Impact of Kaolin and *Beauveria bassiana* Treatments against Olive Fly on the Non-Target Arthropods of the Olive Ecosystem

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Abstract

The study of biodiversity has received a growing interest from the scientific community. Investigations of the efficacy of compounds allowed in organic farming and their risks for human and environmental health represent an increasingly large body of literature. The aim of this study was to evaluate the environmental impact of two compounds that have potential for registration as organic insect management tools against *Bactrocera oleae*. Kaolin clay, a repellent, and a micoinsecticide based on the vital spores of *Beauveria bassiana* were tested in an olive ecosystem. Selected arthropod taxa (*Arachnida*: *Araneae* and *Opiliones*; *Hymenoptera*: *Ichneumonidae*; other *Hymenoptera*; *Coleoptera*: *Coccinellidae*; *Lepidoptera*; *Neuroptera*; *Mecoptera* and *Diptera*: *Syrphidae*) were utilised as bioindicators. Arthropods were collected using yellow sticky traps. The ratio of pest: beneficial arthropods were used to evaluate the sustainability of the agroecosystem. The experimental olive grove was located in Southern Italy, in the middle of Mediterranean Basin. The abundance of *Araneae* and *Hymenoptera* was reduced by both treatments, while *Lepidoptera* were reduced only in the kaolin-treated plots. The ratio of pest: beneficial arthropods were unchanged after treatments. Results demonstrate that both tested products utilised against *Bactrocera oleae* showed few negative effects on beneficial arthropod populations. The taxon *Araneae* was identified as the best bioindicator, as these species were able to indicate well-preserved environmental conditions. More investigation on the efficacy of *B. bassiana* against the olive fly and on its impact on the above ground arthropods is required.

INTRODUCTION

The olive fly, *Bactrocera oleae* (Rossi, 1790) (*Diptera*: *Tephritidae*), is the key pest in olive groves, causing high economic damages for oil and table olive production. Many pest control compounds are available in organic olive farming against this pest, but recently some of them were found to be harmful to both human and environmental health. Iannotta et al. (2007a) found that rotenone and azadirachtin, the most utilised pesticides in organic olive crop protection, cause serious short term effects on non-target insects and ecosystem health. The European community encourages the use of alternative control strategies that have fewer negative impacts on human and environmental health. Repellents and microbiological agents seem to be very promising because of their selectivity and their expected low environmental impact on beneficial insects.

Kaolin (aluminum silicate [$Al_4Si_4O_{10}(OH)_8$], bright white colour, ≤2 mm particle diameter) is a repellent which forms a porous particle film on the olive tree and olive drupes and it seems to be very promising for the control of many invertebrate pests (Glenn et al., 1999; Puterka et al., 2000; Bürgel et al., 2005; Ortu et al., 2009), particularly the olive fly (Saour and Makee, 2004; Speranza et al., 2004; Iannotta et al., 2006). The efficacy of kaolin is explained by mechanisms acting at long and short distances. The long-distance efficacy occurs due to the unrecognisable colour of the white-coated canopy of olive trees (Prokopy and Haniotakis, 1975), while the short-distance efficacy is due to the decrease of oviposition punctures that results from behavioural difficulties of *B. oleae* gravid females (Saour and Makee, 2004). In short, although kaolin clay is chemically inert, its physical and mechanical properties make it efficacious against the olive fly. Although preliminary data about environmental impact of kaolin are available (Iannotta et al., 2007b), these data should be confirmed.

Beauveria bassiana (Bals.) Vuill. (*Moniliales*: *Deuteromycetes*) is an entomopathogenic fungus that grows naturally in soils and acts as a parasite on various insect species, causing white muscardine disease. It has been used as a biological insecticide to control a number of pests (Konstantopoulou and Mazomenos, 2005; Batta, 2007; Sabbahi et al., 2008), including the olive fly. The efficacy of *B. bassiana* against pests is due to two effects: 1) it acts as a contact bioinsecticide; 2) it is an oviposition deterrent, mainly on Tephritid flies. Studies assessing the effects of *B. bassiana* based bio-insecticides on non-target arthropods mainly have been carried out in tropical and desert areas where it is used against locusts (Danfa and van der Valk, 1999; Ivie et al., 2002), or on soil predator arthropods in more tropical environments (Devotto et al., 2007).

