been aims to identify alternatives to organophosphorus pesticides. Those substances include insect growth regulators (IGRs) such as juvenile hormone analogues that disrupt the morphogenesis to the adult, which results in mites with combined adult and juvenile characteristics. Those mites cannot feed or reproduce and die eventually (Dekeyser and Downer, 1994) but the efficacy has not been assessed against storage mites (Collins, 2006). Ecdysone (moulting hormone) agonist showed promising results against *T. putrescentiae*, but the use of such compound resembling steroid hormones, which are found and have important roles in higher animals including a man, would need a very extensive testing (Piccardi et al., 1980). Last of these were chitin synthesis inhibitors which prevent hatching of eggs and affect immature stages, though their exact pathway of action remains unknown (Dekeyser and Downer, 1994). These substances were, nonetheless, quite successful at controlling *A. siro* populations, but the effectiveness against storage mites of all IGRs is limited (Collins, 2006). Some plant parts and extracts also show pesticidal potential like azadirachtin, a component isolated from *Azadirachta indica* A. Juss, also known as the neem tree, or benzyl benzoate present in the resin of *Myroxylon balsamum* (L.) var. *pereirae* Harms, a Peru balsam tree (Collins, 2006), pyrethrum, derived from *Chrysanthemum* sp. which is a natural pyrethroid but is mostly replaced by synthetic pyrethroids that have better insecticidal properties and photostability (Collins, 2006). Also, several novel compounds, such as fatty acids (0.5–0.8% propionic acid caused 100% mortality of *A. siro*); inorganic salts, but those would have to be applied in high doses or are quite expensive or are not environmental-friendly, which prevents their use in practical use; and antibiotics were explored (Collins, 2006).

The most common way of controlling stored product mites is, nonetheless, the use of pesticides. Among tested chemicals, permethrin has shown great potential for controlling mites (Stará et al., 2011) while not increasing oviposition (Ayyappath et al., 1997), which can happen with some pesticides.

Pirimiphos-methyl and etrimfos have also shown their reducing ability when used as dusts on grain that had previously been aerated, so the use of the pesticides was only for eliminating surface infestations. Combination of these two methods also came out as much cheaper option compared to pesticide-only treatment (Armitage, Cogan and Wilkin, 1994). Wilkin (1975) managed to control *A. siro* populations in farm-stored barley with application of lindane and malathion or pirimiphos-methyl dust. Apart from being quite expensive, the issue associated with chemical control is that most products are actually used off-label, meaning they are originally designed to be used for controlling another species – permethrin is primarily targeting house dust mites (Stará et al., 2011) etc., and that mites are able to develop high level of resistance to pesticides (Wilkin, 1973).

2.6 Resistance to pesticides

Since there is a bigger need for sufficient agricultural production worldwide, it is important to acknowledge that Global warming and economic globalization might change the geographical distribution of pests, pesticides will play an important role in future agriculture production. At the same time, though, repeated pesticide use leads to microevolution through selection pressure and results in resistance development (Osakabe et al., 2009). In 2014, 389 cases of two spotted spider mites being resistant to pesticide were reported, which means that they can develop resistance within 2-4 years of pesticide's introduction (Mohankumar et al., 2014).

Over the last decades, there has been an effort made to develop alternatives to the use of pesticides for pest species control like insects and mites on agricultural crops. However, it is very typical for plant-feeding mites to increase their numbers after pesticide application – this is known as pesticide-induced secondary pest outbreak. This pattern is observed with both key mite species (the one pesticide is applied for) as well as secondary pests (those are normally present at minor levels but can experience outbreaks after targeting the key species), the only difference is that it takes longer for a key species to increase in numbers compared to the secondary pests. Such phenomenon is referred to as 'pest resurgence'. Obviously, secondary outbreaks and pest resurgence lead to continuous increased use of pesticides which is usually followed by another outbreak/resurgence. This pattern is called 'pesticide syndrome' or 'pesticide treadmill' (Morse, 1998). What could be responsible for such phenomenon is either natural enemy reduction by used pesticide (Morse, 1998; Sclar et al., 1998), or it might be hormoligosis (Morse, 1998). Hormoligosis occurs when sublethal doses of any stress agent stimulates the organism, which is therefore provided with increased sensitivity to respond to changes in its environment, and also with increased efficiency to develop new or better systems to fit a suboptimum

environment (Luckey, 1968). It has been observed that insecticide-induced hormoligosis occurs in a variety of pests. Morse and Zareh (1991) reported increased fecundity of citrus thrips, *Scirtothrips citri*, after exposing it to leaves treated with sprayed pesticides. The fecundity increased at rates which caused 0.01–1% mortality, whilst it decreased or remained unchanged at higher or lower rates, respectively (Morse and Zareh, 1991). In some cases, mite species *Panonychus ulmi* (Koch) and *Neoseiulus californicus* (McGregor) were proven to lay more eggs when they were exposed to sublethal doses of commonly used pesticides (cypemethrin, imidacloprid, deltamethrin and thiacloprid), while at the same time their lifespan shortened (Saritas and Ay, 2016). Another case of increased fecundity of mite pest *Tetranychus urticae* (Koch) was observed after treated with three neonicotinoids: thiacloprid, acetamiprid and thiamethoxam (Barati and Hejazi, 2015), and also after both direct (spray formulations) and indirect (through ingestion of systematically treated plant leaves) exposure to imidacloprid, where the mite's longevity was prolonged, too, when the individuals ingested the insecticide (James and Price, 2002). On the other hand, pesticide-induced hormoligosis is rarely seen with natural enemies' populations (Morse, 1998).

2.6.1 *A. siro* and resistance

There are recorded cases of *A. siro* being resistant to three different active ingredients – namely etrimfos (Thind and Muggleton, 1998), pirimifos-methyl (Szlendak et al., 2000) and lindane (Wilkin, 1973). Resistance to pesticides found in *A. siro* was at least in one case caused by higher esterase activity, but it is likely that other mechanisms are present also (Szlendak et al., 2000), which suggest that resistance results from gene regulatory changes that are responsible for an increased efficacy of one or more physiological systems used by pests for detoxification, such as oxidation, conjugation to hydrophilic compounds, and excretion. Up-regulation of detoxifying enzymes or gene amplification are possible and used as mechanisms for increasing the amount of protein available to inactivate the pesticide (Heckel, 2012). Lately, it has been revealed that the genes responsible for these enzymes can be transferred horizontally (Van Leeuwen and Dermauw, 2016). Imidacloprid, a very common insecticide, causes different reactions in mites (Sclar et al., 1998; James and Price, 2002; Saritas and Ay, 2016), thus it seems obvious that knowing the mechanism behind developing resistance or behind hormoligosis is crucial. Also, it has been reported, that pesticide-(imidacloprid-) induced increased fecundity only developed within mites fed with leaves treated with imidacloprid, whereas direct exposure (spraying) did not have the same effect (Szczeplaniec and Raupp, 2013). Since digesting pesticides can cause increased fecundity and alter their lifecycle (James and Price, 2002; Szczeplaniec and Raupp, 2013), it is possible that mites' microbiome can alter their answer to pesticide exposure.

2.7 Microbial community in mites

Arthropods host a very diverse bacterial community (Zchori-Fein and Perlman, 2004). Associated microorganisms with synanthropic mites have been studied for many years now, mostly because of microbial potential to cause allergic reactions or asthma (Gregory and Lloyd, 2011), so ever since the 80' there was a focus on isolating and identifying these organisms. The biggest issue with exploring the microbial diversity was that intracellular symbionts are typically very hard or impossible to culture outside of their hosts, but they can be isolated and maintained for a short period of time (Moran and Telang, 1998). The influence of bacterial symbionts on their host varies from protection from natural enemies (Oliver et al., 2003) to the provision of essential nutrients, especially essential amino acids (Douglas, 1998; Moran and Telang, 1998). Some microbial taxa can manipulate reproduction in arthropods (Zchori-Fein et al., 2001; Gotoh et al., 2007), which is supported by the finding that mite-associated bacteria may play an important role in mites' population growth, since growth rates were observed to be altered among populations with variant microorganism composition (Hubert et al., 2021). Apart from host-symbiont interactions, symbiont-symbiont interactions in superinfected hosts seem just as crucial for complete understanding of their role and distribution (Montllor et al., 2002; Oliver et al., 2006).

2.7.1 Primary symbionts

Bacterial endosymbionts in mites can be divided into two main groups: primary and secondary. Primary symbionts are located in specialized cells - bacteriocytes (also mycetocytes). Bacteriocyte symbionts are not known to be obtained from the environment, but they are strictly vertically transmitted via ovary. They are beneficial to their hosts as they alter the host's nutritional status, synthesize specific proteins, and produce antimicrobial substances for protection from microbial pathogens (Douglas, 1989). Enabling the host to meet the nutritional demands of an insufficient diet allows the host to inhabit otherwise unsuitable niches (Akman Gündüz and Douglas, 2009). Number of mycetocytes seems to vary during the host's life, which might regulate the symbiont population (Douglas and Dixon, 1987), also the biomass of symbionts varies with host age, weight and sex within individuals (Douglas, 1989). All maternally transmitted symbionts are quite costly to maintain since they impound resources from their hosts, yet there is a remarkable diversity of them in arthropods. Their presence is usually asymptomatic as they have very few pathogenic effects on their hosts (Haine, 2008). The primary endosymbionts seem to have long co-evolution with their host as they have arisen from a single ancestor many times independently from free-living bacteria (Moran and Telang, 1998).

2.7.2 Secondary symbionts

Secondary symbionts, or facultative symbionts, are present within the host regardless the primary symbionts presence, but their presence is not restricted to bacteriocytes only (Moran and Telang, 1998). They live also within the host cells, host body cavity (Sandstrom et al., 2001) or they can be found free in the hemolymph (Oliver et al., 2003). These bacteria directly enhance the fitness of an infected host lineage, thus increasing the frequency of infection (Oliver et al., 2003). They may play an important role in host's ecological interactions and or mediate the interaction between the host and its natural enemy (Oliver et al., 2003, Oliver et al., 2006). Unlike primary symbionts, facultative symbionts may also undergo horizontal transmission (e.g., through diet containing symbiont cells), even though they are predominantly maternally transmitted (Sandstrom et al., 2001; Haine, 2008). Horizontal transfer among species indicates that some symbionts preserve the ability to infect multiple hosts, even though negative effects on the novel host fitness can follow after new infection (Russell and Moran, 2005). In order to spread and persist, they bestow benefits to the host like increase their reproductive output or survival, or they manipulate the host reproductive system (Haine, 2008). They are unevenly distributed among individuals of the same or related species and thanks to being able to transfer horizontally, they are responsible for coinfections within host and give the opportunity for gene transfer and recombination. Some facultative symbionts are under strong selective pressure to enhance the fitness of the host and can switch from manipulating the host reproduction to successfully enhance host fecundity (Weeks et al., 2007; Moran, McCutcheon and Nakabachi, 2008). Since their presence in species is sporadic, it implies they are not needed by the host for its reproduction or development (unlike most primary symbionts) (Moran and Telang, 1998), yet their distribution among species does not appear random, since there are host groups showing high frequency of a particular symbiont (Moran, McCutcheon and Nakabachi, 2008). Typical representative of secondary symbionts is *Wolbachia*, bacterium responsible for several reproductive disorders such as cytoplasmic incompatibility, inducing parthenogenesis or feminization, thus biasing the sex ratio. *Wolbachia* also undergoes horizontal transmission among different arthropod orders (Moran and Baumann, 1994). Incidence of facultative symbionts within host can also change throughout the year – symbionts who were proven to benefit their host under heat stress were more abundant in individuals collected during hot summer months compared to those collected during spring from the same location (Montllor, Maxmen and Purcell, 2002). Secondary symbionts also seem to play an important role in protecting their host from their natural enemies – especially parasitoids, whose larvae may not develop properly or die within the infected host. This could be either the result of an increased immune response, which could vary with particular symbiont association, or altered metabolic balance of the host; the symbiont may also affect

the survival rate of parasitoid larvae indirectly through toxic secretion or disrupting the way larvae meet their nutritional needs within the host body (Oliver et al., 2003).

2.8 Microbiome of *Acarus siro*

Originally, the bacterial community isolated from the dust mites was represented by *Bacillus* spp., *Staphylococcus* spp., Gram-positive and Gram-negative rods (Oh et al., 1986). Later, as laboratory and molecular methods became more available, bacteria were identified thanks to bacterial 16S ribosomal RNA, revealing the presence of *Bartonella* species and Gram-negative species (Valeiro et al., 2005). Closer examination of microbial composition in *A. siro* found *Bartonella*-like bacteria in postcolonial diverticula and *Cardinium*-like bacteria (Hubert et al., 2012). Up to 12 *Cardinium* sequences (Kopecký et al., 2013) and up to 56 *Bartonella*-like sequences were found in both laboratory and field strains (Kopecký et al., 2014a).

Cardinium, an intracellular symbiotic bacterium, is spread among Acari, Hymenoptera and Hemiptera (Weeks et al., 2003; Zchori-Fein and Perlman, 2004) and is transferred vertically. In some species it is associated with parthenogenesis or feminization (Zchori-Fein et al., 2001). It seems to have the highest prevalence among mites compared to any other arthropod group that tested positive for its presence (Weeks et al., 2003; Kopecký et al., 2013), even though it is estimated that 13 % and more of arthropod species are infected with *Cardinium* (Weinert et al., 2015). Nevertheless, *Cardinium* can also induce cytoplasmic incompatibility (CI), thus affecting reproduction of the host (Gotoh et al., 2007) and is sometimes found along other reproductive bacterial taxa in arthropod species, which include *Wolbachia*, *Arsenophonus* and *Spiroplasma ixodetis*, all of which are likely to affect their hosts' biology (Duron et al., 2008). Along with *Wolbachia*, *Cardinium* is only the second symbiotic bacterium known to induce cytoplasmic incompatibility, although not in all their host species (Gotoh et al., 2007). Its role in host nutrition, since the bacterium harbours complete biotin biosynthesis pathway, remains unexplained (Penz et al., 2012).

Next, *Kocuria* sp. and *Solitalea*-like bacterial taxa were identified (Kopecký et al., 2014b; Hubert et al., 2016b). The body microbiome seems to be dominated by these – either *Bacillus* or *Solitalea*-like with *Kocuria* are the key representative bacterial taxa (Hubert et al., 2021). That being said, various populations of *A. siro* host slightly different microbial communities, which can be unique to given mite population. Moreover, in populations missing the intracellular symbionts (*Cardinium*), environmental bacteria like *Kocuria*, *Staphylococcus* and *Bacillus* were present in the mite body microbiome probably thanks to competitive exclusion of one group of bacteria by another (Hubert et al., 2021).

