

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF ECOLOGY



Bachelor thesis

The impact of neonicotinoids on predation capacity in spiders

Author: Gleb Tugushev

2018

The Bachelor Thesis Supervisor: doc. RNDr. Pavel Saska, Ph.D.

Advisor of thesis: RNDr. Milan Řezáč, Ph.D

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

BACHELOR THESIS ASSIGNMENT

Gleb Tugushev

Applied Ecology

Thesis title

The impact of neonicotinoids on predation capacity in spiders

Objectives of thesis

The majority of studies dealing with the impact of pesticides to spiders focuses on lethal effects. Only little is known about the impact of pesticides to spider behaviour. Considering the function of spiders in regulation of crop pests, the impact of pesticides on spiders' predatory abilities is especially important. Predatory capacity can be expressed as the functional response – the relationship between the density of prey and consumption rate of the predator.

Hypothesis: Particular pesticides differ in their impact on the functional response of spiders.

Methodology

Methods: 160 individuals of wolf spiders of the genus *Pardosa* will be equally divided to eight groups. The individuals will be closed to the paper tubes with residuals of several types of neonicotinoids (and pure water control) for one hour. Then the spiders will be placed to Petri dishes with four different densities of *Drosophila* flies. The results of the experiment will be numbers of killed and consumed prey, in five replicates for each density and pesticide.

Literature review: Sublethal effects of neonicotinoids on arthropods.

The proposed extent of the thesis

dle potřeby

Keywords

neonicotinoids, predation capacity, spiders, sublethal effects, predation

Recommended information sources

Decourtye A., 2007, The sublethal effects of pesticides on beneficial arthropods. *Ann. Rev. Entomol.*52: 81-106.

Pekár S., 2013, Side effects of synthetic pesticides on spiders. in *Spider Ecophysiology*.

Expected date of thesis defence

2017/18 SS – FES

The Bachelor Thesis Supervisor

doc. RNDr. Pavel Saska, Ph.D.

Supervising department

Department of Ecology

Advisor of thesis

RNDr. Milan Řezáč, Ph.D.

Electronic approval: 20. 3. 2017

Ing. Jiří Vojar, Ph.D.

Head of department

Electronic approval: 22. 3. 2017

prof. RNDr. Vladimír Bejček, CSc.

Dean

Prague on 22. 03. 2018

Declaration

I hereby declare that I am the sole author of the thesis entitled:” The impact of neonicotinoids on predation capacity in spiders “, which I wrote under the direction of RNDr. Milan Řezáč, PhD and doc. RNDr. Pavel Saska, PhD. The used literature and sources are stated in the attached list of references.

In Prague on 24.04.2018

Gleb Tugushev

Acknowledgements

I would like to give my thanks and appreciation to RNDr. Milan Řezáč PhD and doc. RNDr. Pavel Saska, PhD. for their support and recommendations regarding my thesis.

In Prague on 24.04.2018

Gleb Tugushev

Abstract

The use of pesticides in agriculture can have a negative impact on the stability of ecological systems. Neonicotinoids are commonly used insecticides, but their impact on the behavior of spiders, which play an important role as predators in agroecosystems, has not been studied enough. The first part of the thesis, literature review, describes the sublethal effects of neonicotinoids on arthropods and shows that pesticides of the neonicotinoids family differently affect behavior, development and internal processes in arthropods. The second part contains a description and results of my own experiment. The spiders *Philodromus cespitum* (Walckenaer, 1802) were divided into groups treated with several types of neonicotinoids. The topical and tarsal contacts were tested. Then the spiders were placed to Petri dishes with three different concentrations of *Drosophila melanogaster* (Meigen, 1830). Killed flies were replaced by living ones. In the topical treatment experiment both factors (treatment and prey density) and their interactions were statistically significant, but effects were very different for each neonicotinoid. Only treatment factor was significant in the tarsal contact experiment.

Keywords: neonicotinoids, predation ability, spiders, sublethal effect

Table of Contents

1. Introduction	8
2. Aims	9
3. Literature review	10
3.1. Metabolism	10
3.2. Behavior	10
3.3. Enzymatic activity	11
3.4. Water loss	12
3.5. Movement.....	12
3.6. Learning.....	14
3.7. Feeding	15
3.8. Development	15
3.9. Predation.....	16
3.10. Sex ratio	17
3.11. Reproduction.....	17
3.12. Defense	18
3.13. Thermoregulation.....	18
3.14. Immunocompetence	19
4. Materials and methods	20
4.1. Spiders	20
4.2. Pesticides	21
4.3. Design of experiments	22
4.4. Data analysis.....	22
5. Results	23
5.1. Mortality	23
5.2. Predation capacity	25
5.2.1. Analysis of the topical application experiment results	25
5.2.2. Analysis of the tarsal application experiment results.....	27
6. Discussion	29
7. Conclusion	31
9. Appendices	37

1. Introduction

Pesticides are chemicals used in agriculture, gardening and forestry to destroy pests. Insecticides as a type of pesticides are used against insects, causing damages to crop, animals and transmitters of different diseases. The main part of manufactured pesticides is used in plant protection (Komínková 2008).

Neonicotinoids are a relatively new class of insecticides which are the most widely used insecticides in the world. Those plant-protecting neuro-active chemicals may have negative physiological and behavioral effects on non-target arthropods such as bees, spiders, and beetles, which play an important role as plant pollinators or natural enemies of pests in greenhouses and in the fields (Pisa 2014).

Neonicotinoids are synthetic insecticides based on nicotine. Their chemical structure belongs to the class of nitromethylene-heterocyclic compounds. The main effect of neonicotinoids - disrupting the transmission of impulses in the nervous system of insects. Neonicotinoids have a selective effect. They are highly toxic to insects and moderately toxic to warm-blooded animals. The active ingredients very quickly metabolize through oxidation by monooxygenases. Several researches have shown that different types of insecticides may have a large number of sublethal effects (Fishel 2015).

The neonicotinoids family includes acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine and thiacloprid.

All the neonicotinoids are promptly moved through plant tissues after applications and can be detected at certain concentrations in tissues such as leaves, vascular fluids, and pollen. Some non-target beneficial arthropods such as honeybees, parasitic wasps, and predaceous ground beetles can be sensitive to imidacloprid. These organisms may be negatively affected by sublethal doses of the insecticide, but the effects vary widely depending on application method and route of intake. Several imidacloprid metabolites have been shown to be equal or greater in toxicity than the parent compound (Fossen 2006).

Sublethal effects of insecticide's treatment can be studied in a laboratory in various ways. These effects are usually differentiated into three groups: biochemical and physiological, behavioral and histological. Assays of biochemical and physiological effects include the monitoring of protein level (changes in enzyme activities and their inhibition, production of stress proteins), respiration, indicators of clinical chemistry and hematological parameters (Komínková 2008).