The aim of this study was to assess the environmental impact of these compounds on non-target arthropods of the olive tree canopy.

MATERIALS AND METHODS

Location and Experimental Field

The study area was located in the municipality of Terranova da Sibari (39°40’11.81” N, 16°23’25.11” E), Cosenza, Italy, at 40 m above sea level (Fig. 1). The experimental olive grove was 20 years old, drop irrigated and tilled. Three plots were chosen for the trials. Each plot was composed of 400 olive trees made up of three Italian cultivars (‘Carolea’, ‘Nocellara del Belice’, ‘Nocellara Messinese’) grown in alternate rows. One plot was treated on the 2nd of August and the 12th of September with kaolin (5 kg hl^{-1}) (Kaolin®, Progetto Geovita, Italy), one plot was treated on the 12th of September with a bioinsecticide based on the vital spores of the fungus *Beauveria bassiana* (150 ml hl^{-1}) (Naturalis®, Intrachem Italia), and one untreated plot was used as a control. The products were sprayed as liquid suspension by conventional spray equipment, in order to provide adequate and uniform canopy coverage.

Data Collection

Arthropods were collected using three yellow sticky traps per plot. This type of trap is usually used to monitor olive fly populations. Traps were placed in the centre of each plot, 20 metres apart, to avoid border effects. Data were collected every 10 days from July to November 2007. The sampled arthropods are known for their sensitivity to environmental alterations (Iannotta et al., 2007a). The abundance of *Araneae*, *Opiliones*, *Ichneumonidae*, other *Hymenoptera*, *Coccinellidae*, *Lepidoptera*, *Neuroptera*, *Mecoptera* and *Syrphidae* was recorded in detail.

Data Analysis

Data of abundance were normalised using the Density of Activity (DAa), taking in account the number of traps and the number of days of trap exposure (Brandmayr et al., 2005). As an abundance value, we also used dominance (Do), expressed as n_i/N, where n_i equalled the number of individuals belonging to the taxon i, N equalled the number of individuals of all taxa registered within a given community. Data were analysed using (a) the index of Coenotic Balance (CB) (Iannotta et al., 2007a), which measured the balance among functional units (feeding habits) of communities, and (b) Abbott’s formula (Abbott, 1925), which evaluated changes in insect populations in response to treatments.

In order to compute the Coenotic Balance, selected taxa were grouped into pest and beneficial arthropods. Among them, *Araneae*, *Opiliones*, *Ichneumonoidae*, *Coccinellidae*, *Neuroptera* and *Syrphidae* were grouped in the beneficials category (*A*), including predators and parasitoids, while other *Hymenoptera*, *Lepidoptera* and *Mecoptera* were grouped in the indifferent category (*I*), which included phytophagous arthropods and pollinators. As previously stated by Iannotta et al. (2007a), natural ecosystems are inhabited by more beneficial than indifferent arthropods, which represent the major part of the beneficial arthropods' prey. We assume that human activities alter this ratio, causing a relatively higher decrease of indifferent arthropods in a short time as compared with beneficial arthropods. Therefore, the Coenotic Balance ($CB_{I/A}$) was coded as follows: $CB_{I/A} = n_I/n_A$, where n_I equals the number of individuals belonging to indifferent arthropod taxa, and n_A equals the number of individuals belonging to beneficial taxa. Higher values are determined by better coenotic balances. A surrogate index of coenotic balance was computed based on *Hymenoptera* only ($CB_{hym/ichn}$). The superfamily *Ichneumonoidae* was chosen as a representative of beneficial taxa because it is relatively easy to identify. As a consequence, the surrogate index was: $CB_{hym/ichn} = n_{hym}/n_{ichn}$, where n_{hym} equalled the number of individuals belonging to *Hymenoptera*, and n_{ichn} equalled the number of individuals belonging to *Ichneumonidae*.