2.9 Microbial interaction with pesticides

The very first report of microbiome-facilitated resistance to pesticides was published more than 50 years ago, when an obligate extracellular bacterial symbiont *Pseudomonas melophthora* of the apple maggot, *Rhagoletis pomonella* (Walsh), showed degradation activities against 3 groups of pesticides (chlorinated hydrocarbons, organophosphates, and carbamates) through a powerful degrading esterase (Boush and Matsumura, 1967). Nowadays, various approaches to describe microbiome-induced resistance are adapted, ranging from molecular investigation to testing the influence of host diet on microbial communities.

2.9.1 Degradation ability of microorganisms

When isolated from the host, 15 out of 25 diamondback moth (*Plutella xylostella*) bacterial symbionts showed esterase activities, which may contribute to insecticide detoxification, hence conferring resistance to the host. Specifically, *Bacillus cereus* degraded indoxacarb and showed ability to use it for metabolism and growth (Ramya et al., 2016). The same bacterium was also reported to degrade synthetic pyrethroids such as chlorpyrifos (Liu et al., 2015), fenvalerate (Selvam, Thatheyus and Vidhya, 2013) and cypermethrin both alone or in co-culture with *Streptomyces aureus*, while the latter was more rapid and effective (Chen et al., 2012). Cypermethrin can also be degraded by *Bacillus subtilis* (Gangola et al., 2018). It is possible that some pesticides can be degraded only when more than one bacterium is involved, indicating that synergistic interactions between different bacteria may play an important role in the process (Sorensen, Ronen and Aamand, 2002).

2.9.2 Diet-induced pesticide resistance

It was shown that diet may play an important role in bacteriome composition – altered dietary composition leads to uneven microbial composition among individuals of the same species. On the other hand, the same diet fed to different species has no to absolutely minimal effect on microbial composition (Chandler et al., 2011). The effect of diet contaminated with pesticides has shown to have significant effect on honeybee bacteriome regarding relative abundances and bacterial structure but did not affect the diversity (Kakumanu et al., 2016). In another case, strains of *Nasonia vitripennis*, parasitoid wasp, were treated with diet containing sub-lethal concentrations of atrazine, a synthetic pesticide. Populations exposed to atrazine had more diverse bacterial structure, and after only single exposure to very small dose of atrazine the overall bacterial load in the host increased. Furthermore, when the offspring of atrazine-exposed populations were switched to control diet, the bacteriome

structure remained most identical to that of the parents, which indicates that single acute exposure to atrazine causes changes in the bacterial microbiome which is inherited across generations even though the exposure is removed. Continual exposure to subtoxic concentration of atrazine diet caused 10-fold higher tolerance by the 36th generation and these populations also demonstrated higher tolerance to glyphosate, despite no prior exposure to the compound. Higher densities of some bacteria were also observed in atrazine-exposed populations; however, it is possible for population densities of symbiotic bacteria to become too high. In that case, the bacterium becomes pathogenic, which results in disrupting the host-microbiome and mortality of the host. The shift in microbial community following continuous atrazine exposure thus provides host resistance and supports symbiont-mediated resistance mechanisms in subsequent generations, therefore affecting the host fitness (Wang et al., 2020). Similar results were obtained with cockroaches, where microbiome-mediated resistance was transferred from resistant strain to a susceptible one through feeding the susceptible strain feces obtained from the resistant one. The transplant altered the distribution of bacterial taxa and introduced new taxa from the resistant strain to the susceptible one (Pietri, Tiffany and Liang, 2018).

Interesting case has been reported by Guo et al. (2020), where two color morphs of the melon aphid *Aphis gossypii* differ in the density of the common insect primary symbiont *Buchnera*. The yellow morph, which has higher density of the symbiont and endosymbiont diversity as such, shows higher level of resistance to pesticides compared to the green morph. Moreover, when the yellow morph was treated with antibiotics to reduce the density of *Buchnera*, its resistance decreased, while various concentrations of antibiotics habitually resulted in similar levels of *Buchnera* (Guo et al., 2020).

What could summarize the broadness of symbiont-mediated resistance issue is the case of the genus *Burkholderia*, whose strains are resistant to organophosphate fenitrothion. Fenitrothion-degrading *Burkholderia* is present in soil at very low levels. However, when fenitrothion was applied, *Burkholderia* drastically increased its levels to >80 % of total cultivable bacterial counts in the soil. When the nymphs of bean bug, *Riptorus pedestris*, were reared on plants growing in such soil to adulthood, they acquired the bacteria and showed resistance to the insecticide. Higher survival rate also appeared when the nymphs infected with fenitrothion-degrading *Burkholderia* were reared on soybean seeds dipped in fenitrothion solution and air-dried. The resistance was observed with percutaneous application, too. Apart from conferring resistance to fenitrothion, *Burkholderia* can degrade other insecticides as well. Insecticide application therefore causes increase of the insecticide-degrading bacteria in the ecosystem and influences the development of resistance even when the pest insects are absent, while pest insects can acquire the symbiont very quickly and establish the resistance within single generation. The symbiont also confers fitness benefits on the host, which may help spread the

resistant trait in the insect populations. In this case, the dispersal of the insecticide-degrading bacteria can be facilitated, since the host amplifies the bacteria in its symbiotic organ and migrates actively, too (Kikuchi et al., 2012).

Since resistance to pirimiphos-methyl has been reported in *A. siro* (Thind and Muggleton, 1998), there is a possibility that such resistance is also mediated by its bacterial symbiont or symbionts.

3 Methods and materials

3.1 Mite strains

In this thesis, the focus was on four strains of *Acarus siro*, each of which had been collected from different location and had been marked accordingly (Table 1).

Table 1: Mite strains with their respective IDs involved in the testing.

ID	Culture	Collector	Year	Diet	Site
6L	laboratory	E. Ždarková	1996	SPMd	grain, Buštěhrad, Czechia
6Tk	Teplice feed	M. Nesvorná	2015	SPMd	horse feed contamination, Teplice, Czechia
6Tu	Tuchoměřice	M. Nesvorná	2016	SPMd	rabbit feed contamination, Tuchoměřice, Czechia
6Z	Zvoleněves	M. Nesvorná	2011	SPMd	oil rape seed debris, Zvoleněves, Czechia

3.1.1 Rearing conditions

Every strain was kept in filter cap cell culture flasks (Cell Culture Flask T25, Eppendorf cat. No. 0030710029) which were placed in closable plastic container in the dark over saturated potassium chloride solution (Solomon, 1951). This solution was prepared from 680 g of potassium chloride and 2 liters of water. It enables to keep stable humidity inside the container – around 85 %. The temperature inside the container was 25 °C, +- 1 °C. The cultures were renewed monthly. The new culture contains 0,3 g of SPMd (stored product mite diet) and cca 0,05 g of mites taken from one month old rearing culture, both are placed into the clean flasks. SPMd is used as a common rearing diet, it consists of 15 g of yeast and 135 g of wheat germ. Both ingredients are mixed by kitchen blender and the mixture is then dried for 30 minutes at 70 °C.

3.2 Pesticides and dilution

The selected pesticides were registered for stored product community and labeled against stored product insects, not mites. The commercial names of biocides and active ingredients are shown in Table 2.

Table 2: Selected pesticides tested, their respective active ingredients and concentrations

Commercial name	Producer	Active ingredient	concentration g/l
Reldan 22	Dow AgroSciences s.r.o.	chlorpyrifos-methyl	225
Actellic 50 EC	Syngenta Limited	pirimiphos-methyl	500
K-OBIOL EC 25	Bayer CropScience GmbH.	deltamethrin piperonyl butoxide	25 250
K-Othrine SC 25	Bayer EnvironmentalScience GmbH.	deltamethrin	25

The pesticides were diluted in water to following concentrations: pirimiphos-methyl 250, 25, 2.5, 0.25, 0.025 mg/mL; chlorpyrifos-methyl 225, 22.5, 2.25, 0.225, 0.0225 mg/ml and both deltamethrin formulations: 25, 2.5, 0.25, 0.025, 0.0025 mg/ml. In short, all solutions were prepared using a ten-fold serial dilution.

3.3 Impregnated filter paper test

For the initial test, glass weighing bottles (cat. No. 264.228.01 Vitrum, a.s., Prague, Czechia) with ground-in stopper and filter paper were used. Filter paper was cut into round shape to fill the bottom of the bottle which has 2,2 cm in diameter. After two layers of the filter paper were placed into the bottle, 50 μ l of diluted biocide were applied. As negative control, 30 μ l of distilled water was applied. Once the filter paper was wet with pesticide or distilled water, respectively, 10 adult mites were placed inside the chamber. The flasks were then closed with glass lid, which ensured there was no room for the mites to escape from the bottle, but at the same time it was not air-tight so the mites couldn't suffocate. After 24 hours of exposure to the biocide (or distilled water) the mites were counted and checked whether alive or dead using stereomicroscope (Olympus SZ51). Every mite was considered dead if it was shriveled and did not show any motion or did not react to stimulation by a few soft paint brush bristles for several seconds. It was counted as alive when it exhibited movement with or without being stimulated by the bristles (Thind and Muggleton, 1998). All four mite cultures were exposed to all four

biocides in every previously obtained concentration in 10 replicates. Concentrations are shown in Table 3.

Table 3: Highest and lowest concentrations of active ingredient present on the filter paper.

active ingredient	concentration of ingredient [$\mu\text{g}/\mu\text{l}/\text{cm}^2$]	
	highest	lowest
pirimiphos-methyl	32883.2527	0.328832527
chlorpyriphos-methyl	29594.92743	0.295949274
deltamethrin	3288.32527	0.032883253
deltamethrin/piperonyl butoxide	3288.32527	0.032883253

3.4 Residual biocide application

Based on the results of previous test we selected pirimiphos-methyl and tested the effect of residual activity to mite population growth. The diet was prepared by mixing 10 g of SPMd with 5 ml of diluted pesticide (Table 4). Both substances were mixed and lyophilized to ensure homogenous distribution of active compound within the diet (Stará, Nesvorná and Hubert; 2014). As the control, distilled water was used instead of the pesticide and the mixture was then treated the same way.

Table 4: The amount of active ingredient present in 1 g of regular rearing diet.

active ingredient/rearing diet [μl]/[g]	pirimiphos-methyl
	12.5
	1.25
	0.125
	0.0125

Into the filter cap cell culture flask, 0,01 g of the diet was applied, next 50 unsexed mites were added. Then the flasks were stored at 25 °C (+–1 °C) at 85% relative humidity in darkness. After three weeks (21 days) the flasks were filled with 10 ml of ethanol, put into air-tight boxes, and placed into the fridge for at least a day (if it was a shorter time period, the mites would be still alive). Later, the flasks were filled up to 35 ml of ethanol. Each flask was well shaken to ensure consistent distribution of mites within the liquid and 3 x 1 ml was moved onto three Petri dishes. Using the dissection microscope, mites in each dish were counted and based on these numbers, the overall number of mites in the flask was determined. Each diluted pesticide was tested in six replicates.

3.5 DNA extraction

For DNA isolations, the mites reared on 0,1% and 0,001% solutions of pirimiphos-methyl (meaning 12.5 and 0.125 $\mu\text{l/g}$, respectively) and on control diet were chosen. The mites were removed from the flask using a micropipette and applied onto sterile 100 μl cell strainer (model 15-1100, Biologix Group Limited). The mites were surface cleaned using 5 ml of sodium chlorine (Savo) water solution in 9:1 ratio (0.47% sodium chlorine solution) and with 2x5 ml of 96% ethanol. Then the mites were washed off with 96% ethanol onto a sterile Petri dish. Next, 30 adult mites were moved from the Petri dish to 6 sterile 1,5ml tubes (Eppendorf Safe-Lock Tubes, cat. No. 0030120086, Eppendorf Quality™) using a micropipette. The design included 6 replicates per treatment. Most of the excess alcohol was removed using micropipette, but to ensure the mites were completely dry, the tubes were placed into SpeedVac Concentrator (SPD111V, Thermo Scientific) for 5-6 minutes. After that, the NucleoSpin® Tissue XS (cat. No. 740901.250) kit was used. First, 180 μl of T1 buffer was applied onto the mites. The mites were homogenized by sterile plastic micropestle. Then, 20 μl of proteinase K was applied directly into the mixture and the tubes were placed into a preheated orbital shaking Incubator (NB-205, N-Biotek) and kept there for 60 minutes (56 °C, 300 rpm). After the incubation, 200 μl of T3 Buffer (from extraction kit NucleoSpin® Tissue XS cat. No. 740901.250 as mentioned above) were added. The tubes were then incubated for 5 minutes at 70 °C and subsequently mixed by vortexing for 5 s twice. Next, 200 μl of 96% ethanol were added, the tubes were shaken by vortexing (again 2 x 5 s) and placed into centrifuge (15 s, 5000 g) right after that. Using a micropipette, each sample was transferred into NucleoSpin Tissue XS Columns placed into 2ml Collection Tubes. These were centrifuged for 1 min at 11,000 x g. Collection Tubes were discarded and replaced with new ones. Next, 50 μl of B5 Buffer were applied to the Columns, centrifuged for 1 min at 11,000 x g and without discarding the flow-through, another 50 μl of B5 were added and centrifuged for 2 min at 11,000 x g. The Collection tubes were discarded, and the Columns were placed into sterile 2ml Eppendorf Safe-Lock tubes. In the last step, using a filter tips for micropipetting, 30 μl of PCR H₂O (Cat. No. P042, Top-Bio s.r.o.) were applied directly onto the center of the silica membrane of the Column. These were centrifuged for 1 min at 11,000 x g, the Columns were discarded, and the isolated DNA samples were stored in the freezer at -40 °C in the tubes for further use.

3.6 qPCR assay

3.6.1 Standard preparation

In order to perform the qPCR, standard and respective standard line had to be prepared. The standard was prepared as follows: first, gene from *Kocuria* colony were amplified in PCR using primers F27 and R1492. The conditions are listed in the table below (Table 5).

Table 5: Initial PCR run conditions for standard preparation and primers used.