Studies revealed that seed-applied neonicotinoids reduced the abundance of arthropod natural enemies similarly to broadcast applications of pyrethroid insecticides. These findings suggest that substituting pyrethroids for seed-applied neonicotinoids will have little net effect on natural enemy abundance. However, seed-applied neonicotinoids are less toxic to spiders and mites, which can contribute substantially to biological control in many agricultural systems than pyrethroids (Douglas & Tooker 2016).

Tests, following the changes in behavior, are based on the premise that the behavior of the test organism is an integrated response corresponding to the complex of biochemical and physiological functions. Chemically induced changes in behavior can be caused by changes in the inner homeostasis of the organism. Changes in behavior can be sensitive indicators of sublethal concentrations. Ecologically significant and often used is the relationship of predator-prey, aggression, territoriality and learning process (Komínková 2008).

2. Aims

The aim of this bachelor thesis is to prepare a brief literature review about the sublethal effects of neonicotinoid pesticides on arthropods. Another part of the thesis are experiments where I test the impact of four different neonicotinoids on the predation capacity in spiders *Philodromus cespitum* (Walckenaer, 1802). In the process of experiments, I analyzed how insecticides affected the amount of flies *Drosophila melanogaster* (Meigen, 1830) killed by the treated spiders at three prey densities (1, 3 and 5 flies). Additionally, I monitored mortality. The main supposition is that there is a negative impact of neonicotinoids on predation ability of tested spiders.

3. Literature review

3.1. Metabolism

Research of Suchail et al. (2004) have shown that Neonicotinoids can be metabolized relatively quickly. They prepared imidacloprid in dimethyl sulfoxide and gave this solute to groups of 20 bees in different concentrations. After total ingestion of $50\mu\text{g imidacloprid kg}^{-1}$ bee, the sum of imidacloprid, 5-hydroxyimidacloprid and olefin residues represented 67% of the actual dose given. Imidacloprid, 5hydroxyimidacloprid and olefin represented respectively, 50%, 9% and 8% of the actual dose. After 6h, only 10.5% of imidacloprid were detected in honeybees. After 24 hours, imidacloprid could not be detected. Imidacloprid had an elimination half-life of 4.5 hours and was quickly metabolized into 5-hydroxyimidacloprid and olefin. These two metabolites reached a peak value 4h after oral intoxication. Olefin and 5-hydroxyimidacloprid represented respectively, 9.5% and 13% of the initial dose 4 hours after intoxication. For both metabolites, the number of residues started to decrease 4 hours after exposure and represented approximately 5% of the ingested dose 48h after exposure. Later the individuals were exposed to a dose of $20\mu\text{g}$ of imidacloprid per 1 kg of bees. The kinetics of metabolization of imidacloprid were similar. Twenty minutes after total ingestion, imidacloprid represented 55% of the actual dose and after 6h only 3% of residues were detected. The imidacloprid half-life was 5 hours after intoxication with a dose of $20\mu\text{g kg}^{-1}$ bee. Olefin metabolite presented a peak value 4 hour after ingestion time at which time it represented 14% of the actual ingested dose. 5-Hydroxyimidacloprid had a different kinetic appearance. It appeared immediately during or after, the ingestion period.

3.2. Behavior

The study of Suchail et al. (2004) has shown that after oral application of imidacloprid on honeybees' early symptoms of poisoning included hyperactivity and tremors. The worker bees became hypoactive in several hours. The retention time of imidacloprid was 9min.

Research of Uhl et al. (2015) investigated behavior of crickets affected by imidacloprid. Distance covered, time spent mobile and highly mobile, time spent

moving served were observed and only nonsignificant changes in cricket's behavior were found. In response to spider presence crickets affected by imidacloprid were more mobile and spent more time moving than the control crickets. However, crickets from the control group without any spiders were more active compared to the control group.

In the spider behavior experiment conducted by the same group of researchers, effects of the pesticide application were also nonsignificant. Spiders covered more distance in the presence of high imidacloprid rate crickets than in the control and spent more time active. Spiders confronted with high imidacloprid rate crickets displayed a tendency to move more than spiders confronted with control crickets.

3.3. Enzymatic activity

As an example of neonicotinoid influence on the enzymatic activity may serve their impact on spiders. The penetration of pesticides into the body can have a negative effect of the central nervous system and lead to disturbances in the functioning of all systems. To survive spiders can produce detoxification enzymes or overproduce acetylcholinesterase, which is an enzyme responsible for the degradation of the neurotransmitter acetylcholine (Pekár 2013). This acetylcholinesterase-based defense system is in arthropods universal (Lee et al., 2015). Acetylcholinesterase terminates synaptic transmission in the cholinergic nervous system of most animals, including arthropods, by hydrolyzing the neurotransmitter acetylcholine at cholinergic synapses and neuromuscular junctions (Toutant et al., 1988). The research of Nielsen et al. (1999) has shown that enzymes, protecting from unpredictable exposure to toxins, remain in the organism for about a year.

In a bee organism pesticide may cause various perturbations, in particular, inhibition of sodium-potassium adenosine triphosphatase (Na^+/K^+ ATPase) which may cause dysfunctions in myocardial cells. Na^+/K^+ ATPase is a transmembrane enzyme that releases energy for cell metabolism and establishes the ionic concentration balance (Desneux 2007).

How pesticides influence an organism depends on individual differences in age, sex, health and nutritional status, and others. For most species, the young and old individuals are more susceptible to harmful substances (Komínková 2008).

3.4. Water loss

Water loss includes passive, evaporative water loss and pesticide induced water excretion. Poisoning of arthropods by insecticides involves a sequence of processes such as the uptake of a pesticide from a substrate, penetration, and distribution of a pesticide in the body tissues, target specific sensitivity, metabolism and excretion of a compound. Caused by a pesticide, this process depends on pesticide dose and temperature and diuretic hormone. Penetration into the hemolymph of neurotoxic pesticides leads to secretion of diuretic hormone which subsequently leads to the loss of water. Additionally, water is lost due to basal metabolism and evaporation, the latter depending on the vapor pressure deficit of the air and the permeability of the cuticle. The results of experiments correspond that the level of water loss depends on temperature and relative humidity (Jagers Op Akkerhuis et al. 1997).

3.5. Movement

One hour after treatment by acetamiprid locomotor activity and the total length walked in the openfield-like apparatus were increased in the experiment of El Hassani et al. (2008). For better understanding of the origin of the differences, each treatment was compared with the others, using Tukey or Scheffe pairwise range post hoc tests. A significant difference was revealed between control and treated bees (0.1 and 0.5 nl/bee), topical acetamiprid application inducing an increase in the distance. Similarly, a significant decrease in the duration of immobility of bees was observed 1 hour after topical, but not oral treatment. Once again, animals treated with the 0.1 and 0.5 nl/bee doses but not with the 1.0 nl/bee dose differed from the control group. The number of activities, such as flying or climbing from one level to a higher one in the apparatus, was not affected by acetamiprid.

