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Repellent Effects of Andiroba and Copaiba Oils against *Musca domestica* (Common House Fly) and Ecotoxicological Effects on the Environment

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ABSTRACT

***Background*:** The main challenge in raising cattle in Brazil is related to ectoparasites, that cause negative effects on milk and meat production, and in severe cases, animal death. Sheds known as crèches attracts large number insects mainly due to milk residues in the environment. The housefly is a major problem due to act as vectors of many other diseases, and so there is the possibility of control of infestations with natural products. Andiroba and copaiba oils may act as natural biocides, there are only a few studies on their effect on biological soil parameters. Therefore, this study aimed to evaluate the repellent effect of andiroba and copaiba oils against flies and on biological soil parameters.

***Materials, Methods & Results*:** The repellency effect of oils of andiroba and copaiba was tested at a concentration of 5% in lambs shed maternity, containing 64 bays (1.8 m^2). It was sprayed 30 mL per pen, where they were housed five lambs each. Pre-treatment counts were taken before the treatment (mean 46 per pen after *Musca domestica*), and post-treatment count was made on 2, 24 and 48 h. The data collected at 2 and 24 h was evaluated and the number of flies was reduced significantly ($P < 0.001$) in the pens treated with oil of copaiba and andiroba compared to control (untreated) pen. After 48 h, no difference was observed between treatments in relation to fly numbers ($P > 0.05$). Ecotoxicological test using increasing concentrations in the soil (0, 1, 5, 10, 25, 50, and 100 mg/kg) regarding changes in basal respiration (C-CO_2), and survival and reproduction of springtails (*Folsomia candida*). It was observed an increased amount of mineralized C-CO_2 until the day 10 of incubation for both oils without inhibition of the microbial respiratory process in any dose. The copaiba oil showed higher amounts of accumulated C-CO_2 compared to andiroba oil in all studied concentrations ($P < 0.05$). In tests with mesofauna organisms, none of the evaluated concentrations of the two oils showed no negative effect on the survival of springtails ($P > 0.05$), the same was observed for the reproduction results, where there was no reduction in the number of juveniles ($P > 0.05$).

***Discussion*:** According literature, andiroba and copaiba oils have repellent effect against domestic fly when sprayed onto infected cow's horn fly, similar results also were reported in vitro tests against *M. domestica* larvae using andiroba oil and noted 80% larval mortality. The use of natural products in disease control is growing, but its impacts on the environment are not known, so in addition to suggesting therapies it is important to be concerned with ecotoxicological tests. Researchers showed an effect of *Eucalyptus globulus* essential oil on *F. candida* and reported 76% reduction in its survival rate at concentration of 60 mg/kg soil. Basal soil respiration is a sensitive indicator that quickly reveals changes in the environmental conditions that affect microbial activity, and the data presented herein reveal an increase in the respiration of microorganisms depending on the amount of oil added to the soil. The essential oils of copaiba and andiroba have repellent effect against *Musca domestica*, and did not show any toxicity to inhibit microbial activity in the soil. In addition, the presence of the oils in the soil did not affect the survival and reproduction of springtails *Folsomia candida*.

Keywords: terrestrial ecotoxicology, essential oils, environment, basal respiration, repellent, flies.

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INTRODUCTION

The main challenge in raising cattle in Brazil is related to parasites [16], that have negative effects on production, and in severe cases, animal death [2,39]. The conventional control of parasites based on the use of acaricides has been ineffective due to commercial formulations [31]. Many residues of these compounds are excreted via faces and urine of treated animals [5,13,36], and the presence of pesticide residues in the environment represent a dangerous risk to the ecosystem, interfering with soil fauna composition [13,42]. Thus, safer and less aggressive methods to humans and the environment have stimulated the search for alternative treatments such as plant extracts to reduce the problems caused by synthetic products [4,14]. Among the oils which have shown satisfactory results in parasite control, andiroba oil and copaiba oil stand out since tests performed by researchers showed promising results against ticks [41]. These same oils have showed repellent effect against horn fly in dairy cows [23] and therefore may be an option against other species of flies. The andiroba and copaiba oils has insecticidal and anti-parasitic effect proven [26,34].

The housefly is a major problem because is a vector/or transmitting of many diseases, and so there is the possibility of control of infestations with natural products [3]. However, even though andiroba and copaiba oils may act as natural biocides, there are only a few studies on their effect on biological soil parameters, unlike commercial pesticides [9,22,32-44]. Thus, the objective of the study was to evaluate the repellent effect of andiroba and copaiba oils against common housefly and on biological soil parameters.