Abbott's formula was developed to calculate the effectiveness of an insecticide on target insects (Abbott, 1925) but has often been utilised to evaluate the impact of spray applications on non-target insects (Michaud and McKenzie, 2004). Abbott's formula compares the number of individuals belonging to a given taxon found after the treatment in a treated plot with the number of individuals found after the treatment in an untreated plot. In other words, this formula measures the percentage reduction of arthropod groups as a consequence of treatments.

RESULTS

Arthropod Abundance

We sampled 667 individuals belonging to the selected taxa. Among them, the individuals belonging to the group other *Hymenoptera* were the most abundant (n = 202), while only one individual belonging to *Opiliones* was collected. The other taxa collected with an appreciable abundance were *Lepidoptera* (n = 176) and *Ichneumonidae* (n = 102). The beneficials (n = 283) were less abundant than indifferent arthropods (n = 384). Although the method was biased toward fliers, an appreciable number of *Araneae* was collected (n = 42). The order *Hymenoptera* (other *Hymenoptera* + *Ichneumonidae*) represented almost the half of the total sampled arthropods (Fig. 2).

The selected taxa were found in all plots, with the exception of *Opiliones*, which was collected only from the untreated plot, but numbers were too low (n = 1) for further consideration. In the untreated and kaolin-treated plots the more abundant arthropods were *Hymenoptera*, followed by *Lepidoptera*, while in the Naturalis-treated plot, *Lepidoptera* were the most abundant, followed by *Hymenoptera* (Table 1). *Araneae* showed a two-fold value of density of activity in the untreated plot in comparison with treated ones, but no significant differences were found among plots for any arthropod group.

Abbott's formula showed the negative effects of kaolin on *Araneae*, other *Hymenoptera* and *Lepidoptera*, whilst the micoinsecticide Naturalis reduced the abundance of *Araneae* and other *Hymenoptera* (Table 2). *Araneae* showed the strongest decrease in the plots treated with Naturalis, while other *Hymenoptera* were more affected by kaolin. *Lepidoptera* were only slightly affected by kaolin, but no negative effects were observed in the Naturalis-treated plot. The other arthropods were not reduced by any treatments.

Coenotic Balance

The coenotic balance computed using all considered groups attained the highest value in the untreated plot and the lowest in the Naturalis-treated plot, but no significant differences were found (Table 3). The coenotic balance computed using only *Hymenoptera* confirmed that the untreated plot showed the highest balance value, but indicated a very low balance in the Naturalis-treated plot (Table 3).

DISCUSSION

The environmental impact of the bioinsecticide based on spores of *Beauveria bassiana* (Danfa and van der Valk, 1999; Ivie et al., 2002; Batta, 2007; Devotto et al., 2007) and of the particle film based on kaolin (Pasqualini et al., 2002; Iannotta et al., 2007b) were not yet available in the literature, but we evaluated the impact of these compounds in the olive ecosystem. This agroecosystem showed a low resilience, and any kind of disturbance of the equilibrium could easily create unbalances that would be difficult to recover. The results confirm that tested compounds can be utilised in organic olive farming. In fact, the total abundance of arthropods was not reduced by treatments. Danfa and van der Valk (1999) found a high mortality rate of hymenopteran parasitoids exposed under laboratory conditions to an isolate of *B. bassiana*. Devotto et al. (2007) showed that under field conditions *B. bassiana* spores had very little effect on soil arthropod predators. Regardless, these results showed fewer negative effects than broad-spectrum insecticide use. Ivie et al. (2002) found only minimal detrimental effects on the biodiversity of non-target beetles for a Malagasy isolate of *B. bassiana*. Our data confirmed these conclusions, based on field evidence of arthropods of the olive tree canopy. In fact, only a detailed analysis was able to detect slight differences between treated and untreated plots; however, the highest coenotic balance was recorded in the untreated plots.