However, after oral or topical delivery of thiamethoxan, the locomotor activity of animals did not significantly differ compared to that of control bees. The way pesticides were applied (orally or topically) had a certain effect on animals behavior.

There was a significant difference between orally treated and topically treated bees, whatever the acetamiprid or the thiamethoxan dose. Topically treated specimens moved less in the box and consequently they covered a shorter distance than orally treated animals.

Effects of neurotoxic insecticide deltamethrin on *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) has been studied by Longley & Jepson (1996). These researches have found that increasing concentrations of the pesticide caused early departure of *A. rhopalosiphi* from honeydew-treated areas.

Pollinators visually use landmarks for navigation and communication to their nest mates. Exposure of pesticides can cause disorientation. For example, a bee, which incorporated a pesticide during the trip may incorrectly acquire or integrate visual pattering (Desneux et al. 2007).

Walking activity is the most common behavior to detect sublethal effects. Higher concentrations can reduce movement, while lower concentrations induce movement. At higher doses or concentrations, movement is usually reduced, while at relatively lower doses or concentrations, it is induced (Pekár 2013).

Impact of pesticides on homing flight is still insufficiently studied because of difficulty of monitoring and measuring direction of flight and the route time between the food source and the hive (Desneux et al. 2007)

The study of (Garcia et al. 2002) has shown that the application of neurotoxic insecticides may lead various consequences such as ataxia, paralysis, reduction of levels of movement which lasted from a few hours to a few days.

Spider behavior is disrupted shortly after treatment with deltamethrin on spiders of *Linyphiidae* family. In analogy to effects in other nerve cells, deltamethrin causes abnormal signaling intensity of humidity sensors in the cuticle, especially at low relative air humidity. The distortion of peripheral information about humidity will affect the coordination of movement, resulting in immobilization and inability to drink (Jagers Op Akkerhuis 1997).

3.6. Learning

In their research, Piironen et al. (2016) test whether bees remembered the learned association. A memory retention test was conducted 2 hours after each proboscis extension reflex assay, where again only the conditioned stimulus was presented, in the absence of the unconditioned stimulus. Ten minutes before and after each proboscis extension reflex assay and after each memory retention test, bees were tested for motivation to respond to sugar stimulus by touching the antennae with a toothpick covered with 60% sugar solution and observing whether extension of proboscis occurred. Bees that showed a negative response to the sugar solution were excluded from the analyses. Also, bees that showed 4 or more sequential negative responses during proboscis extension reflex assays were considered unmotivated and were excluded from analyses. After testing memory retention, bees were released from the harness, returned to their microcolony and their survival was observed.

During the experiment 68% of bees showed a positive extension response in the final test, where bees were presented with the conditioned stimulus-only. There was no difference among treatment groups in the final level of acquisition. In conclusion, 34% (n = 85) of the tested bees remembered the association between the odor and sugar reward when tested 2 hours after proboscis extension reflex conditioning. The treatment groups showed no significant difference in memory retention.

Clothianidin effects on learning of Winter bees were studied by Alkassab & Kirchner (2016). These insects chronically consume sublethal doses of clothianidin, but non-significant impacts on olfactory learning performance were observed in the current study. The sublethal effects of clothianidin on studied behavioral functions including sensory and cognitive functions indicate limited effects on winter bees. Their results showed that daily repeated oral exposure to clothianidin at 10 and 15 ml/kg induced only slight reductions of sucrose responsiveness. Thiamethoxam at concentrations 0.1, 0.5 and 1 ng/bee had no significant effect on the sucrose responsiveness in worker honeybees. Furthermore, a partial decrease of sucrose responsiveness was reported after repeated oral exposure with thiamethoxam at the dose of 1 ng/bee.

Aliouane et al. (2009) showed that the different exposure routes had different subsequent effects. They did not observe a significant effect of thiamethoxam after chronic oral administration at 0.1 and 1 ng/bee on learning and memory performance. Otherwise, they reported that chronic topical application of thiamethoxam at 0.1 ng/bee induced adverse effects on memory performance. 24 h after learning, whereas a significant impairment of learning performance with no effect on memory was observed at 1 ng/bee.

Han et al. (2010) conducted a study investigating the effects of imidacloprid on honeybees. The result of their experiments showed that the number of imidacloprid-exposed bees that successfully navigated through the single T-tube was significantly lower than the number of bees from the control group. Imidacloprid also decreased visual and olfactory learning capacities. Only 40% of pesticide-affected honeybees made the correct decision the reward in the test with T-tubes maze test, indicating that their visual learning ability based on visual orientation may be significantly lower than that of the bees from control group.

3.7. Feeding

In the cricket feeding experiment of Uhl et al. (2015) imidacloprid exposure affected cricket behavior. High rate treated crickets spent 63% less time highly mobile than control crickets. Within 7 days crickets consumed a comparable amount of strawberry leaves in the low rate treated and the control group but they gained 80% less mass in the low rated. The high rate treatment reduced feeding by 87% and resulting in a 120% reduction of cricket mass gain compared to the control and a mean mass loss. Furthermore, high rate treated crickets grew slower than control crickets. Their thorax growth was reduced by 58%. Low mortality did occur in each experiment that was evenly distributed between treatments.

3.8. Development

Sohrabi et al. (2011) corresponded that buprofezin significantly reduced adult longevity in *Bemisia tabaci* (*Hemiptera: Aleyrodidae*). Affected by this insecticide, this period took 4.9 days. In the control group, it took 8.3 days. However, Imidacloprid did not cause any significant effect in comparison with the control group. The sublethal

concentration of buprofezin significantly reduced fecundity and percentage of egg hatch. Females that survive exposure of the sublethal concentration of imidacloprid laid more eggs than control females, but the difference was not significant.

The study demonstrated the variability among *Bemisia tabaci* developmental stages in their susceptibility to insecticides. First and second instars were the most sensitive stages among all larval stages, regardless of the insecticide applied. In this study, both insecticides were less effective on the pupae compared with the larval stages. This trend could be related to size (pupae are larger than larvae), need for a higher amount of insecticide to obtain the same level of mortality and also it could be related to physiological differences between the younger and older larvae such as the presence of defense mechanisms that allow breakdown of insecticide.

3.9. Predation

Predator-prey interactions of arthropods can be significantly affected by neonicotinoids. In the matter of spiders, changes in the web architecture has been studied mainly, because it is an easier method to observe the frequency of web building and the size of webs. However, only a few studies investigate the functional response, which is the relationship between prey capture and prey density. It allows to explore sublethal effects of pesticides on searching efficiency and handling time (Pekár 2013).