MATERIALS AND METHODS

Andiroba oil (*Carapa guianensis*) RF3150[1] and Copaiba oil (*Copaifera reticulata*) RF3350[1]. The three main components of andiroba oil are α-humulene, bicyclogermacrene, and germacrene-D, which together account for 53.34%. Already the copaiba oil has αcopaene as its major component, representing 40.09% of the oil. The complete chemical composition of these oils has been previously published [41].

The repellency effect of oils of andiroba and copaiba was tested at a concentration of 5% [23] in lambs shed maternity, containing 64 bays (1.8 m^2). The tests were done in triplicate for each treatment, and the each bay considered one replication. The treatments were well defined and control group (no treatment), for andiroba, and copaiba group. Importantly, it was the groups were divided randomly into the shed, being spaced as a buffer zone by two pens of each. It was sprayed 30 mL treatment per pen (used manual sprayer) for animals and the environment, where they were housed five lambs of each. Fly counts (*Musca domestica*) was taken before the treatment (mean 46 per pen), and after the treatment at 2, 24 and 48 h.

During the soil experiment, increasing concentrations of andiroba and copaiba oils on the microbiological and ecotoxicological soil parameters were tested through standardized ISO tests. The treatments consisted of control (Ctrl), solvent control with Triton 100XL[2] (Ctrl Solv.) and concentrations of 1, 5, 10, 25, 50, and 100 mg/kg soil for both oils. It was necessary to carry out the dilution of the oil, since both showed hydrophobic characteristics. For this reason an oil dilution using Triton was mixed using 1 mL of oil to 5 mL of Triton. After homogeneization the solution was mixed with 44 mL of water resulting as a stock solution. Three tests were conducted to identify changes in soil biological parameters such as microbial activity through the basal respiration assay, and survival and reproduction tests with springtails *Folsomia candida*, using standardized methodology.

For the determination of microbial activity, soil with a natural community of microorganisms was used. Oxisol was extracted from the soil (0 - 0.20 m depth). In the laboratory the soil was sieved on a 2 mm mesh, and stored at 3°C until the beginning of analysis. More details on the physicochemical characteristics of the soil can be found at researchers [37].

The experiment was conducted for 10 days in a controlled environment (no light and temperature of 28 ± 1°C). Each oil concentration was applied to 50 g of moist soil (65% of the maximum water holding capacity (WHC)), and packed in airtight sealed glass with three replications. In order to measure the amount of released carbon, each replicate received 25 mL of sodium hydroxide (NaOH 0.05 M) that was changed periodically according to the evaluation times: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days of incubation. The carbon released in the form of $C\text{-}CO_2$ was quantitated by the titration of NaOH, with standardized hydrochloric acid solution (0.05 M HCl) using phenolphthalein as an indicator [1].

The test for evaluating the survival and reproduction of springtails *Folsomia candida* was performed with Tropical Artificial Soil (TAS), which is a soil adaptation of OECD 207 [30]. This soil consists of a mixture of 70% sand, 20% white clay (kaolin) and 10% coconut fiber (sieved on a 2 mm mesh). To perform the test, TAS pH was adjusted to 6.0 ± 0.5 by the addition of $CaCO_3$ [19], and the water content was initially adjusted to 65% WHC [21].

The test with springtails was conducted based on the ISO [20], with a completely randomized design with four replications, and lasted 28 days. Each replicate consisted of a plastic pot filled with 30 g of soil with the respective concentration of andiroba and copaiba oils. Each pot received 10 juvenile springtails (aged 10-12 days) on the beginning of the test. After 14 days the organisms were fed with yeast, and weekly the flasks were opened for airing. After 28 days, each pot was emptied into another container with water and a few drops of black ink. After a gentle agitation, adult individuals on the surface were counted, and those alive were photographed for subsequent counting.

The data on the number of flies was normally distributed, and data analysed using analysis of variance (ANOVA) followed by Tukey's test. The data on accumulated basal respiration were submitted to ANOVA and means compared by the LSD test ($P < 0.05$) and *t* test ($P < 0.05$). Analyses were performed using Statistica 7.0 software. For the survival and reproduction test with *Folsomia candida* the results were evaluated by analysis of variance (ANOVA one way) followed by Dunnett test ($P < 0.05$) using the Statistica software 7.0.