Primer name	pattern		
F27	5'-AGAGTTTGATCMTGGCKCAG- 3'		
R1492	5'-TACGGYTACCTTGTTACGACTT- 3'		
Step	Temperature [°C]	Time	No. of cycles
Initial denaturation	94	5 min	30
Denaturation	94	1:50 min	
Annealing	50	1:50 min	
Elongation	72	1 min	
Final extension	72	10 min	

The gene was integrated into vector which was subsequently integrated into bacteria through transformation of competent cells. The bacteria were then cultivated. These three steps were performed in accordance with Promega A1360, pGEM-T Easy Vector System kit. Next, the clones containing the desired DNA were identified – the cloned bacteria were white colored, and the gene was amplified in PCR using primers pUCM13R and pUCM13F. Run conditions are listed in Table 6.

Table 6: PCR conditions and primers used for gene amplification from cloned bacteria.

Primer name	pattern		
pUCM13R	5'-CACAGGAAACAGCTATGAC-3'		
pUCM13F	5'-GTTTTCCCAGTCACGAC-3'		
Step	Temperature [°C]	Time	No. of cycles
Initial denaturation	94	5 min	30
Denaturation	94	1 min	
Annealing	54	50 s	
Elongation	72	1:30 min	
Final extension	72	5 min	

To verify the cloned DNA sequence, samples were sequenced. The cloned DNA was then isolated from the bacteria (plasmid DNA being the vector) according to the Promega A1330, Wizard Plus SV Mini preps DNA Purification System kit. In the next step, the circular DNA had to be cleaved in specific

recognition site using restriction enzyme Sal I, recombinant. This was performed according to the New England Biolabs R0138S kit. In the last step, the DNA was purified using GeneAll Expin Combo kit, specifically TE buffer, which is used to melt the samples.

3.6.2 qPCR

For qPCR assay, a mixture of reagents was prepared. This mixture included specific SP1 and SP2 primers (Table 8), PCR H₂O and TP SYBR 2x Master Mix. SP1 and SP2 primers were first diluted with PCR H₂O in 1:10 ratio (Table 7). This mixture was present in every tube (samples, standard, negative control) which underwent the qPCR in the volume of 20 μ l. Depending on the number of tubes involved, the mixture was prepared accordingly, based on the known ratio of chemicals present in one tube. The desired overall volume (along with individual volumes) was hence calculated by simply multiplying the respective volumes of each chemical by the number of tubes involved. The mix was prepared for 96 samples, including 10% pipetting mistake. As mentioned before, 20 μ l of the mixture was applied into desired number of tubes and 5 μ l of each DNA sample were added.

Table 7: Chemicals and their respective volumes needed for the qPCR reaction.

Chemicals	Volume [μ l] per 25 μ l tube	Volume [μ l] for 96 samples
TP SYBR 2x Master Mix	12,5	1200
SP1 primer (solution)	1,25	120
SP2 primer (solution)	1,25	120
PCR Water	5	480
DNA sample	5	-

Table 8: Primers used in the qPCR reaction to amplify bacterial RNA.

Primer name	pattern
SP1_515pF	ACACTGACGACATGGTTCTACA GA GTGYCAGCMGCCGCGGTAA
SP2_806aR	TACGGTAGCAGAGACTTGGTCT AC GGACTACNVGGGTWTCTAAT

For the purpose of creating a standard line, the standard itself had to be diluted: first, 50 μ l of the standard were applied into 0.5ml tube, while 45 μ l of PCR water were applied into another six 0.5ml tubes. 5 μ l of standard were moved from the concentrated 50 μ l to the first tube with water and mixed using a micropipette, then 5 μ l of this mixture were moved to another tube etc. Thanks to this simple procedure we managed to obtain decimal dilution in the range between 1E+08 to 1E+02 copies of gene, in other words, approximately from 1E-01 to 1E-07 ng of the standard DNA.

Just as the DNA sample, 5 μ l of each concentration were also mixed with 20 μ L of reagents mix. The qPCR plate therefore held 7 concentrations of diluted standard, samples themselves and negative

control. The qPCR conditions were 95 °C for 2 minutes for initial denaturation, followed by 10 s of denaturation at 95 °C, 40 cycles at 55 °C for 30 s, elongation at 72 °C for 30 s (first reading of fluorescence signal) and eventually 15 s 95 °C, 30 s 60 °C and 15 s 95°C for melting conditions. The fluorescence signal is read every +0,5 °C. Melting curves were also recorded to ensure qPCR specificity (Table 9).

Table 9: qPCR Run conditions.

Step	Temperature [°C]	Time	No. of cycles/notes
Initialization	95	2 min	
Denaturation	95	10 s	
Annealing	55	30 s	40
Elongation	72	30 s	Fluorescence signal reading
Melt curve	95	15 s	Fluorescence signal reading every 0.5 °C
	60	30 s	
	95	15 s	

3.7 Barcode sequencing

The resulting amplicons were then prepared for sequencing with the second-stage PCR. During this second stage, Illumina sequencing adapters and sample-specific barcodes were incorporated into amplicons by PCR amplification with Fluidigm Access Array for Illumina primers. The Fluidigm PCR amplification started at 95 °C for 5 min, followed by 8 cycles at 95 °C for 30 s, 60 °C for 45 s, 72 °C for 30 s, and terminated at 72 °C for 7 min. This barcoding PCR step and amplicon sequencing were performed at the Genome Research Core, Research Resources Center, University of Illinois (Chicago, IL, USA) on a MiniSeq platform (Illumina, San Diego, CA, USA) and employing paired-end 2 × 153 bp reads. Forward and reverse sequences were aligned and processed using a combination of MOTHUR 1.42.0 and UPARSE 11 following previously used protocol (Hubert et al., 2019). Briefly, reads were merged, and quality filtered in MOTHUR, and then processed according to the MiSeq SOP protocol. Sequences were then mothur-formatted to RDP (Ribosome Database Project) reference dataset. Chimeras were detected using the chimera.vsearch command. Chimeras, ambiguous sequences, chloroplast, and mitochondrial DNA sequences were discarded. Processed FASTA file was used for OTUs (operational taxonomy units) identification and taxonomic descriptions using RDP reference dataset in UPARSE. OTUs were identified at a 97% similarity threshold using the Sintax command in UPARSE and discarding chimeras. Representative sequences of each OTU were taxonomically classified according to RDP identification and using BLASTn. The OTUs were blasted against the

reference 16S RNA and/or nucleotide collection databases and the taxon with the highest match similarity was selected. The OTU representative sequences were aligned in T-Coffee (<http://tcoffee.crg.cat/>) (Hubert et al., 2021). The list of samples, factors and files deposited at NCBI describing the microbiome of *Acarus siro* is shown in Table S1.

3.8 Statistical methods

Mortality data has binominal distribution and were calculated according using GLM (generalized linear models) (Pekár and Brabec, 2009). The tested variables were concentration of pesticide (LOG +0.00001) mg/ml, mite population, type of biocide and their interaction. The dependent variable was mite mortality from filter paper test. Because all factors were important, the effect of pesticide was tested separately for all analyzed population. The analyses were done in R using MASS library (Pekár and Brabec, 2009; Ripley et al., 2021). The effect of pesticide was expressed as LC50 and LC95 doses, i.e., fitted values for 50 and 95% mortality with 95% confidence limits.

The population growth was analyzed using bootstrap ANOVA (Mangiafico, 2015), because the condition of linearity for pesticide concentration was redeemed in data set. The dependent variable was number of mites, the tested variables included pesticide concentration and mite population. The data were visualized as Huber M-estimators and confidence intervals by group using rcompanion package (Mangiafico, 2022), then one/two-way bootstrap ANOVA was applied med1way and post-hot comparison by lincon function using package WRS2 (Mair and Wilcox, 2020).

4 Results

4.1 Filter paper test

The mortality of observed populations of *Acarus siro* was significantly ($P>0.05$) influenced by all tested variables and their interactions. In the next step the effect of pesticide to mite mortality was tested separately for each pesticide (Figures 1 – 4). The concentration of pirimiphos-methyl (Actellic 50 EC) significantly influenced mortality, there were also differences among the population and the interaction between concentration and population was significant. The most tolerant was 6Z population followed by 6Tk and 6Tu populations. The most sensitive was 6L population, when concentration of pirimiphos-methyl was 1.6-fold lower than for 6Z population. The effect of deltamethrin (K-Othrine SC 25) concentration to mite mortality was significant, as well as the effect of population and interaction

population x concentration. The most tolerant was 6Z population followed by the other three populations without remarkable differences. When the piperonyl-butoxide was combined with deltamethrin, the toxicity was higher than for deltamethrin alone, as showed LC₅₀ and LC₉₅ values. Although the effect of population was still significant in the model ($P > 0.05$), there were no remarkable differences in the fitted LC₉₅ values. Chlorpyrifos-methyl (Reldan 22) showed similar trend. Although all tested variables and their interaction had significant effect on mite mortality, no remarkable differences in LC₉₅ among mite populations were found. LC₅₀ and LC₉₅ are shown in Figures 5 and 6.

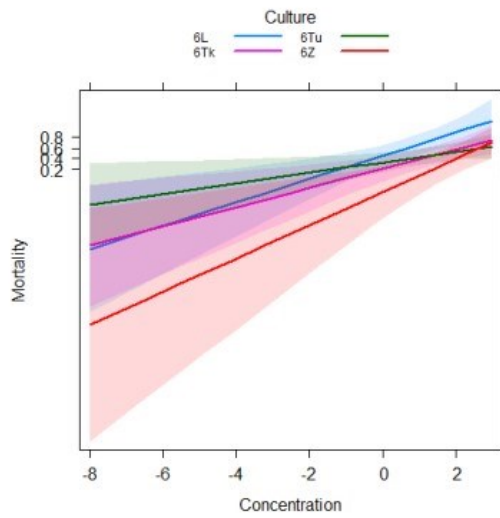


Figure 1: Dependence of mortality on concentration of pitimiphos-methyl.

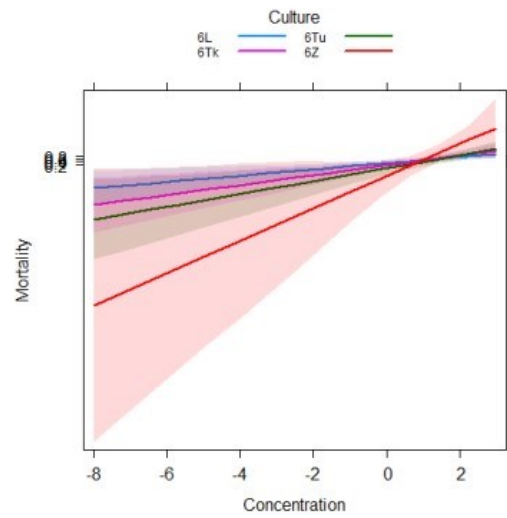


Figure 2: Dependence of mortality on concentration of chlorpyrifos-methyl.

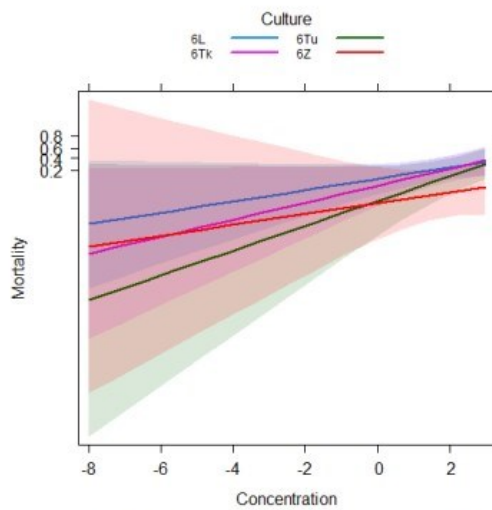


Figure 3: Dependence of mortality on concentration of deltamethrin.

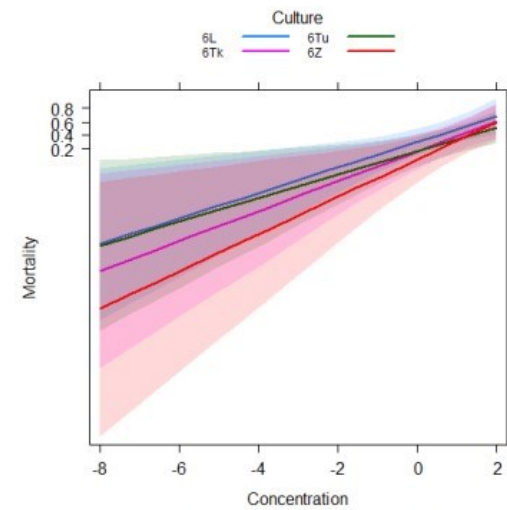


Figure 4: Dependence of mortality on concentration of deltamethrin piperonyl-butoxide.

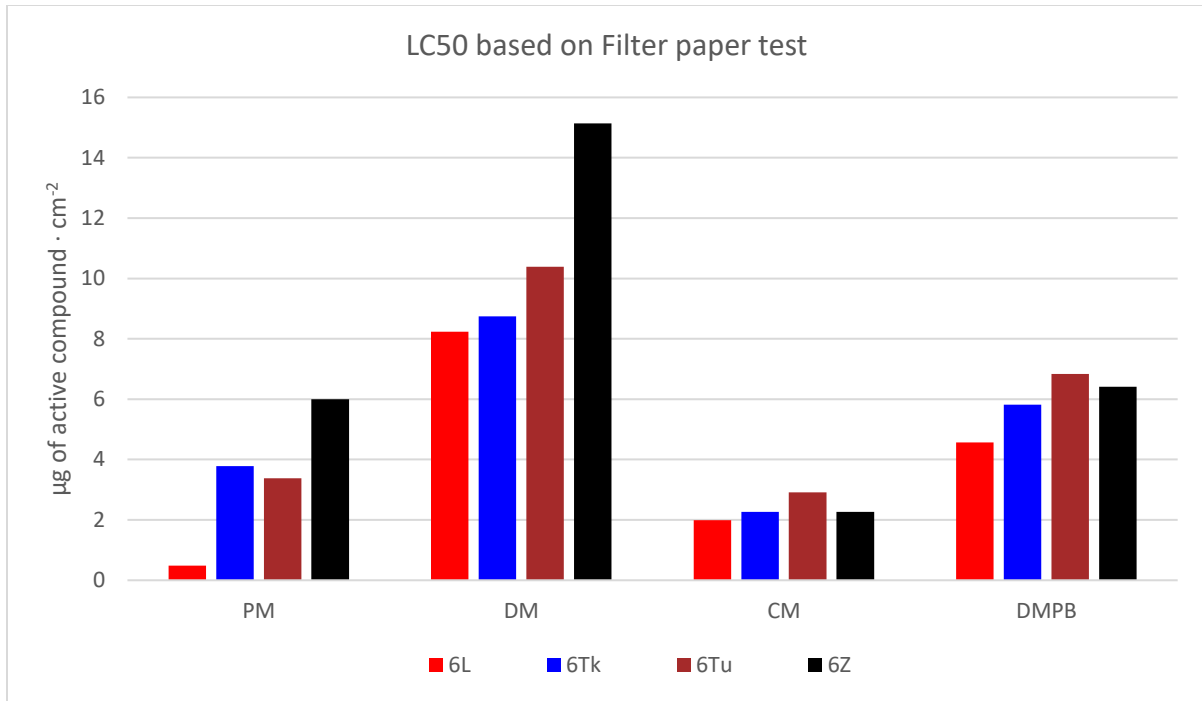


Figure 5: LC_{50} values. PM – pirimiphos-methyl, DM – deltamethrin, CM – chlorpyrifos-methyl, DMPB – deltamethrin + piperonyl butoxide.