The results of the study of Deng et al. (2007) show that methamidophos, which is a highly toxic acaricide, had positive effects on the predation behavior of male spiders and short-term negative effects on that of female spiders.

Another study demonstrated that neonicotinoids (acetamiprid, thiamethoxam, and imidacloprid) effect predaceous arthropods differently and can cause significant violation of ecological balance (Kilpatrick et al. 2005).

In the predation experiment of Uhl et al. (2015) differences in survival between treatments occurred predominantly within the first 24 h of the experiment. Thereafter, cricket mortality was minor in all treatments. Crickets in the low imidacloprid rate survived spider predation twice as long as those from the control group. Cricket behavior was not affected by imidacloprid in contrast to the cricket feeding experiment

with its longer exposure time of 7 d. Neither high imidacloprid rate, nor low rate crickets were less highly mobile than control crickets.

Another study by Englert et al. (2012) shows how the predation by *Gammarus fossarum* (Crustacea: Amphipoda) upon mayflies increases with increasing thiacloprid (neonicotinoid) concentration, up to a concentration of 1.00 mg/l, which might thereby have short-term implications for mayfly populations during insecticide peak exposure events. Also, thiacloprid increases predation by *Gammarus fossarum* while reducing its leaf consumption, which hence might alter species interactions at environmentally relevant concentrations.

3.10. Sex ratio

Comparison of the sex ratio of the offspring treated by imidacloprid and buprofezin by Sohrabi et al. (2011) showed that there were more males than females for buprofezin and imidacloprid insecticides than in the control group, but the differences between these neonicotinoids were not significant.

There were significant differences observed in sex ratio between the treatments and the control. There are few studies reporting sublethal effects of imidacloprid on biological parameters of parasitoids. There are also other researchers which reported that imidacloprid was harmless on preimaginal stages of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae), however, it was considerably toxic to parasitoid adults.

3.11. Reproduction

Neurotoxic substances affect all processes of reproduction involves coordinated by the nervous and hormonal systems, namely, mate finding, chemical or sound communication, courtship, mating, egg sac production, spermatogenesis, oogenesis, and brood care (Pekár 2013).

Deng et al. (2008) reported that synthetic pesticides caused a reduction in egg sac production in some linyphiids, production of fewer eggs quantity and lower fertility.

In the research of Chaimanee et al. (2016) honey bee queens were treated with different doses of imidacloprid. No bees died in any of the treatments over a 7 days period. In the queen groups treated with imidacloprid, sperm viability was significantly

decreased in queens treated with all four doses of imidacloprid (0.02, 0.1, 0.2 and 0.4 ppm). There was no significant difference in sperm viability with increased imidacloprid doses (0.1, 0.2, and 0.4 ppm). Low doses of imidacloprid (0.02 ppm) caused down-regulation of P450 sub-family genes, antioxidant, immunity and development genes 1 day after treatment. Imidacloprid reduced CYP306A1 expression. Also, the expression of genes encoding antioxidative enzymes superoxide dismutase and thioredoxin peroxidase was significantly down-regulated. Further, coumaphos and imidacloprid were found to have significantly negative impacts on development genes.

Neither pesticide exposure nor parasite treatment affected total number of males, eggs, larvae and pupae produced by workers in microcolonies. Fecundity was on average for control, pesticide, parasite and pesticide and parasite groups, respectively (Piiroinen et al. 2016).

3.12. Defense

Secondary defense strategies (for example escape) are based on movement abilities. In cursorial spider species, reduced speed of movement increases susceptibility to predation. In web-building species, spraying causes them to leave their webs. Inhibition of pesticides may have an effect on the secondary defense strategies and makes spiders more defenseless because of reduced speed of movement and reduced reaction to kairomenes, signaling the presence of predators in a certain area (Pekár 2013). Studies of Deng et al. (2007) have shown that a low dose of organophosphates stimulated predation in *Hylyphantes graminicola* (Araneae: Linyphiidae),

3.13. Thermoregulation

The investigation of Tosi et al. (2016) has found that there is a significant effect of thiamethoxam (a neonicotinoid pesticide) treatment on the thorax temperature of African honey bees (*Apis mellifera scutellata*). The thorax temperature of medium-dose treated bees (25.95 ± 0.09 °C) and high-dose treated bees (26.06 ± 0.011 °C) were significantly lower compared to low-dose treated bees (26.22 ± 0.010 °C). There was a significant effect of treatment on forager thorax temperatures at 60, 70, 80 and 90 min after the treatment during the first day of experiment. There was a significant

effect of the neonicotinoid treatment on the thorax temperature of the honey bees 20 and 30 min after the cold shock.

Other researcher correspondes that forager bees under colder environmental conditions maintain their thorax above ambient temperature during the foraging cycle using the flight muscles, while under warmer conditions foragers use evaporative cooling for the same purpose (Coelho 1991) . In other experiment the thorax surface temperature of dancing honeybees *Apis mellifera carnica* was measured using thermography. It was found that foraging modulated dancing temperature. Furthermore, dancing temperature was increased with the amount of brood and decreased with the amount of honey and pollen (Stabentheiner 2001).

The thorax temperature of *Apis mellifera scutellata* foragers was significantly different after acute oral thiamethoxam (neonicotinoid) treatment from one hour after exposure and for at least 24 hours. This change may affect colony foraging efficiency through an alteration of both in-hive tasks and outside-hive tasks (for example recruitment dance, food unloading, flight performance). Furthermore, bees treated with higher doses of thiamethoxam showed reduces ability to recover from a 5-min cold shock at 4°C. The effects of thiamethoxam on individual honey bee thermoregulation may involve a broad range of processes such as flight and homing ability and task allocation affecting overall colony fitness and could contribute to colony exhaustion and loss (Tosi et al. 2016).

3.14. Immunocompetence

Brandt et al. (2016) reported that there are significant effects of thiacloprid, imidacloprid and clothianidin on general immune parameters of honey bees. As measures of individual immunocompetence Tosi et al. (2016) used three different aspects of honey bee immunity, total hemocyte count, wound healing response, and antimicrobial activity of the hemolymph. The results of this study show that all three aspects of immunity are affected by sublethal concentrations of neonicotinoids.

It was found that neonicotinoids at field realistic concentrations on immunocompetence in individual adult worker honey bees. However, it remains to be shown whether the observed alterations of the immune system have consequences for the disease resistance capacity of honey bees. Furthermore, researchers claim that thiacloprid, which is classified as “not harmful for bees” because of its lower acute

toxicity, showed similar sublethal effects on immune parameters at a field realistic concentration as more toxic neonicotinoids such as imidacloprid, clothianidin and thiamethoxam. They also correspond that significant piece of information to the ongoing discussion of the role of neonicotinoid insecticides in colony losses. The results we report clearly indicate the need for more detailed laboratory and long-term field studies, aiming to assess how insecticides interfere with pathogen propagation and disease susceptibility.