RESULTS

The number of flies on treatment was shown in Figure 1. It was observed repellency of flies sprayed with bays copaiba and andiroba oils in periods 2 and 24 h of experiment ($P < 0.001$). After 48 h, no difference was observed between the number of flies by treatment ($P > 0.05$).

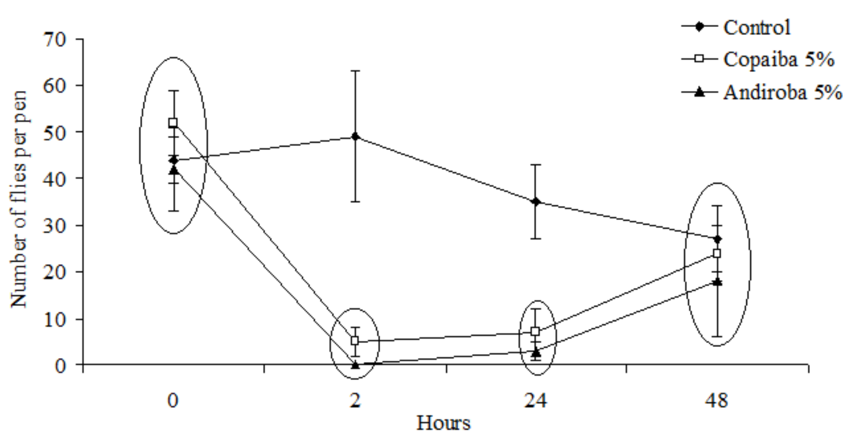

Figure 1. Average number of flies on treatment (control, copaiba and andiroba) in each bay 0, 2, 24 and 48 h after spraying. *Mean within the same circle, no difference ($P < 0.001$).

The amount of mineralized CO_2 on day 10 of incubation increased for oils, and andiroba and copaiba no inhibition of microbial respiratory process on any of the doses evaluated, even at the highest. The basal respiration remained constant in the first two days, changing most significantly on the third day (Figure 2). This behavior can be attributed to the initial adaptation of microorganisms in the presence of oil in the soil, and after that the community is established and begins the most intense process of compound mineralization.

The results for andiroba oil shows that there was a significant difference ($P < 0.05$) on microbial soil respiration when 5 mg/kg soil was used, while for copaiba oil increased respiration rate was significant at 1 mg/kg soil, and higher concentrations (50 and 100 mg/kg soil) showed higher respiration rates for both oils (Figure 3).

The differences found between the amounts of released $C\text{-}CO_2$ according to increased oil concentration compared to the control group, indicate that there was an increase on microbial respiration. The copaiba oil showed higher amounts of C-CO_2 accumulated in 10 days when compared to andiroba oil at all concentrations evaluated ($P < 0.05$). The concentration tested that showed higher differences was 1 mg/kg soil, where the copaiba oil showed accumulated carbon of 9.56 mg CO_2/g soil, a finding clearly higher than the of observed for andiroba oil (5.49 mg CO_2/g soil) [Figure 4].

The *Folsomia candida* survival test fulfilled the validation criterion required by ISO 11267 [20], since the mortality in the control group was lower than 20%. The validity criteria for the reproduction test were also completed, where the number of juvenile springtails was greater than 100 individuals in the control, and coefficient of variation was lower than 30%, in fact 23.07%. There was no statistical difference between the control and the solvent control, confirming that the dilution of oils in Triton did not influence the results. The results show no significant reduction ($P > 0.05$) on the survival and reproduction rates of springtails *F. candida* in any of the concentrations evaluated for both oils (Figure 5).