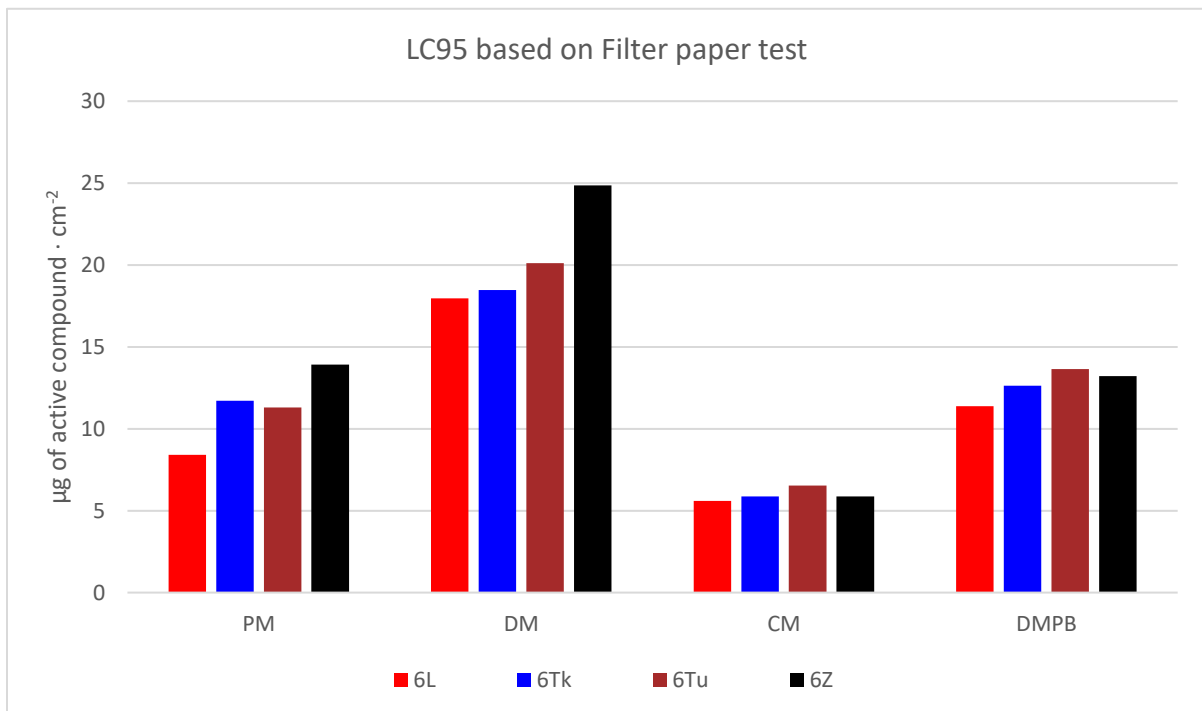


Figure 6: LC_{95} values. PM – pirimiphos-methyl, DM – deltamethrin, CM – chlorpyrifos-methyl, DMPB – deltamethrin + piperonyl butoxide.

4.2 Population growth test

Because pirimiphos-methyl (Actellic) showed the highest differences among populations in the filter paper test, this compound and its dilution was analyzed by population growth test as diet additive. However, the linear decrease of number of mites as the response to pesticide concentration was expected. The obtained response showed hormoligosis effect, when low pesticide concentration accelerates the population growth of mites in comparison to the control.

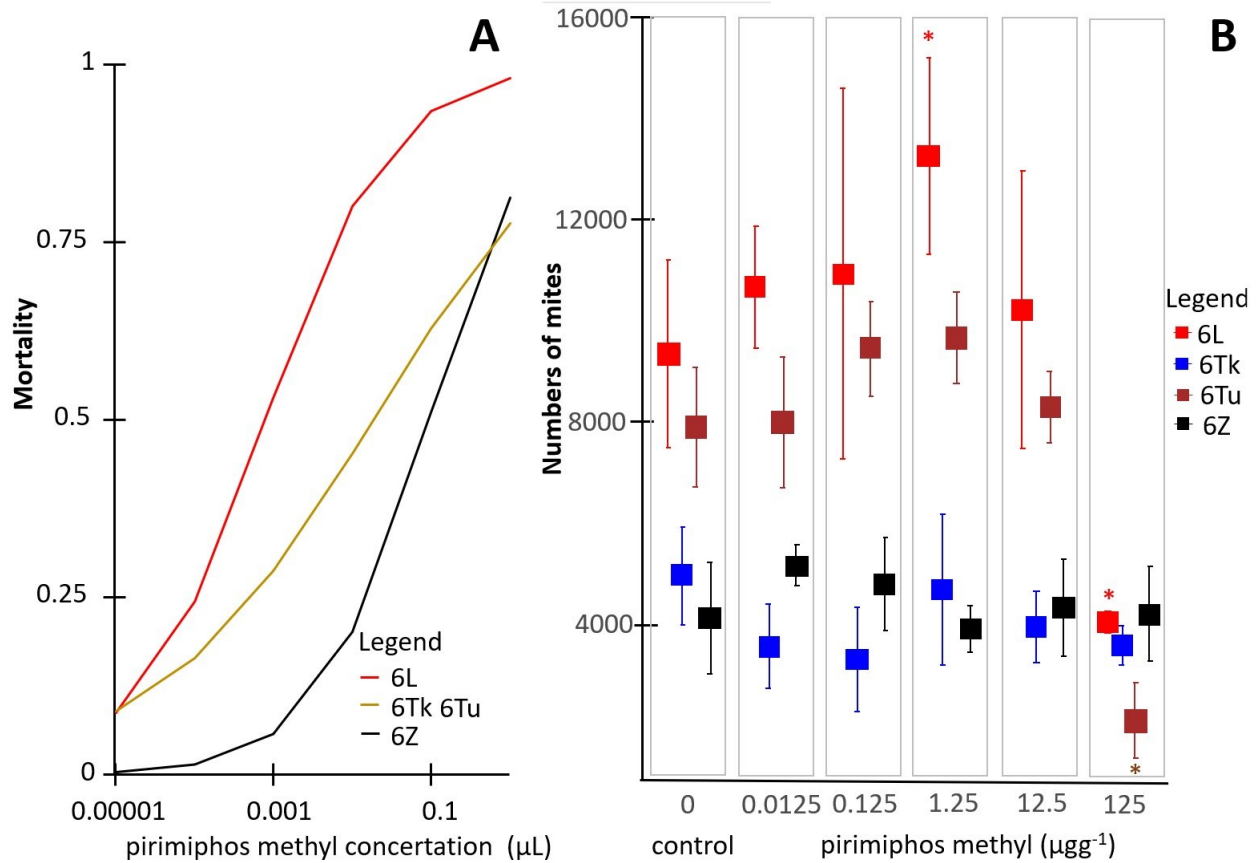


Figure 7: A - mortality of mites in relation to concentration of pirimiphos-methyl; B - growth test results showing final count of mites after 3 weeks on treated diet and control.

In control situation the population growth was dependent on mite population (med1way: $F = 14.637$, $P < 0.05$), the highest growth was for 6L and 6Tk (not different according to lincon function), followed by population 6Z and 6Tu with two-fold lower growth. When tested concentration 1.25 mg/g pirimiphos-methyl diet was compared to control, the effect of concentration was not significant, but the effect of population and interaction was significant (med2way: $F = 3.284$, $P = 0.069$, $F = 32.83$, $P < 0.05$, $F = 19.44$, $P < 0.05$). The populations 6Tu, 6Z and 6Tk were not affected by feeding on pirimiphos-methyl-treated diet, while population growth of 6L was significantly higher on pirimiphos-methyl-treated diet. The growth on pirimiphos-methyl was 1.3-fold higher for 6L than on control

(Figure 7). The next analysis was done for the highest pirimiphos-methyl concentration, i.e., 125 mg/g diet. All factors, i.e., population, pesticide treatment and their interaction significantly influenced population growth (med2way: $F=40.08$, $P < 0.05$, $F=7.53$, $P < 0.05$, $F = 45.684$, $P < 0.05$). However, feeding on pesticide-treated diet did not influence population growth of 6tu and 6Z populations, while significantly (lincon: $P < 0.05$) suppressed population growth on 6L and 6Tu population. The number of mites was two-fold lower for pesticide treated 6L population and 3-fold lower for pesticide treated 6Tu population. Hormoligosis was apparent in 6L and slightly in 6Tu strains, while there was no response to pesticide concentration in 6Z and 6Tk (Figure 7).

4.3 Microbiome description

4.3.1 Characterization of microbial profiles

The microbiome has been characterized with operational taxonomic units (OTUs). The predominant OTU in 6L strain was OTU1, which represents *Solitalea*-like bacterium, despite the different diets it had been treated with. Next, OTU11 (*Kocuria*) has been found in the 6L samples in noteworthy amount along with OTU6, *Staphylococcus* bacterium. The 6L strain also appears to host OTU2, OTU11, OTU15 and OTU55 (*Bacillus*, *Kocuria*, *Moraxella* and another *Solitalea*-like bacterium, respectively). The remaining reads were mostly bacteria marked as REST, which means their identification was either not precise enough because of insufficient identification of the genome or the abundance was scarce. OTU1 was on the other hand basically absent in the 6Z strain, it only appeared to be present in the samples treated with the diet that contained 1,25 $\mu\text{g/g}$ of pirimiphos-methyl. However, the amount of the symbiont was rather small compared to the other taxa and to the amount of it in 6L. The predominant OTU in the 6Z strain in treated samples was OTU2, *Bacillus*, especially in the samples treated with 0.0125 $\mu\text{g/g}$ of pirimiphos-methyl, where it represented about 90 % of all bacterial taxa found in the samples. OTU3, which represents *Sodalis*-like bacterium, was present in these samples as well, but in rather small amount, while the rest of the bacterial profile of these samples treated with mentioned diet contained small amount of OTU55, OTU2 and REST. 6Z samples treated with 1,25 $\mu\text{g/g}$ pirimiphos-methyl diet showed more diverse profile compared to the less “aggressive” diet. OTU2 and OTU3 were present at almost same amount and together these two formed most of the profile. It was also possible to identify OTU1, OTU11, OTU6 (*Staphylococcus*), OTU55, OTU5 (*Lactobacillus*) and OTU17 (*Sphingomonas*) and REST contributed to this particular profile. Interestingly, there was a significant difference between all three 6Z tested samples. As mentioned, OTU2 and OTU3 were vastly present in the treated samples, but they were only a small part of the rich profile of the 6Z control group. 6Z control profile revealed the presence of OTU6, OTU10 (*Klebsiella*), OTU9 (*Escherichia*), OTU8

(*Stenotrophomonas*), OTU11, OTU17, OTU16 (*Micrococcus*), OTU17, OTU19 (*Lactobacillus*), OTU2, OTU3, OTU30 (*Leuconostoc*), OTU14 (*Corynebacterium*) and in the mean about 40 % of all the bacterial diversity was identified as REST, which means that the microbiome of 6Z strain is very diverse, whilst basically lacking *Solitalea*-like symbiont and only hosting *Sodalis*-like symbiont in very small amount. The shift in the microbial composition, when the control is compared to both types of the diet, is more than notable. 6Tu, on the other hand, seemed to rely on OTU1 as it represented almost 60 % of the entire bacterial profile, while the second most abundant bacterium was OTU2. It also showed to host OTU3 and OTU6 in slightly bigger densities, OTU55, OTU9 and OTU5 were also identified but in very small amount, and the profile was completed with other less abundant taxa (REST). The profile did not differ in the three tested groups. 6Tk control group hosted mostly OTU1 accompanied by OTU10, OTU11, OTU8, OTU6, OTU55, OTU12 (unidentified *Actinomycetales*), OTU14, OTU19 and REST. In the samples treated with 1.25 µg/g of pirimiphos-methyl the profile consisted of OTU1, but its amount decreased to a third of the amount present in the control group. About 60 % of reads were represented by OTU3 with additional OTU55, OTU5, small amount of OTU11, OTU6, OTU2 and REST (Figures 8 and 9).

4.3.2 Quantification of OTUs

The number of OTUs discovered in strains differed in control and pirimiphos-methyl-treated diets (Figure 10). For 6Z, the number of OTUs was the highest in control (mean count 143 OTUs), but it dropped once the mites were exposed to treated diet – for 0.0125 µg diet the number lowered dramatically by approximately 100 OTUs, in 1.25 µg/g diet the count was slightly higher (mean count 71 OTUs). In 6Tu, the number of OTUs in control group was the lowest (mean 70) and it grew with the concentration of pirimiphos-methyl in the diet. There was a mild growth from the amount of OTUs in control to 0.0125 µg diet (mean 85) but there was a great upsurge in the second treated diet, meaning the highest mean count of OTUs was found in 1.25 µg diet (mean 163). It was also the highest number of OTUs measured of all samples. 6Tk strain had similar count in control (mean 102) and 0.0125 µg diet (mean 88), though there was a bigger drop in 1.25 µg diet (mean 60). 6L strain showed exactly opposite trend – OTUs found in control were the lowest (mean 80) and the number grew with the concentration of the pesticide in the diet (to mean 137 OTUs) (Figure 10).