4. Materials and methods

4.1. Spiders

Spiders *Philodromus cespitum* (Walckenaer, 1802) live mainly on the vegetation of forest steppe, forest edge, heaths, parks and orchards. In the Czech republic, they are abundant except for higher places (Kůrka 2015).

Spiders were collected by beating tree branches and captured by aspirator in an apple orchard at the Crop Research Institute, Prague-Ruzyně, Czech Republic. Animals were transferred to the laboratory. Subsequent determination of the genus was carried out using the European spiders determination key (Nentwig et al. 2018) with the help of RNDr. Milan Řezáč, Ph.D.

Each spider was put into 1.5-ml Eppendorf vials containing a piece of wet cotton wool to maintain humidity. For air exchange in the lid of each vial a small hole was previously made. A total of 284 spiders were used in the experiment.

4.2. Pesticides

Four neonicotinoids of different commercial brands containing various active substances were chosen for the laboratory experiment. The pesticides formulations were diluted in water to the manufacturer`s recommended concentrations.

Commercial brand	Active substance	Manufacturer	Concentration
Actara	thiamethoxam	Syngenta Crop Protection AG	0,00854
			0,00976
Biscaya	thiacloprid	Bayer CropScience AG	0,0244
			0,0366
Confidor	imidacloprid	Bayer CropScience AG	0,0732
Mospilan	acetamiprid	Nisso Chemical Europe GmbH	0,00732
			0,0305

Table 1. Used insecticides

Actara 25 WG is an insecticide containing the active substance thiamethoxam. Thiamethoxam is a synthetic insecticide. It is intended for the control of sucking and chewing pests (mostly aphids (*Aphidoidea*) and *Leptinotarsa decemlineata*), in which the insecticide has Stomach and contact action. In affected insects thiamethoxam acts by interfering with the nicotinic acetyl choline receptor of the nervous system. The insecticide is effective against adult and larvae pests (Anonymous 2016).

The active substance of Biscaya is thiacloprid (22.97 % w/w). It is an insecticide to control aphids and pollen beetles (*Nitidulidae*) in crops and reduce damage from orange blossom midge (*Sitodiplosis mosellana*) in wheat. Biscaya may have adverse effects on some non-target beneficial arthropods, but it would be unlikely (Anonymous 2017).

Confidor 70 WG contains imidacloprid (chloro-nicotinyl). The insecticide has an acute, contact and stomach effect as acetylcholine receptor agonist. It is used mainly against aphids, leafminer (*Phyllocnistis citrella*), thrips (*Scirtothrips aurantii*), whitefly nymphs (*Trialeurodes vaporariorum*) and mealybugs (*Planococcus ficus*) (Anonymous 2015).

Mospilan 20 SP is a systemically active selective insecticide designed to control a wide range of animal pests (mainly aphids, colorado potato beetle (*Leptinotarsa decemlineata*), weevil (*Ceutorhynchus*), midges (*Didymomyia*)). The active substance is acetamiprid (Anonymous 2010).

4.3. Design of experiments

For particular treatments spiders were selected randomly. In the first experiment neonicotinoids and distilled water were sprayed directly to the animals. During the second experiment same fluids were sprayed to the areas into which the spiders were later placed so they were in tarsal contact with the pesticides or control. For application Burkard scientific auto-load Potter Precision Laboratory Spray Tower was used. For one spraying, 3 milliliters of liquid were applied, the pressure was 5 lb./sq.in.

The exposition duration was always 60 minutes. For the second experiment spiders were separately placed to the treated arenas, so they were mainly in tarsal contact with the insecticide or distilled water. In both experiments after the exposition spiders were separately placed to Petri dishes (diameter = 54 mm) with three different densities of wingless *Drosophila melanogaster* (one, three or five flies). I was replacing killed flies by living ones for ten hours, so their density was constant throughout the whole experiment. The number of captured fruit flies was checked every 10 minutes. The predatory activity was compared by recording the number of killed prey. Moreover, the mortality of spiders during the experiment was also recorded. Dead specimens were not included into the functional response data analysis.

4.4. Data analysis

Data were analyzed using R-statistical program (version 3.4.2). For mortality analysis, I created two generalized linear models with a binomial distribution for topical and tarsal treatments. In case of occurrence of insignificant factors, the model was gradually updated. To further simplify the model, individual pesticides (levels of treatment factor) that did not have a significant effect on mortality and had similar coefficients, were combined into one level.

Data obtained as a result of experiments are the number of *Drosophila melanogaster* killed by individual spiders. In the case of some spiders this number was zero. I created a generalized linear model with Poisson error distribution for each experiment. Every model contains a response variable (quantity of fruit flies killed) and two categorical explanatory variables (treatment and prey density). If a model included insignificant factors, it was gradually simplified by forward selection. The final model included only explanatory categorical variables and their interactions which had a significant impact on functional response. The null hypothesis was determined as follows: predatory performance doesn't depend on type of neonicotinoid and prey density.

5. Results

5.1. Mortality

The percentage mortalities observed in topical treatment and tarsal treatment experiments are summarized in Table 1 and Table 2, respectively.

In the experiment with topical application of pesticides mortality was relatively similar for all except for Confidor, where 32 % of tested spiders died within eleven ours after application. Unexpectedly, lower concentrations of Actara, Biscaya and Mospilan caused higher mortality rate than higher concentrations, however there was no statistically significant effect of any pesticide tested. The mortality data are shown in the table 1. In the control group (spiders treated with distilled water) one spider died during the experiment, which could be caused by mechanical influence of water sprayed.

Treatment	n	Mortality (%)
Distilled water	23	4
Actara – 0.00854	22	14
Actara – 0.00976	15	0
Biscaya – 0.0244	23	9
Biscaya – 0.0366	15	0
Mospilan – 0.00732	23	9
Mospilan – 0.0305	15	0
Confidor – 0.0732	22	32

Table 2. Mortality for *Philodromus* after topical application of pesticides and distilled water

As in the first experiment, lower concentrations of neonicotinoids caused higher mortality rate than higher concentrations (Table 2). The statistical model could not be simplified completely due to a lack of variability in the data. Mortality rate after application of Confidor and Mospilan (0.00732) was significantly higher, than in the topical application experiment (Mospilan: p-value<0.01; Confidor: p-value<0.001).