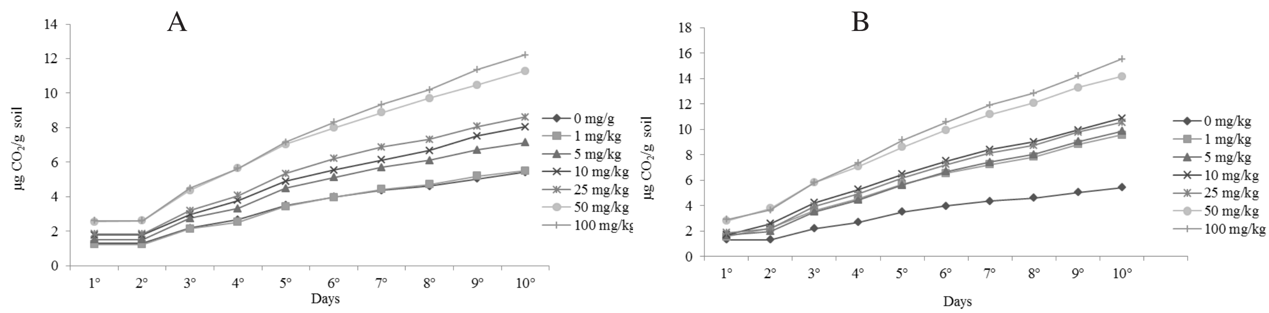

Figure. 2. Daily amounts of released carbon as C-CO_2 when applied increasing doses of (A) andiroba oil, and (B) copaiba oil, in Oxisol (n = 3).

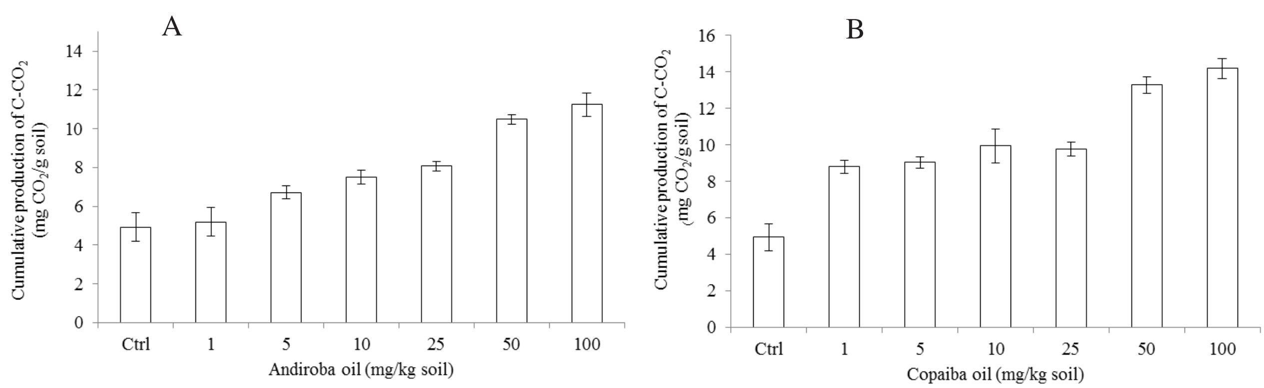

Figure 3. Quantity of released carbon after 10 days of accumulation in the form of C-CO_2 under different doses andiroba oil (A) and copaiba oil (B) in Oxisol. Treatments with different letters are statistically significant by average test LSD ($P > 0.05$).

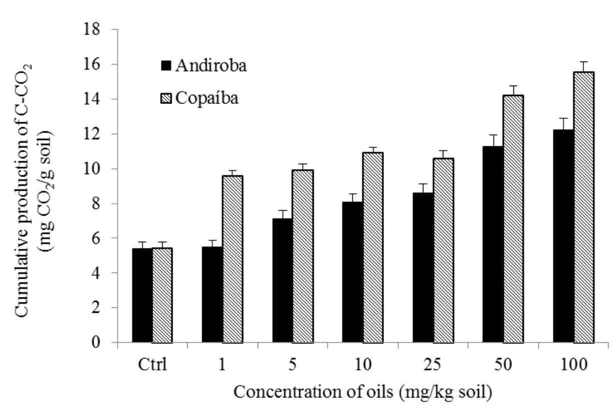

Figure 4. Quantity of released carbon after 10 days of accumulation in the form of C-CO_2 under different doses of andiroba oil (black bars), and copaiba (striped bars) in Oxisol, comparing oils. Treatments with different letters are statistically significant by *t*-test ($P > 0.05$).