4.3.3 Quantification of copies

In comparison to total numbers of OTUs, the levels of copies per mite were also counted (Figure 10). For 6Z, the number of copies were the highest in control but lowered in both pesticide-treated diets to similar levels. In 6Tu, just as in 6Z, the highest number of copies was measured in control, but unlike in 6Z the levels dropped in 1.25 µg diet, and they were the lowest in 0.0125 µg diet. In 6Tk strain there

was a big decline in copies from control to 0.0125 μg diet, on the other hand the number increased when control and 1.25 μg diet were compared, leaving the treated diet with the highest value for this strain. In 6L, the number of copies reduced according to the concentration of the pesticide (Figure 10).

4.3.4 Characterization of microbial diversity

Inverse Simpson index was used to describe diversity in the samples. In the control group, 6Z strain revealed the highest value of all tested samples with the mean result being 0.86 but reaching almost to 1 in several samples, showing a very rich diversity. This also corresponds with the highest number of OTUs found in the respective group. On the other hand, 6L strain showed completely opposite results with the value being about 6 times lower than for 6Z (mean 0.13). 6Tk control group was characterized by somewhat higher diversity than 6L (mean 0.22). The samples treated with 0.0125 μg pirimiphos-methyl diet showed similar diversity as in the control group in 6Tk (mean 0.16) and 6Tu (mean 0.29) strains, there was an increase to above 0.25 on average in 6L (mean 0.32) but there was a massive drop in the values for 6Z, where the results showed values close to 0.10. In 1.25 μg pirimiphos-methyl-treated diet samples, the values grew for all four strains with 6Tk (mean 0.47) exceeding the levels of the other three – 6L and 6Tu values were around 0.41, 6Z had mean 0.35. For 6L, it was on this diet where the highest diversity index was registered of all three tested diets. The same applies to 6Tk and 6Tu strains. 6Tu levels on all three diets were quite similar, whilst the growth of diversity in 6Tk on 1.25 μg diet was notably bigger. The greatest differences, though, were found in 6Z as the index values were considerably different for every diet. In this case, however, the index values corresponded with the amount of OTUs found in the samples – the highest values were in control and the lowest in 0.0125 μg diet. For 6Tk there was a similarity for control and 0.0125 μg diet, but unlike for the number of OTUs found, which decreased in 1.25 μg diet, the diversity index actually grew in the respective samples. 6Tu strain demonstrated growth of both OTUs and the diversity index as the concentration of pirimiphos-methyl in the diet increased, but the upsurge of the diversity index was not as notable as it was for OTUs. 6L values for number of OTUs resembled those for diversity index even more precisely than 6Z values did. 6Tk was thus the only strain whose diversity index did not correspond with the number of OTUs (Figure 10). Heatmaps were also created to visualize abundance (Figure 12). Data obtained from samples showing number of copies per mite, diversity indexes and amount of OTUs found in each sample are listed in Table S2.

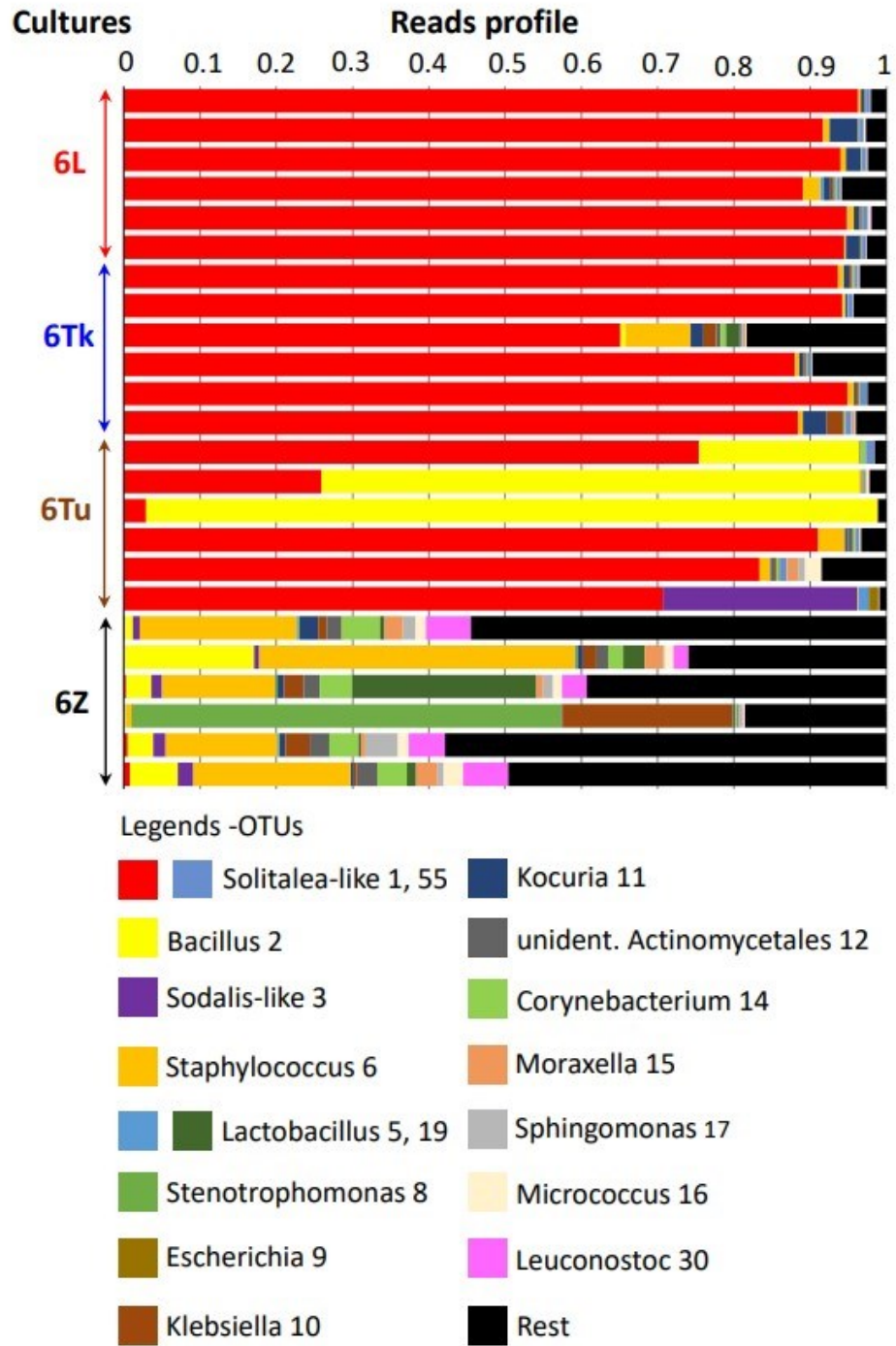


Figure 8: Microbial profiles of *A. siro* strains reared on SPMd showing the percentage of present OTUs.

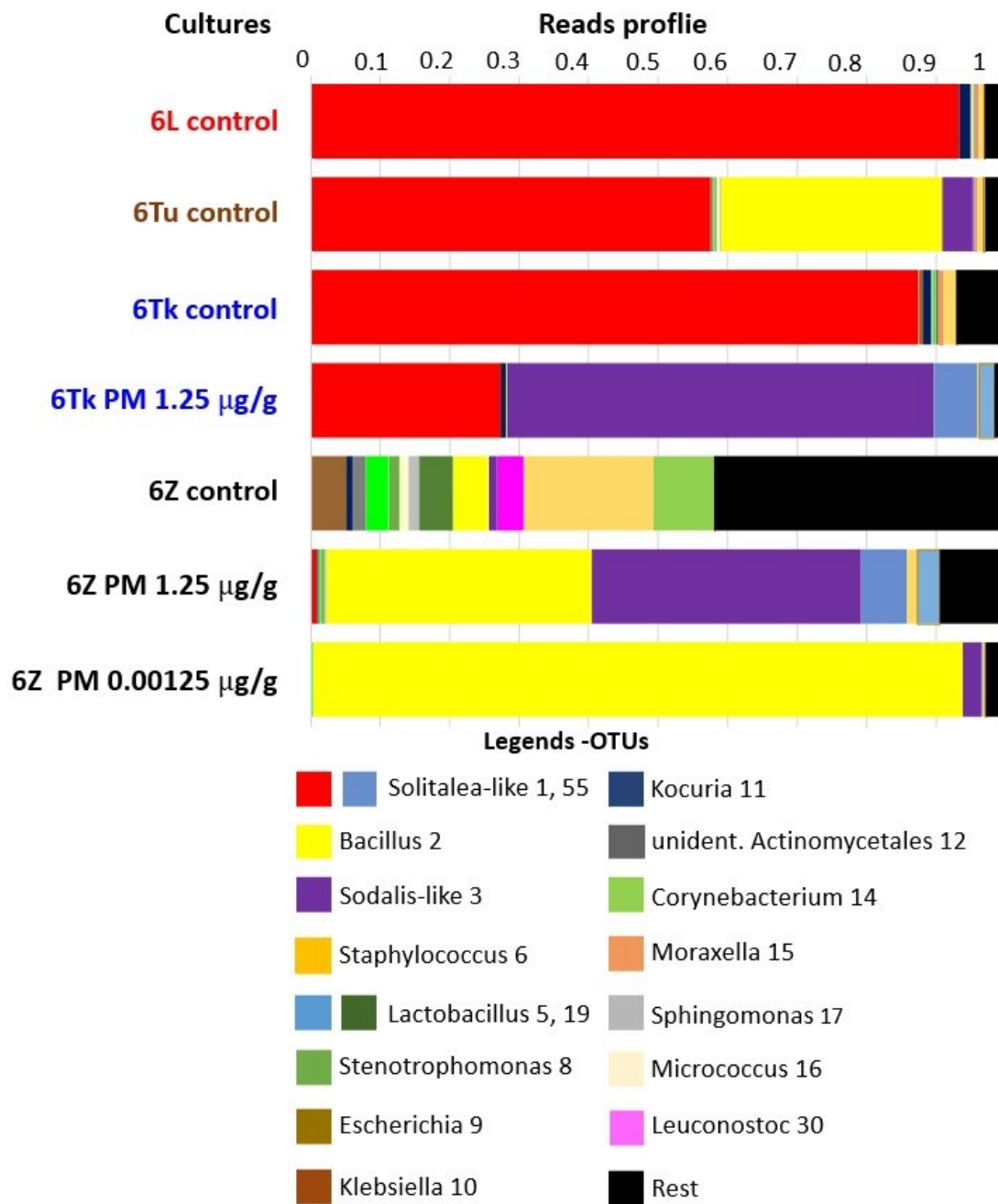


Figure 9: Microbial profiles of *A. siro* strains and respective changes (if there were any) after exposure to pirimiphos-methyl-treated diet.

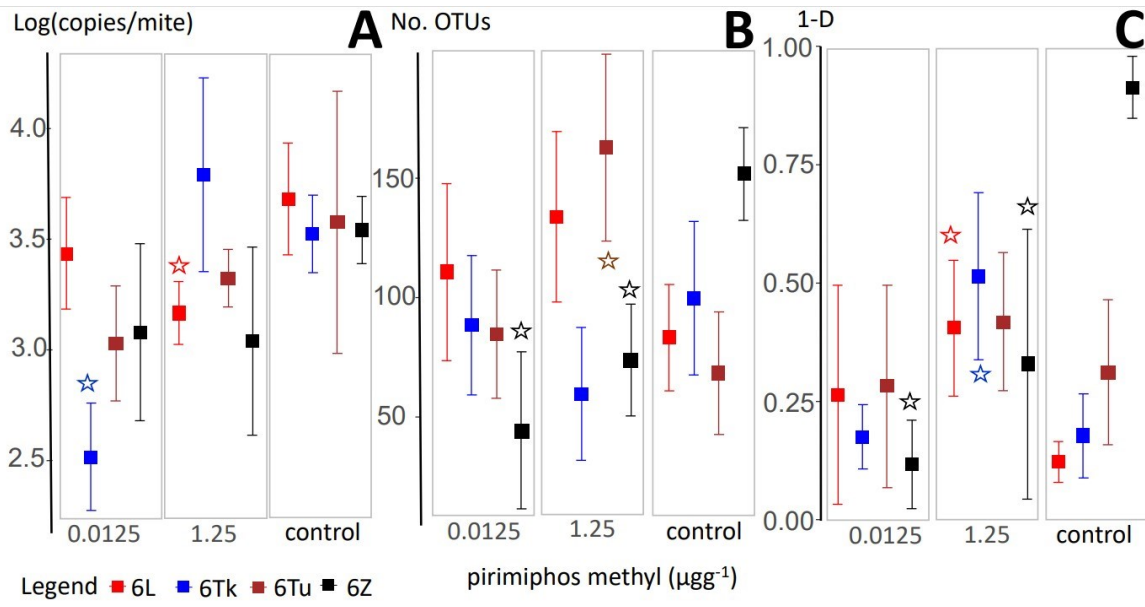


Figure 10: A - number of bacterial copies found in individual mite calculated based on qPCR analysis; B - number of OTUs found in all tested *A. siro* cultures; C - inverse Simpson diversity index of tested samples.