Treatment	n	Mortality (%)
Distilled water	21	0
Actara – 0.00854	15	7
Actara – 0.00976	15	0
Biscaya – 0.0244	15	7
Biscaya – 0.0366	15	0
Mospilan – 0.00732	15	40
Mospilan – 0.0305	15	7
Confidor – 0.0732	15	60

Table 3. Mortality for *Philodromus* after tarsal application of pesticides and distilled water

5.2. Predation capacity

Data obtained as a result of experiments are the number of *Drosophila melanogaster* killed by individual spiders. In the case of some spiders this number was zero. I created a generalized linear model with Poisson error distribution for each experiment. Every model contains a response variable (quantity of fruit flies killed) and two categorical explanatory variables (treatment and prey density).

5.2.1. Analysis of the topical application experiment results

Spiders treated with different neonicotinoids clearly differed in their ability to hunt, as shown in the Figure 1. Moreover, there was a consequential contrast between different concentrations of the same insecticides. The lowest number of killed flies had Mospilan (0.0305), Actara (0.00854) had the most diverse influence on predation capacity in spiders. On the average, specimens treated by distilled water (control group) killed the greatest number of fruit flies.

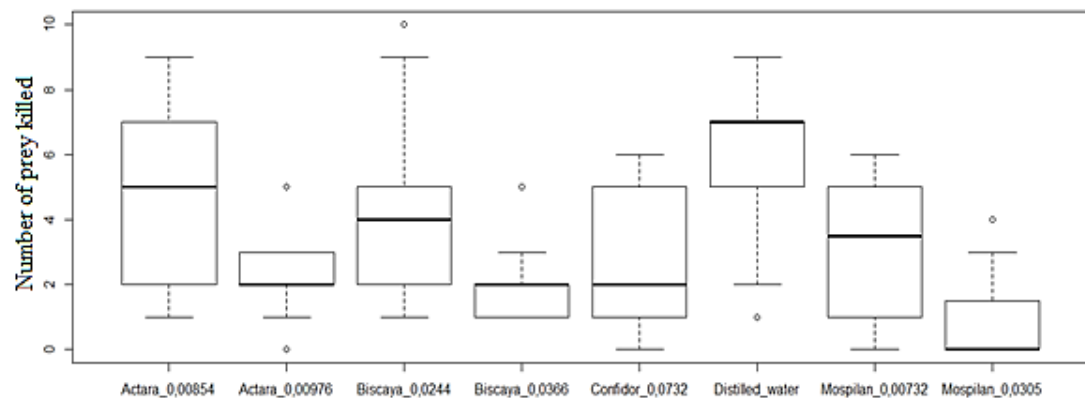


Figure 1. Comparing the number of *Drosophila melanogaster* individuals killed by insecticide-treated spiders in the topical application experiment

The way how specific pesticides differed in their effect with different fruit-flies concentrations is displayed in Figure 2. Speaking of prey density, I could not trace the general pattern of the influence.

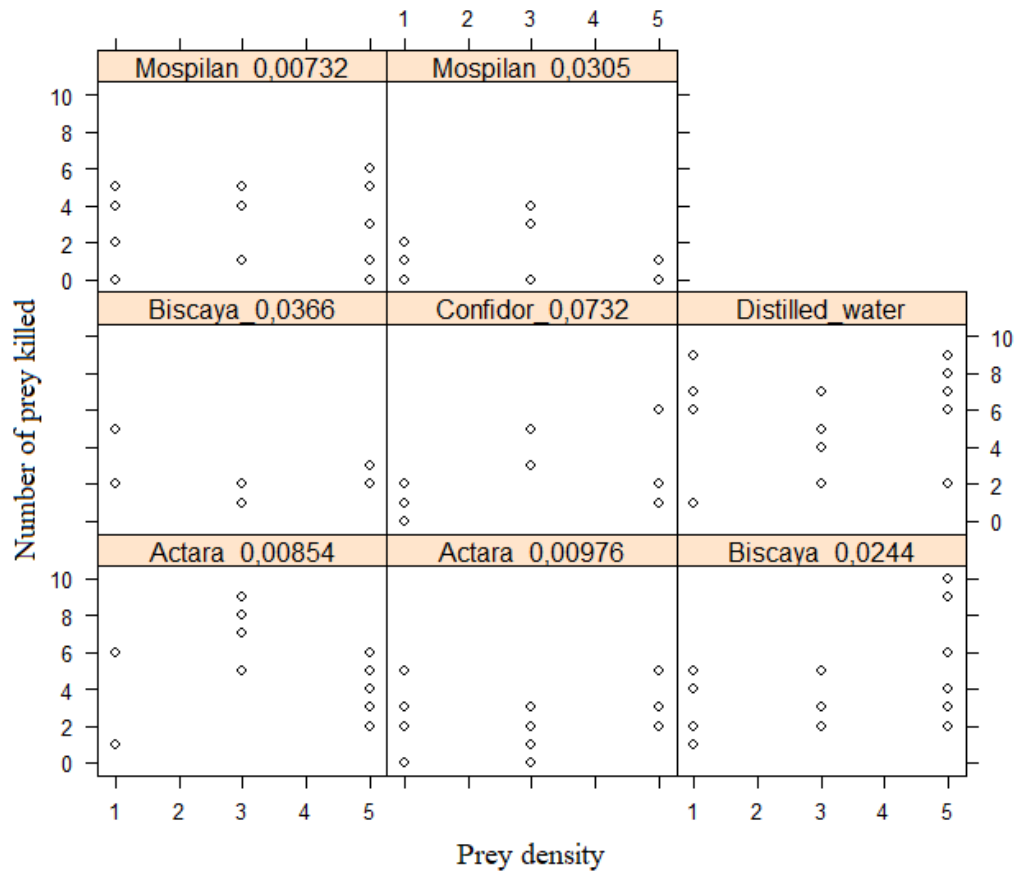


Figure 2. Dependence of the number of individuals killed in *Drosophila melanogaster* at 3 different densities of prey for each treatment

By statistical analysis I found that the pesticide treatment had more influence on predation capacity of spiders than prey density and interaction of these factors (Table 3.). Nevertheless, all factors had a significant impact. A more detailed analysis revealed that Confidor (P-value=0.06097), distilled water (P-value=0.03155), prey density (3 fruit flies) (P-value=0.01105) and interactions of this prey density with Actara (0.00976) (P-value=0.01974), Biscaya (0.0366) and distilled water can be considered as statistically significant factors.

Factor	<i>Df</i>	Deviance	Resid. Df	Resid. Dev	P
NULL			108	236.96	
Treatment	7	76.50	101	160.46	<0.001
Prey density	2	6.12	99	154.33	<0.05
Treatment * Prey density	14	34.25	85	120.08	<0.01

Table 4. The results of the Generalized Linear Model explaining the dependence of the number of killed *Drosophila melanogaster* on treatment and prey density.