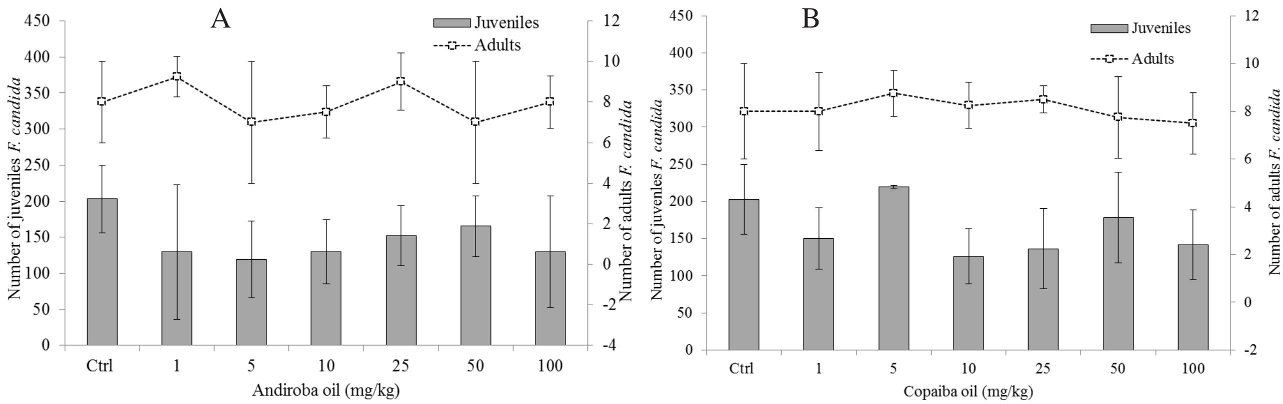

Figure 5. Mean and standard deviation of *Folsomia candida* in tropical artificial soil (TAS) contaminated with increasing concentrations of (A): andiroba oil and (B): copaiba oil. Control (Ctrl), (n = 4).

DISCUSSION

The andiroba and copaiba oils have repellent effect against domestic fly, however this effect is short-lived, because in our study the repellency effect was seen only in the first 24 h. A similar result was observed for both oils when sprayed onto infected cow's horn fly [23], similar results also were reported by other researchers [12], who conducted in vitro tests against *Musca domestica* larvae using andiroba oil and noted 80% larval mortality. Our study also showed that these oils do not have negative effects on soils and microbial fauna of the soil.

Basal soil respiration is a sensitive indicator that quickly reveals changes in the environmental conditions that affect microbial activity [18]. The data presented herein reveal an increase in the respiration of microorganisms depending on the amount of oil added to the soil. However, the interpretation of the respiration data should be cautiously, since an increase in respiratory activity could be initiated by the high productivity of a particular ecosystem, as by environmental stress disorders.

An explanation for the increase on respiration rate when copaiba oil was used may be related to factors such antimicrobial compound since it is capable of inhibiting bacteria growth [15,29]. This same antimicrobial mechanism may explain the results found regarding andiroba oil [35,40] . This study showed no bacterial inhibition effect, but the high respiration rate found when higher oil concentrations were used might be due to higher energy consumption by microorganisms to ensure survival [27]. Similar results to this study were shown by researchers [17], where basal respiration in soils treated with birch tar oils extracted in two forms caused significant increase on respiratory rate after one day of incubation of the treated soil with a concentration of 5 mL/m^2 of oil.

Environmental changes in soil, such as changes in the management or presence of xenobiotic compound, affects microorganisms that can be detected through changes in the activity of these communities [38]. In this way, we can highlight some factors that influence microbial activity, especially waste composition added, physical state, and form of application [10,24], which may explain the differences on released C-CO_2 between copaiba and andiroba treatments. There are some other studies in the literature evaluating the effect of other xenobiotic compounds in the soil over basal respiration parameters, i.e. Castro Jr. *et al*. [6] showed that the use of glyphosate in soil did not cause significant changes on the amounts of released CO_2, unlike Dallmann *et al*. [8] that have observed increased CO_2 production in soil contaminated with glyphosate.

According to literature [25], CO_2 production is also related to glyphosate decomposition in soil since microorganisms may act as bioremediators of contaminated soil, transforming xenobiotic compound an in inert substances such as CO_2 and water [11], moreover, the rate of metabolism of the compounds may vary according to soil type [43].

Other important parameter to be evaluated in the presence of xenobiotics coumponds in soil, is its impact on edaphic soil fauna. The springtail species *Folsomia candida* are soil organisms very sensitive to chemicals [7], and often used as biological indicators in ecotoxicological tests. In this study, these essential oils did not cause any effect *F. candida* survival and reproduction.