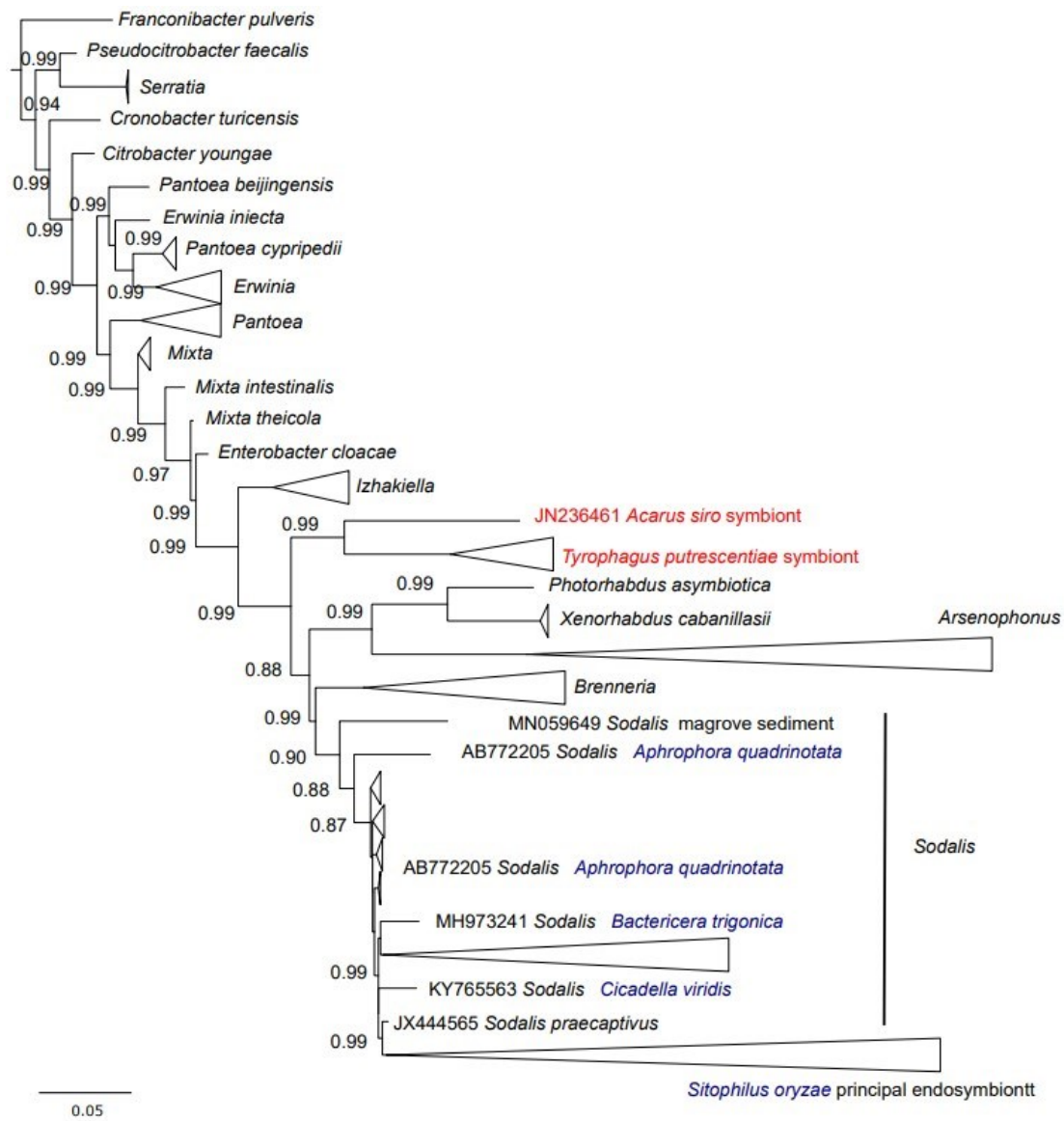


Figure 11: Relative proximity of Solitalea-like bacteria in *A. siro* to symbiotic bacteria found in house dust mite *Tyrophagus putrescentiae* and *Sodalis*-like bacteria found in insects..

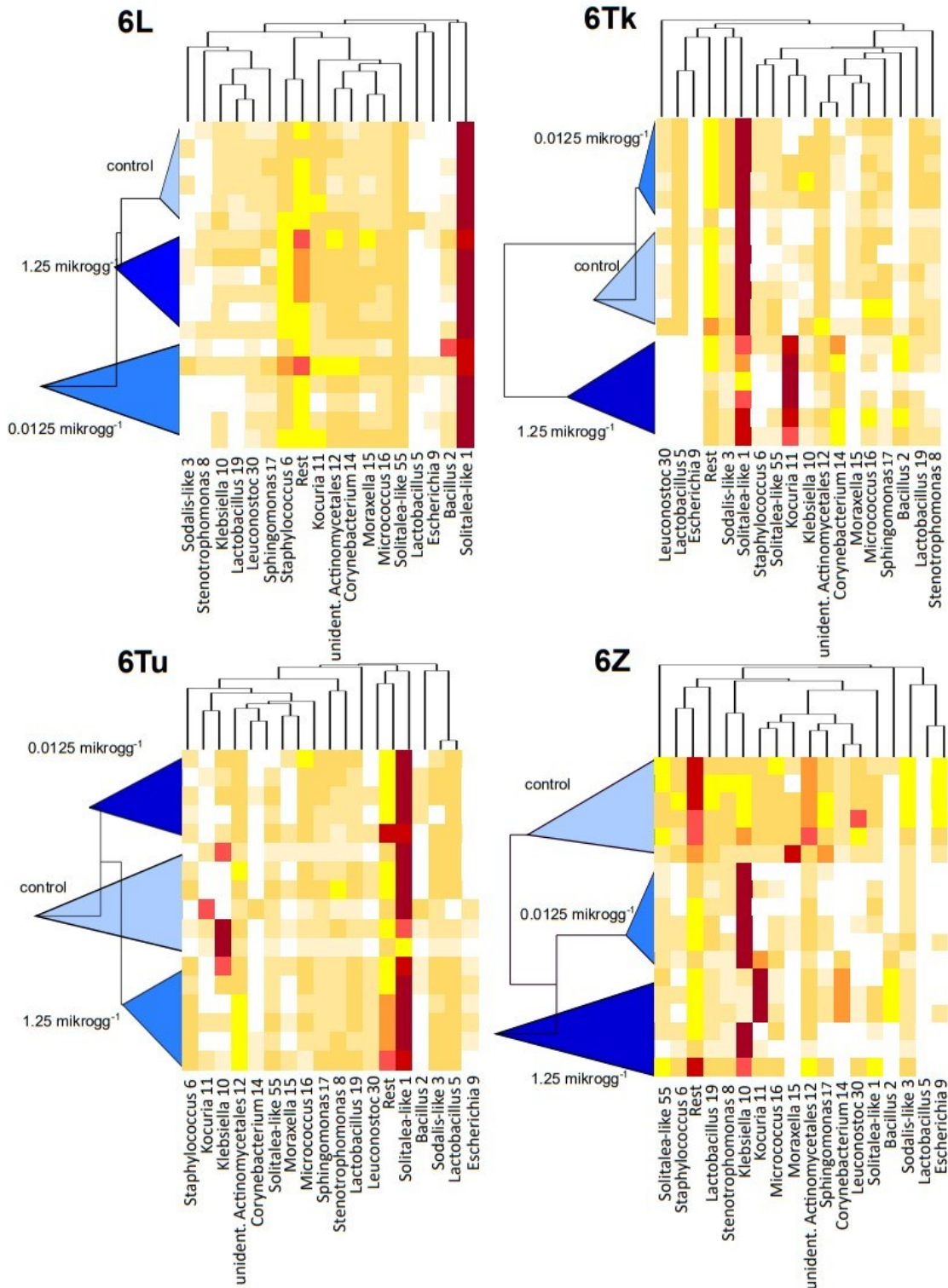


Figure 12: The heatmaps of standardized bacterial relative abundance in samples of all tested *A. siro* cultures in control and pirimiphos-methyl-treated samples. The length of triangles in a heatmap indicates the variability inside the samples from the same residues, the same applies for OTUs clusters. Both clusters were constructed using UPGMA method in Bray-Curtis distance.

5 Discussion

In this thesis, the filter paper test was performed first to determine discriminating dose of pesticides (Thind and Muggleton, 1998) and also to test whether the response to the four selected pesticides would differ. It was found that pirimiphos-methyl showed greatest differences among the mite populations. 6L strain seemed to be the most sensitive while 6Z was the least susceptible. Therefore pirimiphos-methyl was selected for the following growth test. The results of this test revealed that for 6Tu and 6L strains the exposure to the pesticide-treated diet induced hormoligosis, as it was defined by Luckey (1968), as a response. The increase in fecundity was observed several times (e.g., Lowery and Sears, 1986, Zanuncio et al., 2003) who demonstrated that the phenomenon was caused directly by the pesticide because they ruled out the possibility of the sole diet to be responsible. James and Price (2002) confirmed that use of pesticide increases oviposition when the pests are exposed to it by both spraying and ingestion. The results obtained here are in accordance with the finding of Yu et al. (2010), who found that it requires low or mild concentrations of pesticide to induce hormoligosis, while high doses lead to suppressed population growth or inhibition of fecundity. Since it was found that long-term exposure to pesticides can also alter fecundity in subsequent generations, it is worth considering that a field use of such substances might end up in ongoing growth of pest populations (Cutler et al., 2009). 6Z and 6Tk strains showed no signs of pesticide-induced hormoligosis. Based on these findings, the microbiome analysis was performed.

It has been reported several times that microbial communities of arthropods differ among species. Such observations were made in spider mites (Acari: Tetranychidae) when screened for the presence of reproduction-manipulative bacteria (Zhang et al., 2016, Zélé et al., 2018), but there appears to be microbial variability among populations of the same species originating from various geographical areas (e.g., Hubert et al., 2017). Lee et al. (2019) showed differences among populations of *Dermatophagoides farinae*, *D. pteronyssinus* and *T. putrescentiae*. Since the microbiome of storage mites seems to be influenced by the species and mite populations (Hubert et al., 2021), microbial profiles of *A. siro* strains were thus expected to differ. The profiles of 6L and 6Tu strains were dominated by *Solitalea*-like bacteria. That did not change for both despite being exposed to pesticide-treated diet. Approximately 30 % of reads in 6Tu strain belonged to *Bacillus*. When the hormoligosis effect is considered, it may be just the reason why 6L exhibited such an intense response while 6Tu's reproduction increased only mildly. 6Tk strain showed shift from the control profile, where nearly 90 % of reads were represented by *Solitalea*-like bacteria, but once exposed to pesticide-treated diet, *Sodalis*-like bacteria became predominant, forming basically 60 % of the microbiome. Its population

growth remained unaffected by the pesticide, showing that this change in abundance of several taxa probably contribute to the lack of stress response. Proximity of *Solitalea*-like bacteria found in *A. siro* to other *Solitalea*-like symbionts found in *T. putrescentiae* and *Sodalis*-like bacteria found in insects is shown in Figure 11. 6Z control strain showed the richest profile among all, practically lacking *Solitalea*-like and *Sodalis*-like bacteria, but hosting taxa like *Staphylococcus*, *Bacillus*, *Stenotrophomonas*, *Leuconostoc* etc. Once exposed to the treated diet, *Bacillus* became the dominant taxa, forming over 90 % of the profile and less than 5 % of reads belonged to emerging *Sodalis*-like bacteria. Once the concentration of pesticide in the diet increased, *Sodalis*-like bacteria became more prevalent and reduced the amount of *Bacillus* to approximately 37 % of reads. Together, they were responsible for nearly 80 % of all reads, which shows that 6Z strain responds to the diet and adjusts to the conditions induced by the dietary change to maintain its population size. Just like 6Tk, 6Z did not respond with hormoligosis. It is thus likely, that the presence of *Bacillus* and *Sodalis*-like bacteria and the ability to change their abundance ratios enables the mites to tolerate pesticide exposure, with *Sodalis*-like bacteria taking over with the raising concentration of pesticide. The population growth of 6L was strongly influenced once the pesticide was added to rearing diet and also the susceptibility of 6L to pesticide in filter paper test was higher than it was among the other three strains, while 6Z strain with no notable number of *Solitalea*-like bacteria was the least susceptible to pirimiphos-methyl in filter paper test, thus it seems that persistence of *Solitalea*-like bacteria levels limits the mite's ability to deal with the pesticide. Microbial composition thus may be responsible for resistance to pirimiphos-methyl (Thind and Muggleton, 1998). However, deeper research on this topic should be conducted since *Solitalea*-like bacteria numbers in *A. siro* 6L and 6Z populations did not correlate with population growth when treated with antibiotics. In fact, antibiotic treatment failed to remove *Solitalea*-like bacteria (Hubert et al., 2016a). While the diet may play a significant role in obtaining new bacterial symbionts, thus as a driving force in microbial change, in insects (Pietri, Tiffany and Liang, 2018; Kikuchi et al., 2012), it is unlikely that the diet itself induced changes in microbiome in storage mites as the effect of diet on microbial composition in these mites was previously observed as low (Hubert et al., 2016b; Hubert et al., 2020).

Lee et al. (2019) found that strains of *D. farinae* collected from distant sites showed nearly identical microbial pattern after being bred under the same conditions. When the variability among species is considered, it is possible that microbial profiles reflect unique symbiont patterns rather than variability caused by different origin site (Lee et al., 2019). On the other hand, when natural populations of *Tetranychus* mites (*T. urticae*, *T. ludeni* and *T. evansi*) were tested for prevalence and composition of endosymbiotic bacteria (*Wolbachia*, *Cardinium* and *Rickettsia*), it was found that after 6 months of laboratory rearing the populations lost their diversity. *Rickettsia* and *Cardinium* were suppressed or

eliminated, while *Wolbachia* was either fixed or lost. It was therefore suggested that the symbiont microbial diversity might be caused and maintained by environmental heterogeneity which cannot be obtained in laboratory (Zélé et al., 2020). In *A. siro*, *Solitalea*-like bacteria was reported to replace *Cardinium* infection in 6L strain, which happened to occur after long-term cultivation under laboratory conditions (Hubert et al., 2016a). Apart from that, the loss of diversity under laboratory conditions does not seem to be the case for *A. siro* as control profiles showed similar results as Hubert et al. (2021).

Microbial change induced by dietary additive was obtained with antibiotics, specifically when *Dermatophagoides* and *Tyrophagus* strains were treated with medium containing ampicillin in 100:1 ratio. The amount of bacteria in mites was reduced by 25-fold, the proportion of *Bartonella*, otherwise dominant, also decreased and the bacterial endotoxin concentration was reduced by 100-fold. Along with those changes, antibiotic-treated mites had lower ability to cause allergic reaction in human tissues (Lee et al., 2019). Bearing in mind, that antibiotics like rifampicin and tetracycline failed to eliminate *Solitalea*-like bacteria, it is hence obvious, that sufficient concentration of proper active compound should provide efficient treatment to reduce the impact of mite presence on human health.

Since some strains of microbial symbionts confer pesticide resistance for their host, it is important to consider the use and dosage of appropriate chemical compound as one control method might work efficiently for nonsymbiotic populations, while failing to control the symbiotic ones (Haine, 2008). The host may also acquire symbiont genes which may eventually help them to defend themselves from their natural enemies (Haine, 2008). Finding the effective compound may, nonetheless, be more difficult because the microbial profile undergoes changes as the populations ages. These changes usually involve replacement of bacterial and fungal taxa and their respective quantity in mites. In *D. farinae*, young cultures show higher amount of *Cardinium* which is replaced by *Staphylococcus*. Fungal part of internal microbiome was represented by *Saccharomyces cerevisiae* in young populations, which is used as a nutrient source. As the culture grows older, *Cardinium* is replaced by *Staphylococcus* and yeasts are replaced by *Aspergillus* and *Candida*, that are antagonists to *S. cerevisiae*. Relative abundance of *Kocuria* also grows with the age of the culture (Hubert et al., 2019; Klimov et al., 2019).