5.2.2. Analysis of the tarsal application experiment results

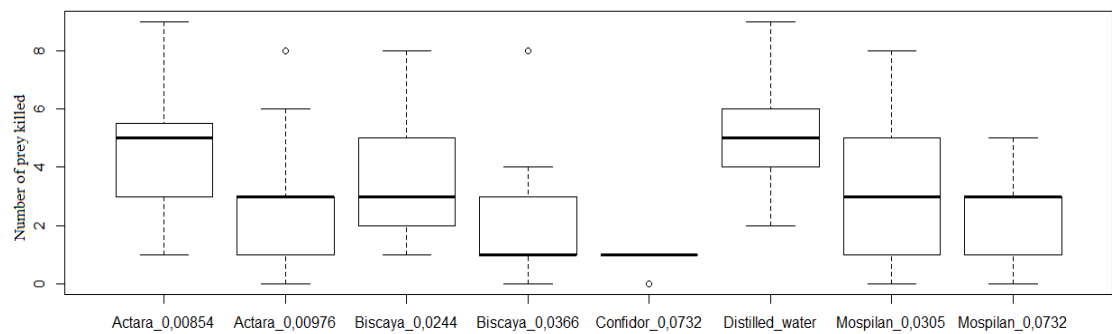


Figure 3. Comparing the number of *Drosophila melanogaster* individuals killed by insecticide-treated spiders

Figure 4 clearly shows that there is no general trend in the influence of prey density. However, in the control group predation activity was relatively high in all prey densities. By contrast in the group treated by Confidor, functional response was always low.

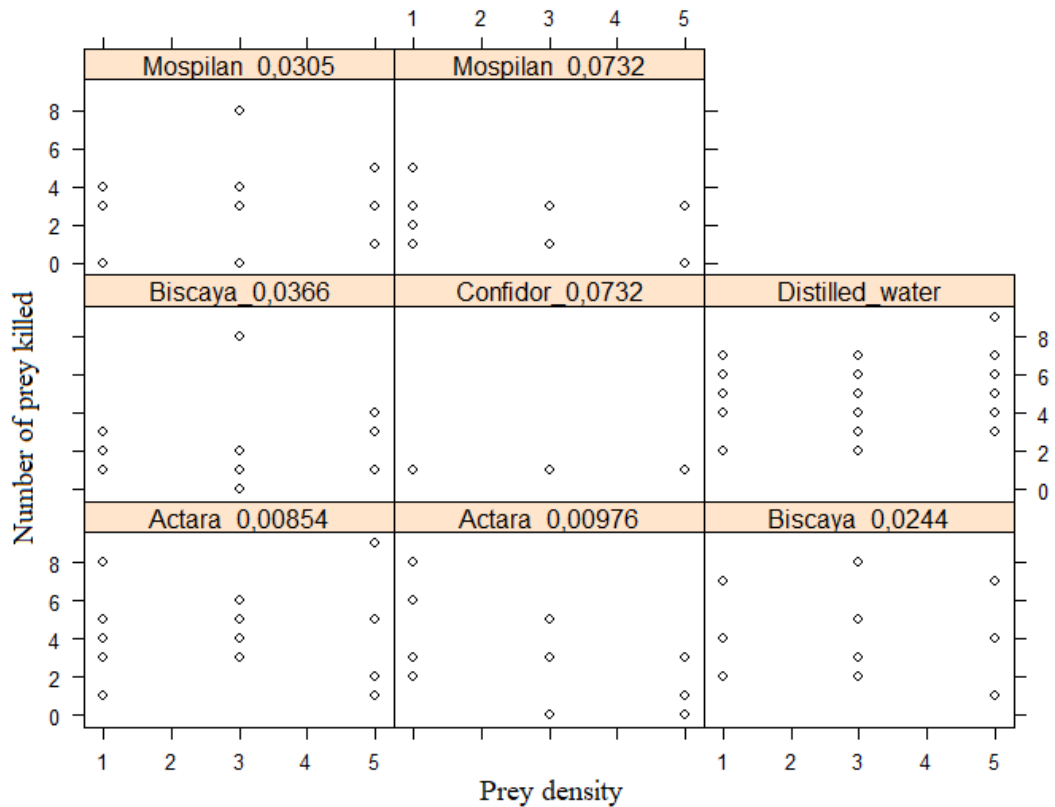


Figure 4. Dependence of the number of individuals killed in *Drosophila melanogaster* at 3 different densities of prey for each treatment

The only statistically significant factor was treatment (Table 4.), namely Biscaya (0,0366) (P-value=0.0219), Confidor (P-value=0.05247), Mospilan (0.0305) (P=0.04486). However, interaction of Actara (0.00976) (P-value=0.00278) and Mospilan (0.0305) (P-value=0.07948) with prey density (5 fruit flies) was also significant.

Confidor was the only level of treatment factor which had a significant effect on the number of killed flies in both statistical models. Interactions of Actara (0.00976 mg/l) and prey density (3 flies in topical and 5 flies in tarsal treatments) were significant for both treatments. Considering that Confidor caused the highest mortality, it can be concluded that this pesticide had the greatest impact on predation capacity of spiders tested.

Factor	Df	Deviance	Resid. Df	Resid. Dev	P
NULL			106	195.38	
Treatment	7	48.544	99	146.83	<0.001
Prey density	2	0.244	97	146.59	0.8851
Treatment * Prey density	14	20.029	83	126.56	0.1292

Table 5. The results of the Generalized Linear Model explaining the dependence of the number of killed *Drosophila melanogaster* on treatment and prey density.

6. Discussion

In the current study I investigated the impact of four neonicotinoid pesticides on predation capacity of *Philodromus cespitum* spiders. I found only few researches studying the sublethal effects (predation capacity changes in particular) in spiders caused by the insecticides tested in my experiment, because the impact on target organisms in the most frequently studied.

In my experiment Confidor, Biscaya, and Mospilan significantly reduced predation capacity in the spiders studied. Confidor caused the highest mortality of all pesticides tested and negatively influenced predation activity after both topical and tarsal treatments. The significant influence of imidacloprid (active substance of Confidor) was observed in the study of Chen et al. (2012). In their research spiders *Pardosa pseudoannulata* were placed into containers with 10, 20, 30, 40, 50, and 60 fruit flies per container. Two days later spiders were treated with Imidacloprid at five different concentrations (12.5, 25, 50, 100, 200 mg/l). Dipping method of treatment was applied. They were recording number of prey killed every 24 hours for 5 days. As a result, it was found that, at a concentration 25 mg/l predation ability was significantly reduced, however at a lower concentration (12.5 mg/l) predation ability was increased. Researchers consider one of the possible explanations for these results to be the increased activity of some enzymes (for example CarE) in the low dose treated spiders. Similar results were revealed by Malaquias et al. (2014) in their experiment, in which imidacloprid was applied to *Podisus nigrispinus* (Hemiptera, Pentatomidae) fed on *Spodoptera frugiperda* (Lepidoptera, Noctuidae) larvae and significantly reduced

predation at a prey density of 16 larvae, however there was no significant difference between predation activities in treated females of *Podius nigrispinus*.