Contrary to what has been reported in this study, Martins *et al*. [28] evaluated the effect of *Eucalyptus globulus* essential oil on *Folsomia candida* and reported 76% reduction in its survival rate at concentration of 60 mg/kg soil. These authors also reported a reduction in the reproduction rate where the estimated concentration to reduce 50% of the reproduction rate was EC_{50} 35 mg/kg. Oil toxic effect in springtails occurs due to the formation of an impermeable film surrounding its body, and consequently, inhibiting breathing [28]. Another factor might be due to the presence of amphiphilic compounds present in the oils, which may penetrate the invertebrate cells affecting their physiology. Hagner *et al*. [17] have studied different birch tar oil concentrations and the survival and reproduction rate of springtails *F. candida*, and observed EC_{50} value of 5100 mg/kg. However, this value is very high, and possibly not found in normal field conditions, so that authors claimed that the risks caused by birch tar oil are insignificant in the short term.

CONCLUSION

These results suggest that andiroba and copaiba essential oils can be used as an alternative treatment against ectoparasites as flies, and other infectious diseases since they do not appear to have toxic effect able to inhibit microbial activity. However, they may increase the respiratory activity of soil microorganisms, which may contribute to its rapid degradation. The results on survival and reproduction rates of springtails *Folsomia candida* also showed no toxic effect. Therefore, in general, this study concludes that andiroba and copaiba oils has repellent effect against *Musca domestica*, and show no risk to springtails and soil microbial population, well known terrestrial toxicity indicators.

MANUFACTURERS

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***Ethical approval*.** The procedure was approved by the Animal Welfare Committee of Universidade do Estado de Santa Catarina.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Alef K. & Nannipieri P. 1995.** *Methods in applied soil microbiology and biochemistry*. London: Academic Press, 576p.

2 **Antunes S., Merino, O., Lérias J., Domingues N., Mosqueta J. & Fuente de la J. 2015.** Artificial feeding of *Rhipicephalus microplus* female ticks with anti calreticulin serum do not influence tick and *Babesia bigemina* acquisition. *Ticks and Tick-borne Diseases*. 6(1): 47-55.

3 **Barreiro C., Albano H., Silva J. & Teixeira P. 2013.** Role of flies as vectors of foodborne pathogens in rural areas. *ISRN Microbiology*. 2013(1): 1-7.

4 **Borges L.M.F., Sousa L.A.D. & Barbosa C.S. 2011.** Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. *Revista Brasileira de Parasitologia Veterinária*. 20(1): 89-96.

5 **Boxall A.B.A. 2008.** Fate of Veterinary Medicines Applied to Soils. In: Kümmerer K. (Ed). *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks*. 3rd.edn. Freiburg: Springer, pp.103-117.

6 **Castro Jr. J.V., Selbach, P.A. & Zachia Ayub M.A. 2006.** Glyphosate herbicide evaluation on soil microflora. *Pesticicidas: Revista de Ecotoxicologia e Meio Ambiente*. 16(1): 21-30.

7 **Chelinho S., Domene X., Campana P., Natal-da-Luz T., Scheffczyk A., Römbke J., Andrés P. & Sousa J.P. 2011.** Improving ecological risk assessment in the Mediterranean area: Selection of reference soils and evaluating the influence of soil properties on avoidance and reproduction of two oligochaete species. *Environmental Toxicology Chemistry*. 30(5): 1050-1058.

8 **Dallmann C.M., Scheneider L., Bohm G.M.B. & Kuhn C.R. 2010.** Glyphosate application of the impact on soil microflora grown with genetically modified soybeans. *Revista Thema*. 7(1):1-11

9 **Diao X., Jensen J. & Duus Hansen A. 2007.** Toxicity of the anthelmintic abamectin to four species of soil invertebrates. *Environmental Pollution*. 148(2): 514-519.

10 **Ding C. & He J. 2010.** Effect of antibiotics in the environment on microbial populations. *Applied Microbiology and Biotechnology*. 87(3): 925-941.

11 **Ding W., Reddy K.N., Zablotowicz R.M. & Bellaloui N. 2011.** Physiological responses of glyphosate-resistant and glyphosate-sensitive soybean to aminomethylphosphonic acid, a metabolite of glyphosate. *Chemosphere*. 83(4): 593-598.

12 **Farias M.P.O., Barros F.N., Alves L.C. & Faustino M.A.G. 2009.** Eficácia do óleo da semente de andiroba (*Carapa guianensis*) sobre larvas de *Musca domestica* (Diptera: Muscidae) por meio do teste de imersão. Jornada de ensino, pesquisa e extensão da UFRPE. 3p. Disponível em: <http://www.eventosufrpe.com.br/jepex2009/cd/resumos/R0209-1.pdf>. [Acessed online in September 2016].