Thanks to excessive use of some pesticides and their toxicity to the environment, it is hence important to explore the microbial ability to degrade these pesticides and use them for detoxification of water, soil, etc., be it by a single bacterium (e.g., Selvam, Thatheyus and Vidhya, 2013; Gangola et al., 2018; Sharma et al., 2016; Zhang et al., 2019) or by a consortium of bacteria (e.g., Sørensen, Ronen and Aamand, 2002; Chen et al., 2012). *Klebsiella pneumoniae*, for example, was reported to be able to degrade deltamethrin (Tang et al., 2019), which was tested in the filter paper test here, but *Klebsiella* was present in all four strains of *A. siro*. Several bacterial species can degrade the same pesticide, which

widens the spectrum of organisms that can be used (Lin et al., 2020). Moreover, endosymbionts might be used to interfere with virus infections spread by insects (Brownlie and Johnson, 2009), since some symbionts such as *Wolbachia* have shown antiviral effect protecting their host from diverse viruses (Hedges et al., 2008). Microbial metabolic abilities thus provide a simple, effective, environmental-friendly and cheap way of bioremediation of contaminated soils and other environments that are contaminated with pesticides due to their excessive use (Chen and Zhan, 2019; Zhan et al., 2020).

6 Conclusions

It was possible to observe variations in response to pesticide exposure among *A. siro* populations in both filter paper test and the subsequent growth test. Filter paper test revealed inconsistency in response to pirimiphos-methyl among populations. In the growth test, mild concentrations of pirimiphos-methyl in diet caused hormoligosis in 6L and 6Tu strains, while the growth of 6Tk and 6Z strains remained unaffected. Population growth of 6L was higher than of 6Tu. Analyses of microbial profiles showed that adding the pesticide to the diet leads to changes in bacterial taxa present in the microbiome, diversity, relative abundances and absolute numbers of copies in individual mites in comparison to control. For 6L, pesticide-treated diet affected number of OTUs, copies per mite and diversity, but the microbial composition did not change significantly. The most prevalent OTU was *Solitalea*-like bacteria. For 6Tk, there were notable changes in copies per mite, number of OTUs, diversity and there was also a shift in microbial community once the mites were exposed to high concentrations of pirimiphos-methyl. *Solitalea*-like bacteria was replaced with *Sodalis*-like bacteria. For 6Tu, the addition of the pesticide did not induce microbial changes, but it did affect number of OTUs, diversity and number of copies found in individuals. 6Z showed massive change of microbial profile once exposed to the pesticide-treated diet and also between the profiles of both treated groups. Number of OTUs and diversity changed along with the profile, but copies per mite were similar in both treated groups. Pirimiphos-methyl thus affects population dynamics and microbial communities of some *Acarus siro* strains.

7 References

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8 Supplementary materials

Table S1. The list of samples, factors and files deposited at NCBI describing the microbiome of *Acarus siro*

Accession	ID	factor.A	factor.B	file_A	file_B	file_C	file_D
SAMN22566697	6LA2_1	PM 1.25 microg/g	6L	6La_A2_1_R1_001.fastq.gz	6La_A2_1_R2_001.fastq.gz	6Lb_A2_1_R1_001.fastq.gz	6Lb_A2_1_R2_001.fastq.gz
SAMN22566698	6LA2_2	PM 1.25 microg/g	6L	6La_A2_2_R1_001.fastq.gz	6La_A2_2_R2_001.fastq.gz	6Lb_A2_2_R1_001.fastq.gz	6Lb_A2_2_R2_001.fastq.gz
SAMN22566699	6LA2_3	PM 1.25 microg/g	6L	6La_A2_3_R1_001.fastq.gz	6La_A2_3_R2_001.fastq.gz	6Lb_A2_3_R1_001.fastq.gz	6Lb_A2_3_R2_001.fastq.gz
SAMN22566700	6LA2_4	PM 1.25 microg/g	6L	6La_A2_4_R1_001.fastq.gz	6La_A2_4_R2_001.fastq.gz	6Lb_A2_4_R1_001.fastq.gz	6Lb_A2_4_R2_001.fastq.gz
SAMN22566701	6LA2_5	PM 1.25 microg/g	6L	6La_A2_5_R1_001.fastq.gz	6La_A2_5_R2_001.fastq.gz	6Lb_A2_5_R1_001.fastq.gz	6Lb_A2_5_R2_001.fastq.gz
SAMN22566702	6LA2_6	PM 1.25 microg/g	6L	6La_A2_6_R1_001.fastq.gz	6La_A2_6_R2_001.fastq.gz	6Lb_A2_6_R1_001.fastq.gz	6Lb_A2_6_R2_001.fastq.gz
SAMN22566703	6LA4_1	PM 0.0125 microg/g	6L	6La_A4_1_R1_001.fastq.gz	6La_A4_1_R2_001.fastq.gz	6Lb_A4_1_R1_001.fastq.gz	6Lb_A4_1_R2_001.fastq.gz
SAMN22566704	6LA4_2	PM 0.0125 microg/g	6L	6La_A4_2_R1_001.fastq.gz	6La_A4_2_R2_001.fastq.gz	6Lb_A4_2_R1_001.fastq.gz	6Lb_A4_2_R2_001.fastq.gz
SAMN22566705	6LA4_3	PM 0.0125 microg/g	6L	6La_A4_3_R1_001.fastq.gz	6La_A4_3_R2_001.fastq.gz	6Lb_A4_3_R1_001.fastq.gz	6Lb_A4_3_R2_001.fastq.gz
SAMN22566706	6LA4_4	PM 0.0125 microg/g	6L	6La_A4_4_R1_001.fastq.gz	6La_A4_4_R2_001.fastq.gz	6Lb_A4_4_R1_001.fastq.gz	6Lb_A4_4_R2_001.fastq.gz
SAMN22566707	6LA4_5	PM 0.0125 microg/g	6L	6La_A4_5_R1_001.fastq.gz	6La_A4_5_R2_001.fastq.gz	6Lb_A4_5_R1_001.fastq.gz	6Lb_A4_5_R2_001.fastq.gz
SAMN22566708	6LA4_6	PM 0.0125 microg/g	6L	6La_A4_6_R1_001.fastq.gz	6La_A4_6_R2_001.fastq.gz	6Lb_A4_6_R1_001.fastq.gz	6Lb_A4_6_R2_001.fastq.gz
SAMN22566709	6LK_1	control	6L	6La_K_1_R1_001.fastq.gz	6La_K_1_R2_001.fastq.gz	6Lb_K_1_R1_001.fastq.gz	6Lb_K_1_R2_001.fastq.gz
SAMN22566710	6LK_2	control	6L	6La_K_2_R1_001.fastq.gz	6La_K_2_R2_001.fastq.gz	6Lb_K_2_R1_001.fastq.gz	6Lb_K_2_R2_001.fastq.gz
SAMN22566711	6LK_3	control	6L	6La_K_3_R1_001.fastq.gz	6La_K_3_R2_001.fastq.gz	6Lb_K_3_R1_001.fastq.gz	6Lb_K_3_R2_001.fastq.gz
SAMN22566712	6LK_4	control	6L	6La_K_4_R1_001.fastq.gz	6La_K_4_R2_001.fastq.gz	6Lb_K_4_R1_001.fastq.gz	6Lb_K_4_R2_001.fastq.gz
SAMN22566713	6LK_5	control	6L	6La_K_5_R1_001.fastq.gz	6La_K_5_R2_001.fastq.gz	6Lb_K_5_R1_001.fastq.gz	6Lb_K_5_R2_001.fastq.gz
SAMN22566714	6LK_6	control	6L	6La_K_6_R1_001.fastq.gz	6La_K_6_R2_001.fastq.gz	6Lb_K_6_R1_001.fastq.gz	6Lb_K_6_R2_001.fastq.gz
SAMN22566715	6TKA2_1	PM 1.25 microg/g	6TK	6Tk_A2_1_R1_001.fastq.gz	6Tk_A2_1_R2_001.fastq.gz		
SAMN22566716	6TKA2_2	PM 1.25 microg/g	6TK	6Tk_A2_2_R1_001.fastq.gz	6Tk_A2_2_R2_001.fastq.gz		
SAMN22566717	6TKA2_3	PM 1.25 microg/g	6TK	6Tk_A2_3_R1_001.fastq.gz	6Tk_A2_3_R2_001.fastq.gz		
SAMN22566718	6TKA2_4	PM 1.25 microg/g	6TK	6Tk_A2_4_R1_001.fastq.gz	6Tk_A2_4_R2_001.fastq.gz		
SAMN22566719	6TKA2_5	PM 1.25 microg/g	6TK	6Tk_A2_5_R1_001.fastq.gz	6Tk_A2_5_R2_001.fastq.gz		
SAMN22566720	6TKA2_6	PM 1.25 microg/g	6TK	6Tk_A2_6_R1_001.fastq.gz	6Tk_A2_6_R2_001.fastq.gz		
SAMN22566721	6TKA4_1	PM 0.0125 microg/g	6TK	6Tka_A4_1_R1_001.fastq.gz	6Tka_A4_1_R2_001.fastq.gz	6Tkb_A4_1_R1_001.fastq.gz	6Tkb_A4_1_R2_001.fastq.gz
SAMN22566722	6TKA4_2	PM 0.0125 microg/g	6TK	6Tka_A4_2_R1_001.fastq.gz	6Tka_A4_2_R2_001.fastq.gz	6Tkb_A4_2_R1_001.fastq.gz	6Tkb_A4_2_R2_001.fastq.gz
SAMN22566723	6TKA4_3	PM 0.0125 microg/g	6TK	6Tka_A4_3_R1_001.fastq.gz	6Tka_A4_3_R2_001.fastq.gz	6Tkb_A4_3_R1_001.fastq.gz	6Tkb_A4_3_R2_001.fastq.gz
SAMN22566724	6TKA4_4	PM 0.0125 microg/g	6TK	6Tka_A4_4_R1_001.fastq.gz	6Tka_A4_4_R2_001.fastq.gz	6Tkb_A4_4_R1_001.fastq.gz	6Tkb_A4_4_R2_001.fastq.gz
SAMN22566725	6TKA4_5	PM 0.0125 microg/g	6TK	6Tka_A4_5_R1_001.fastq.gz	6Tka_A4_5_R2_001.fastq.gz	6Tkb_A4_5_R1_001.fastq.gz	6Tkb_A4_5_R2_001.fastq.gz
SAMN22566726	6TKA4_6	PM 0.0125 microg/g	6TK	6Tka_A4_6_R1_001.fastq.gz	6Tka_A4_6_R2_001.fastq.gz	6Tkb_A4_6_R1_001.fastq.gz	6Tkb_A4_6_R2_001.fastq.gz
SAMN22566727	6TKK_1	control	6TK	6Tka_K_1_R1_001.fastq.gz	6Tka_K_1_R2_001.fastq.gz	6Tkb_K_1_R1_001.fastq.gz	6Tkb_K_1_R2_001.fastq.gz
SAMN22566728	6TKK_2	control	6TK	6Tka_K_2_R1_001.fastq.gz	6Tka_K_2_R2_001.fastq.gz	6Tkb_K_2_R1_001.fastq.gz	6Tkb_K_2_R2_001.fastq.gz
SAMN22566729	6TKK_3	control	6TK	6Tka_K_3_R1_001.fastq.gz	6Tka_K_3_R2_001.fastq.gz	6Tkb_K_3_R1_001.fastq.gz	6Tkb_K_3_R2_001.fastq.gz
SAMN22566730	6TKK_4	control	6TK	6Tka_K_4_R1_001.fastq.gz	6Tka_K_4_R2_001.fastq.gz	6Tkb_K_4_R1_001.fastq.gz	6Tkb_K_4_R2_001.fastq.gz
SAMN22566731	6TKK_5	control	6TK	6Tka_K_5_R1_001.fastq.gz	6Tka_K_5_R2_001.fastq.gz	6Tkb_K_5_R1_001.fastq.gz	6Tkb_K_5_R2_001.fastq.gz
SAMN22566732	6TKK_6	control	6TK	6Tka_K_6_R1_001.fastq.gz	6Tka_K_6_R2_001.fastq.gz	6Tkb_K_6_R1_001.fastq.gz	6Tkb_K_6_R2_001.fastq.gz
SAMN22566733	6Tua2_1	PM 1.25 microg/g	6Tua	6Tua_A2_1_R1_001.fastq.gz	6Tua_A2_1_R2_001.fastq.gz	6Tub_A2_1_R1_001.fastq.gz	6Tub_A2_1_R2_001.fastq.gz
SAMN22566734	6Tua2_2	PM 1.25 microg/g	6Tua	6Tua_A2_2_R1_001.fastq.gz	6Tua_A2_2_R2_001.fastq.gz	6Tub_A2_2_R1_001.fastq.gz	6Tub_A2_2_R2_001.fastq.gz
SAMN22566735	6Tua2_3	PM 1.25 microg/g	6Tua	6Tua_A2_3_R1_001.fastq.gz	6Tua_A2_3_R2_001.fastq.gz	6Tub_A2_3_R1_001.fastq.gz	6Tub_A2_3_R2_001.fastq.gz
SAMN22566736	6Tua2_4	PM 1.25 microg/g	6Tua	6Tua_A2_4_R1_001.fastq.gz	6Tua_A2_4_R2_001.fastq.gz	6Tub_A2_4_R1_001.fastq.gz	6Tub_A2_4_R2_001.fastq.gz
SAMN22566737	6Tua2_5	PM 1.25 microg/g	6Tua	6Tua_A2_5_R1_001.fastq.gz	6Tua_A2_5_R2_001.fastq.gz	6Tub_A2_5_R1_001.fastq.gz	6Tub_A2_5_R2_001.fastq.gz
SAMN22566738	6Tua2_6	PM 1.25 microg/g	6Tua	6Tua_A2_6_R1_001.fastq.gz	6Tua_A2_6_R2_001.fastq.gz	6Tub_A2_6_R1_001.fastq.gz	6Tub_A2_6_R2_001.fastq.gz
SAMN22566739	6Tua4_2	PM 0.0125 microg/g	6Tua	6Tua_A4_2_R1_001.fastq.gz	6Tua_A4_2_R2_001.fastq.gz	6Tub_A4_2_R1_001.fastq.gz	6Tub_A4_2_R2_001.fastq.gz
SAMN22566740	6Tua4_3	PM 0.0125 microg/g	6Tua	6Tua_A4_3_R1_001.fastq.gz	6Tua_A4_3_R2_001.fastq.gz	6Tub_A4_3_R1_001.fastq.gz	6Tub_A4_3_R2_001.fastq.gz
SAMN22566741	6Tua4_4	PM 0.0125 microg/g	6Tua	6Tua_A4_4_R1_001.fastq.gz	6Tua_A4_4_R2_001.fastq.gz	6Tub_A4_4_R1_001.fastq.gz	6Tub_A4_4_R2_001.fastq.gz
SAMN22566742	6Tua4_5	PM 0.0125 microg/g	6Tua	6Tua_A4_5_R1_001.fastq.gz	6Tua_A4_5_R2_001.fastq.gz	6Tub_A4_5_R1_001.fastq.gz	6Tub_A4_5_R2_001.fastq.gz
SAMN22566743	6Tua4_6	PM 0.0125 microg/g	6Tua	6Tua_A4_6_R1_001.fastq.gz	6Tua_A4_6_R2_001.fastq.gz	6Tub_A4_6_R1_001.fastq.gz	6Tub_A4_6_R2_001.fastq.gz
SAMN22566744	6TuaK_1	control	6Tua	6Tua_K_1_R1_001.fastq.gz	6Tua_K_1_R2_001.fastq.gz		
SAMN22566745	6TuaK_2	control	6Tua	6Tua_K_2_R1_001.fastq.gz	6Tua_K_2_R2_001.fastq.gz	6Tub_K_2_R1_001.fastq.gz	6Tub_K_2_R2_001.fastq.gz
SAMN22566746	6TuaK_3	control	6Tua	6Tua_K_3_R1_001.fastq.gz	6Tua_K_3_R2_001.fastq.gz	6Tub_K_3_R1_001.fastq.gz	6Tub_K_3_R2_001.fastq.gz