The effect of imidacloprid and thiamethoxam (active substance of Actara) on predation activity of *Popillia japonica* (Coleoptera, Scarabaeidae) was tested by Peck & Olmstead (2010). The results showed that rate of egg consumption was 28.3 – 76.1 % in imidacloprid-treated insects. For thiamethoxam-treated beetles the effect was similar. This is different from my results, where by itself, Actara did not have a significant effect on predation in any of the applications.

In the research of Řezáč et al. (2010) Mospilan topically applied to *Philodromus cespitum* was found harmless and predation capacity was reduced only slightly. Higher prey density increased number of *Drosophila melanogaster* killed. These results are in general similar to the results of my study with the topical application of Mospilan, however in my study the influence of prey density on the number of prey killed was not significant.

Little is known about the effect of Biscaya or its active substance thiacloprid on predation capacity of arthropods. After a triple exposure treatment (contact with spray droplets, orally through food, and residually through walking on sprayed plant leaves) thiacloprid significantly reduced predation rate of *Macrolophus pygmaeus* (Heteroptera, Miridae) and caused 100% mortality during 24 hours of experiment Martinou et al. (2014). The study of Englert et al. (2012) displayed that thiacloprid at 0.50 – 1.00 g/l significantly increased predation by *Gammarus fossarum* (Crustacea, amphipoda) on *Baetis rhodani* (Ephemeroptera, Baetidae) in an experiment with aqueous contact and contact through food. In my experiment Biscaya at higher concentration (0.0366) significantly reduced predation rate of spiders and caused no mortality.

Further research is required to better understand the significance of this impact and the importance of adjustments in the pesticide management in orchards and plantations.

7. Conclusion

Gardens, fields, plantations and other agroecosystems are common habitats of spiders. These arthropods are very typical and diverse predators. Therefore, their role in plant protection against various pests is consequential. *Philodromus cespitum* studied in this thesis is an important inhabitant of agroecosystems in Europe.

The main purpose of my work was to obtain more information about how neonicotinoid treatment will affect the number fruit-flies killed by spiders *Philodromus cespitum*, however mortality and the prey density factor were also analyzed. It was confirmed that the pesticide treatment and interaction of certain treatment with different prey densities had a significant impact on predation capacity.

Based on the data presented in my research and various other studies, it can be concluded that some popular neonicotinoid pesticides can not only reduce spider populations, but also have a significantly negative effect on their predation capacity which definitely matters in predator-prey relationship in agroecosystems. This impact may cause economic damage in agriculture.

There is a need for further research aimed to minimizing the negative impact of neonicotinoids on agroecosystems.

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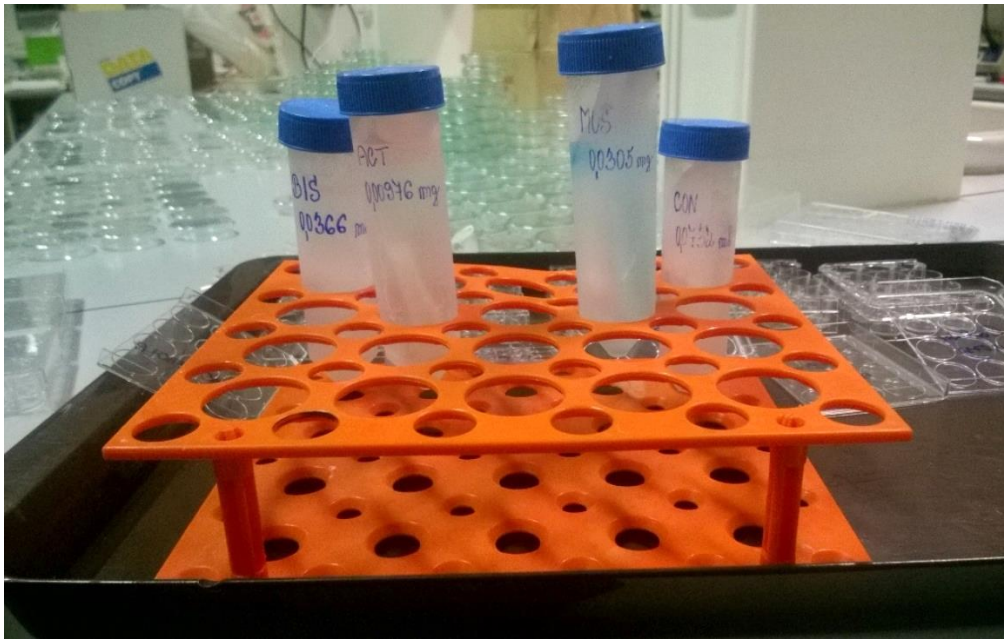
9. Appendices

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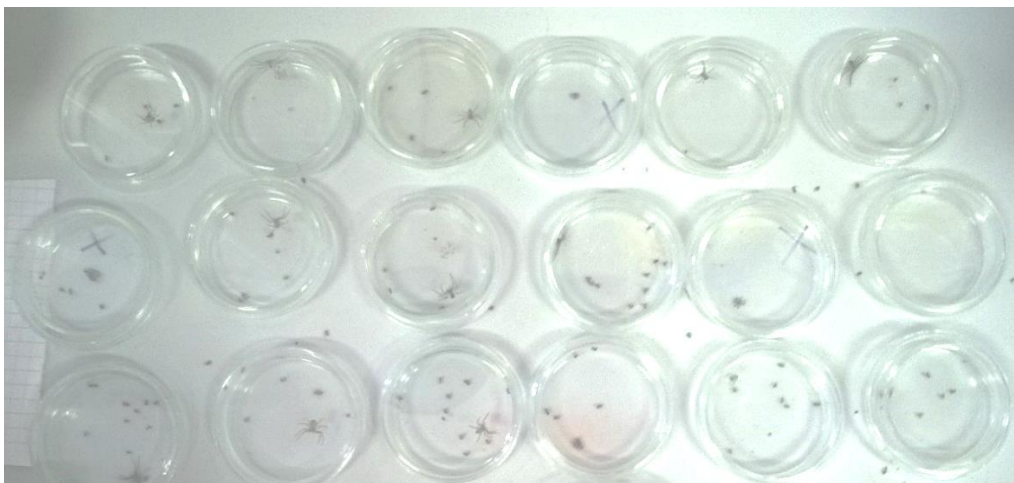
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Appendix 2: Spiders and fruit flies on petri dishes

Appendix 3: Burkard scientific auto-load Potter Precision Laboratory Spray Tower



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