13 **Floate K.D. 2006.** Endectocide use in cattle and fecal residues: environmental effects in Canada. *Canadian Journal of Veterinary Research*. 70(1): 1-10.

14 **Gomes G.A., Monteiro C.M.O., Serna T.O.S S., Zeringota V., Calmon F., Matos R.S., Daemon R., Gois R.W.S., Santiago G.M.P. & Carvalho M.G. 2012.** Chemical composition and acaricidal activity of essential oil from *Lippia sidoides* on larvae of *Dermacentor nitens* (Acari: Ixodidae) and larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). *Parasitology Research*. 111(6): 2423-2430.

15 **Goren A.C., Piozzi F., Akcicek E., Kiliç T., Çarikçi S., Mozioglu E. & Setzer W.N. 2011.** Essential oil composition of twenty-two *Stachys* species (Mountain tea) and their biological activities. *Phytochemistry Letters*. 4(4): 448-453.

16 **Grisi L., Leite R.C., Martins J.R.S., Barros A.T.M., Andreotti R., Cançado P.H.D., de León A.A.P., Pereira J.B. & Villela H.S. 2014.** Reassessment of the potential economic impact of cattle parasites in Brazil. *Brazil Journal Veterinary Parasitology.* 23(2): 150-156.

17 **Hagner M., Pasanen T., Bengt Lindqvist B., Lindqvist B., Tiilikkala K., Penttinen O.P. & Setälä H. 2010.** Effects of birch tar oils on soil organisms and plants. *Agricultural and Food Science*. 19(1): 13-23.

18 **Hund-Rinke, K. & Simon M. 2008.** Bioavailability assessment of contaminants in soils via respiration and nitrification tests. *Environmental Pollution.* 153(2): 468-475.

19 **ISO 10390. 2005.** Soil quality – determination of pH. International Organization For Standardization. v.1. Geneva: ISO, 7p.

20 **ISO 11267. 1999.** Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants.International Organization For Standardization. v.1. Geneva: ISO, 19p.

21 **ISO 11274. 1998.** Soil quality – Determination of the water-retention characteristic - laboratory methods. International Organization For Standardization. v.1. Geneva: ISO, 20p.

22 **Jensen J. & Scott-Fordsmand J.J. 2012.** Ecotoxicity of the veterinary pharmaceutical ivermectin tested in a soil multi-species (SMS) system. *Environmental Pollution*. 171(1): 133-139.

23 **Klauck V., Pazinato R., Stefani L.M., Santos R.C., Vaucher R.A., Baldissera M.D., Raffin R., Athayde M., Baretta D., Machado G. & Silva A.S. 2014.** Insecticidal and repellent effects of tea tree and andiroba oils on flies associated with livestock. *Medical and Veterinary Entomology*. 28(1): 33-39.

24 **Kotzerke A., Sharma S., Schauss K., Heuer H., Thiele-Bruhn S., Smalla K., Wilke B.M. & Schloter M. 2008.** Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure. *Environmental Pollution*. 153(2): 315-322.

25 **Kryuchkova Y.V., Burygin G.L., Gogoleva N.E. & Gogolev Y.V. 2014.** Isolation and characterization of a glyphosate degrading rhizosphere strain, *Enterobacter cloacae* K7. *Microbiological Research*. 169(1):99-105.

26 **Leandro L.M. & Vargas F.S. 2012.** Chemistry and biological activities of terpenoids from Copaiba (*Coparfera* SPP). *Oleoresins*. 17(4): 3866-3889.

27 **Leita L., Nobili M. de, Muhlbachova G., Mondini C., Machiol L. & Zerbi G. 1995.** Biovailability and effects of heavy metals on soil microbial biomass during laboratory incubation. *Biology and Fertility of Soils.* 19(2):103-108.

28 **Martins C., Natal-da-Luz T., Sousa J.P., Gonçalves J., Salgueiro L. & Canhoto C. 2013.** Effects of essential oils from *Eucalyptus globulus* lleaves on soil organisms involved in leaf degradation. *PLoS One*. 8(4):1-7.