SAMN22566747	6TuK_4	control	6Tu	6Tua_K_4_R1_001.fastq.gz	6Tua_K_4_R2_001.fastq.gz	6Tub_K_4_R1_001.fastq.gz	6Tub_K_4_R2_001.fastq.gz
SAMN22566748	6TuK_5	control	6Tu	6Tua_K_5_R1_001.fastq.gz	6Tua_K_5_R2_001.fastq.gz	6Tub_K_5_R1_001.fastq.gz	6Tub_K_5_R2_001.fastq.gz
SAMN22566749	6TuK_7	control	6Tu	6Tua_K_R1_001.fastq.gz	6Tua_K_R2_001.fastq.gz		
SAMN22566750	6ZA2_2	PM 1.25 microg/g	6Z	6Za_A2_2_R1_001.fastq.gz	6Za_A2_2_R2_001.fastq.gz	6Zb_A2_2_R1_001.fastq.gz	6Zb_A2_2_R2_001.fastq.gz
SAMN22566751	6ZA2_5	PM 1.25 microg/g	6Z	6Za_A2_5_R1_001.fastq.gz	6Za_A2_5_R2_001.fastq.gz	6Zb_A2_5_R1_001.fastq.gz	6Zb_A2_5_R2_001.fastq.gz
SAMN22566752	6ZA2_6	PM 1.25 microg/g	6Z	6Za_A2_6_R1_001.fastq.gz	6Za_A2_6_R2_001.fastq.gz	6Zb_A2_6_R1_001.fastq.gz	6Zb_A2_6_R2_001.fastq.gz
SAMN22566753	6ZA2_7	PM 1.25 microg/g	6Z	6Za_A2_7_R1_001.fastq.gz	6Za_A2_7_R2_001.fastq.gz		
SAMN22566754	6ZA2_8	PM 1.25 microg/g	6Z	6Za_A2_8_R1_001.fastq.gz	6Za_A2_8_R2_001.fastq.gz		
SAMN22566755	6ZA2_9	PM 1.25 microg/g	6Z	6Za_A2_9_R1_001.fastq.gz	6Za_A2_9_R2_001.fastq.gz		
SAMN22566756	6ZA4_1	PM 0.0125 microg/g	6Z	6Za_A4_1_R1_001.fastq.gz	6Za_A4_1_R2_001.fastq.gz	6Zb_A4_1_R1_001.fastq.gz	6Zb_A4_1_R2_001.fastq.gz
SAMN22566757	6ZA4_2	PM 0.0125 microg/g	6Z	6Za_A4_2_R1_001.fastq.gz	6Za_A4_2_R2_001.fastq.gz	6Zb_A4_2_R1_001.fastq.gz	6Zb_A4_2_R2_001.fastq.gz
SAMN22566758	6ZA4_4	PM 0.0125 microg/g	6Z	6Za_A4_4_R1_001.fastq.gz	6Za_A4_4_R2_001.fastq.gz	6Zb_A4_4_R1_001.fastq.gz	6Zb_A4_4_R2_001.fastq.gz
SAMN22566759	6ZA4_5	PM 0.0125 microg/g	6Z	6Za_A4_5_R1_001.fastq.gz	6Za_A4_5_R2_001.fastq.gz	6Zb_A4_5_R1_001.fastq.gz	6Zb_A4_5_R2_001.fastq.gz
SAMN22566760	6ZA4_6	PM 0.0125 microg/g	6Z	6Za_A4_6_R1_001.fastq.gz	6Za_A4_6_R2_001.fastq.gz	6Zb_A4_6_R1_001.fastq.gz	6Zb_A4_6_R2_001.fastq.gz
SAMN22566761	6ZA4_7	PM 0.0125 microg/g	6Z	6Za_A4_R1_001.fastq.gz	6Za_A4_R2_001.fastq.gz		
SAMN22566762	6ZK_1	control	6Z	6Za_K_1_R1_001.fastq.gz	6Za_K_1_R2_001.fastq.gz	6Zb_K_1_R1_001.fastq.gz	6Zb_K_1_R2_001.fastq.gz
SAMN22566763	6ZK_2	control	6Z	6Za_K_2_R1_001.fastq.gz	6Za_K_2_R2_001.fastq.gz	6Zb_K_2_R1_001.fastq.gz	6Zb_K_2_R2_001.fastq.gz
SAMN22566764	6ZK_3	control	6Z	6Za_K_3_R1_001.fastq.gz	6Za_K_3_R2_001.fastq.gz	6Zb_K_3_R1_001.fastq.gz	6Zb_K_3_R2_001.fastq.gz
SAMN22566765	6ZK_4	control	6Z	6Za_K_4_R1_001.fastq.gz	6Za_K_4_R2_001.fastq.gz	6Zb_K_4_R1_001.fastq.gz	6Zb_K_4_R2_001.fastq.gz
SAMN22566766	6ZK_5	control	6Z	6Za_K_5_R1_001.fastq.gz	6Za_K_5_R2_001.fastq.gz	6Zb_K_5_R1_001.fastq.gz	6Zb_K_5_R2_001.fastq.gz
SAMN22566767	6ZK_6	control	6Z	6Za_K_6_R1_001.fastq.gz	6Za_K_6_R2_001.fastq.gz	6Zb_K_6_R1_001.fastq.gz	6Zb_K_6_R2_001.fastq.gz

Table S2. The list of samples of *Acarus siro* microbiome, diversity indexes and numbers of reads based on qPCR (universal primers). The reads were recalculated per mite.

Sample_ID	factor.B	factor.A	copies N	copies LogN	Taxa_S	Simpson_1-D	Shannon_H
6LA2_1	6L	PM 1.25 microg/g	12088	4,08	144	0,2973	1,038
6LA2_2	6L	PM 1.25 microg/g	15931	4,20	103	0,2289	0,774
6LA2_3	6L	PM 1.25 microg/g	5345	3,73	104	0,469	1,596
6LA2_4	6L	PM 1.25 microg/g	12558	4,10	152	0,4596	1,583
6LA2_5	6L	PM 1.25 microg/g	12255	4,09	186	0,6481	2,293
6LA2_6	6L	PM 1.25 microg/g	12842	4,11	118	0,3716	1,202
6LA4_1	6L	PM 0.0125 microg/g	20838	4,32	79	0,5093	0,9123
6LA4_2	6L	PM 0.0125 microg/g	21591	4,33	319	0,7636	2,665
6LA4_3	6L	PM 0.0125 microg/g	22642	4,35	116	0,1257	0,4802
6LA4_4	6L	PM 0.0125 microg/g	22680	4,36	127	0,1646	0,5966
6LA4_5	6L	PM 0.0125 microg/g	18469	4,27	71	0,09349	0,3326
6LA4_6	6L	PM 0.0125 microg/g	12537	4,10	113	0,2512	0,8528
6LK_1	6L	control	20715	4,32	76	0,07215	0,2735
6LK_2	6L	control	17375	4,24	101	0,1574	0,5128
6LK_3	6L	control	13753	4,14	83	0,1164	0,414
6LK_4	6L	control	12516	4,10	100	0,2062	0,7236
6LK_5	6L	control	14742	4,17	71	0,1011	0,3787
6LK_6	6L	control	9757	3,99	50	0,1074	0,3672
6TKA2_1	6Tk	PM 1.25 microg/g	32120	4,51	62	0,5366	0,9872
6TKA2_2	6Tk	PM 1.25 microg/g	34890	4,54	69	0,613	1,207
6TKA2_3	6Tk	PM 1.25 microg/g	30484	4,48	88	0,6316	1,278
6TKA2_4	6Tk	PM 1.25 microg/g	20692	4,32	90	0,5564	1,276
6TKA2_5	6Tk	PM 1.25 microg/g	17932	4,25	24	0,1121	0,2532
6TKA2_6	6Tk	PM 1.25 microg/g	10188	4,01	26	0,3467	0,6187
6TKA4_1	6Tk	PM 0.0125 microg/g	6722	3,83	70	0,184	0,6525

6TKA4_2	6Tk	PM 0.0125 microg/g	21297	4,33	96	0,1331	0,4982
6TKA4_3	6Tk	PM 0.0125 microg/g	18466	4,27	47	0,05556	0,2095
6TKA4_4	6Tk	PM 0.0125 microg/g	16947	4,23	125	0,2335	0,8361
6TKA4_5	6Tk	PM 0.0125 microg/g	13754	4,14	98	0,1556	0,5702
6TKA4_6	6Tk	PM 0.0125 microg/g	5679	3,75	89	0,2243	0,7667
6TKK_1	6Tk	control	13976	4,15	93	0,1211	0,4675
6TKK_2	6Tk	control	13955	4,14	62	0,1119	0,4103
6TKK_3	6Tk	control	10590	4,02	168	0,5663	1,925
6TKK_4	6Tk	control	15858	4,20	113	0,224	0,7478
6TKK_5	6Tk	control	31840	4,50	101	0,09762	0,3705
6TKK_6	6Tk	control	12141	4,08	73	0,2163	0,7005
6TuA2_1	6Tu	PM 1.25 microg/g	25049	4,40	213	0,566	2,105
6TuA2_2	6Tu	PM 1.25 microg/g	17560	4,24	137	0,1948	0,7162
6TuA2_3	6Tu	PM 1.25 microg/g	23542	4,37	142	0,3183	1,136
6TuA2_4	6Tu	PM 1.25 microg/g	29065	4,46	184	0,5973	1,531
6TuA2_5	6Tu	PM 1.25 microg/g	16645	4,22	122	0,3689	1,266
6TuA2_6	6Tu	PM 1.25 microg/g	29109	4,46	181	0,4287	1,482
6TuA4_2	6Tu	PM 0.0125 microg/g	3748	3,57	73	0,3719	1,175
6TuA4_3	6Tu	PM 0.0125 microg/g	9421	3,97	87	0,2144	0,7499
6TuA4_4	6Tu	PM 0.0125 microg/g	9372	3,97	61	0,1061	0,401
6TuA4_5	6Tu	PM 0.0125 microg/g	14552	4,16	90	0,1931	0,6907
6TuA4_6	6Tu	PM 0.0125 microg/g	25314	4,40	115	0,587	1,299
6TuK_1	6Tu	control	10897	4,04	47	0,3868	0,7495
6TuK_2	6Tu	control	32572	4,51	123	0,4359	0,8487
6TuK_3	6Tu	control	24983	4,40	43	0,07861	0,2191
6TuK_4	6Tu	control	6281	3,80	79	0,1689	0,5667
6TuK_5	6Tu	control	9432	3,97	75	0,3032	1,021
6TuK_7	6Tu	control	30106	4,48	54	0,4349	0,8013
6ZA2_2	6Z	PM 1.25 microg/g	1451	3,16	80	0,8798	3,17
6ZA2_5	6Z	PM 1.25 microg/g	18702	4,27	60	0,05734	0,2228
6ZA2_6	6Z	PM 1.25 microg/g	16938	4,23	30	0,02237	0,08121
6ZA2_7	6Z	PM 1.25 microg/g	21264	4,33	68	0,4027	0,8887
6ZA2_8	6Z	PM 1.25 microg/g	14664	4,17	89	0,3586	0,8893
6ZA2_9	6Z	PM 1.25 microg/g	18030	4,26	101	0,4184	0,9568
6ZA4_1	6Z	PM 0.0125 microg/g	20285	4,31	36	0,02882	0,1076
6ZA4_2	6Z	PM 0.0125 microg/g	21204	4,33	39	0,02377	0,08945
6ZA4_4	6Z	PM 0.0125 microg/g	1378	3,14	19	0,09112	0,3033
6ZA4_5	6Z	PM 0.0125 microg/g	12791	4,11	96	0,1157	0,461
6ZA4_6	6Z	PM 0.0125 microg/g	7080	3,85	87	0,1587	0,6067
6ZA4_7	6Z	PM 0.0125 microg/g	26262	4,42	37	0,3081	0,6096
6ZK_1	6Z	control	4783	3,68	155	0,9447	3,996
6ZK_2	6Z	control	8334	3,92	142	0,7953	2,76
6ZK_3	6Z	control	5244	3,72	167	0,9112	3,562
6ZK_4	6Z	control	28798	4,46	152	0,6263	1,618
6ZK_5	6Z	control	3780	3,58	137	0,9611	3,943
6ZK_6	6Z	control	1936	3,29	107	0,9403	3,727