29 **Morelli C.L., Mahrous M., Balgacem M.N., Branciforti M.C., Brtas R.E.S. & Bras J. 2015.** Natural copaiba oil as antibacterial agent for bio-based active packaging. *Industrial Crops and Products*. 70(3): 134–141.

30 **OECD 207. 2008.** OECD-guideline for testing of chemicals. Earthworm acute toxicity test. Organisation for Economic Co-operation and Development, Paris. 3p.

31 **Rajaput Z.I., Hu S., Chen W., Arijo A.G. & Xiao C. 2006.** Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science*. 7(11): 912-921.

32 **Römbke J., Höfer H., Garcia M.V.B. & Martius C. 2006.** Feeding activities of soil organisms at four different forest sites in Central Amazonia using the bait lamina method. *Journal of Tropical Ecology*. 22(3): 313-320.

33 **Römbke J., Krogh K.A., Moser T., Sheffczyk A. & Liebig M. 2010.** Effects of the Veterinary Pharmaceutical Ivermectin on Soil Invertebrates in Laboratory Tests. *Archives of Environmental Contamination and Toxicology*. 58(2): 332-340.

34 **Santos A.O., Izumi E., Ueda-Nakamura T., Dias-Filho B.P., Veiga-Junior V.F.V. & Nakamura. 2013.** Antileishmanial activity of diterpene acids in copaiba oil. *Memórias do Instituto Oswaldo Cruz*. 108(1): 59-64.

35 **Santos R.C., Alves C.F.S., Schneider T., Lopes L.Q., Aurich C., Giongo J.L., Brandelli A. & Vaucher R.A. 2012.** Antimicrobial activity of Amazonian oils against *Paenibacillus* species. *Journal of Invertebrate Pathology*. 109(3): 265-268.

36 **Sarmah A.K., Meyer M.T. & Boxall A.B.A. 2006.** A global perspective on the use, sales, exposure pathways, ocurrence, fate and effects of veterinary antibiotics (Vas) in the environment. *Chemosphere*. 65(5): 725-759.

37 **Segat J.C., Alves P.R., Baretta D. & Cardoso E.J.B.N. 2015.** Ecotoxicological evaluation of swine manure disposal on tropical soils in Brazil. *Ecotoxicology Environmental Safety*. 122: 91-97.

38 **Sinha S., Chattopadhyay P., Pan I., Chatterjee S., Chanda P., Bandyopadhyay D., Das K. & Sen S.K. 2009.** Microbial transformation of xenobiotics for environmental bioremediation. *African Journal of Biotechnology*. 8(22): 6016-6027.

39 **Sutherst W., Maywald G.F. & Bourne A.S. 2007.** Including species interactions in risk assessments for global change. *Global Change Biology*. 13(9): 1-17.

40 **Vaucher R.A., Giongo J.L., Bolzan L.P., Corrêa M.S., Fausto V.P., Alves C.F.S., Lopes L.Q.S., Boligon A.A., Athayde M.L., Moreira A.P., Brandilli A., Raffin R.P. & Santos R.C.V. 2015.** Antimicrobial activity of nanostructured Amazonian oils against *Paenibacillus* species and their toxicity on larvae and adult worker bees. *Journal of Asia-Pacific Entomology*. 18(2): 205-210.

41 **Volpato A., Grosskopf R.K., Santos R.C., Vaucher R.A., Raffin R.P., Boligon A.A., Athayde A.L., Stefani L.M. & Silva A.S. 2015.** Influence of essential oils of rosemary, andiroba and copaiba on stages of the tick *Rhipicephalus* (*Boophilus*) *microplus*. *Journal of Essential Oil Research*. 27(3): 244-250.

42 **Wall R. & Beynon S. 2012.** Area-wide impact of macrocyclic lactone parasiticides in cattle dung. *Medical and Veterinary Entomology*. 26(1): 1-8.

43 **Yu X.M., Yu T., Yin G.H., Dong Q.L., An M., Wang H.R. & Ai C.X. 2015.** Glyphosate biodegradation and potential soil bioremediation by *Bacillus subtilis* strain Bs-15. *Genetics and Molecular Research*. 14(4): 14717-14730.

44 **Zortéa T., Baretta D., Maccari A.P., Segat J.C., Boiago E.S., Sousa J.P. & Silva A.S. 2015.** Influence of cypermethrin on avoidance behavior, survival and reproduction of *Folsomia candida* in soil. *Chemosphere*. 122: 94-98.