The structure of arthropod assemblage collected in this study confirms data already available in the literature for olive groves (Petacchi and Minnocci, 1994). *Hymenoptera* was the most abundant taxonomic group as reported by Iannotta et al. (2007a). Although the collection method was biased towards flying arthropods that were attracted to the colour yellow, enough *Araneae* (walkers) were also collected to perform an analysis of abundance.

Naturalis and Kaolin had a negative impact only on *Araneae* and other *Hymenoptera*, two groups which live in a close relationship with the substratum. *Araneae* are visual predators which have difficulty moving due to kaolin, and more easily come in contact with *B. bassiana* spores. *Hymenoptera*, similar to *Lepidoptera*, are mainly flower-visiting insects that have difficulties in finding and utilising alimentary sources within the kaolin-treated plot.

The tested preparations had no evident negative effects on the arthropods of olive groves. The high efficacy against the olive fly and the low environmental impact of kaolin are confirmed by several authors (Iannotta et al., 2007b); however, further studies on the efficacy against the olive fly and impact at the soil level for *B. bassiana* in olive groves are needed. Furthermore, this research confirms *Araneae* as useful bioindicators of sustainable agroecosystem management.

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Literature Cited

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265−267.

Batta, Y.A. 2007. Biocontrol of almond bark beetle (*Scolytus amygdali* Geurin-Meneville, *Coleoptera*: *Scolytidae*) using *Beauveria bassiana* (Bals.) Vuill. (*Deuteromycotina*: *Hyphomycetes*). J. Appl. Microbiol. 103(5):1406−1414.

Brandmayr, P., Zetto, T. and Pizzolotto, R. 2005. I Coleotteri Carabidi per la valutazione ambientale e la conservazione della biodiversità [Carabid beetles for environmental evaluations and biodiversity conservation]. APAT, Manuali e linea guida, 34, Roma, Italy. (in Italian)

Bürgel, K., Daniel, C. and Wyss, E. 2005. Effects of autumn kaolin treatments on the rosy apple aphid, *Dysaphis plantaginea* (Pass.) and possible modes of action. J. Appl. Entomol. 129:311−314.

Danfa, A. and van der Valk, H. 1999. Laboratory testing of *Metarhizium* spp. and *Beauveria bassiana* on Sahelian non-target arthropods. Biocontrol Sci. Techn. 9:187−198.

Devotto, L., Carrillo, R., Cisternas, E. and Gerding, M. 2007. Effects of lambda-cyhalothrin and *Beauveria bassiana* spores on abundance of Chilean soil surface predators, especially spiders and carabid beetles. Pedobiologia 51(1):65−73.

Glenn, D.M., Puterka, G.J., Vanderzwet, T., Byers, R.E. and Feldhake, C. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92:759−771.

Iannotta, N., Belfiore, T., Noce, M.E., Perri, L. and Scalercio, S. 2006. Efficacy of products allowed in organic olive farming against *Bactrocera oleae* (Gmel.). Proc. Olivebioteq 2006, 5−10 Nov., Mazara del Vallo, Marsala, Italy. 2:323-326.

Iannotta, N., Belfiore, T., Brandmayr, P., Noce, M.E. and Scalercio, S. 2007a. Evaluation of the impact on entomocoenosis of active agents allowed in organic olive farming against *Bactrocera oleae* (Gmelin, 1790). J. Environ. Sci. Hlth. B 42:783−788.

Iannotta, N., Belfiore, T., Noce, M.E., Scalercio, S. and Vizzarri, V. 2007b. Environmental impact of kaolin treatments on the arthropod fauna of the olive ecosystem. p.291−298. In: A.A.M. Del Re, E. Capri, G. Fragoulis and M. Trevisan (eds.), Environmental Fate and Ecological Effects of Pesticides. La Goliardica Pavese, Pavia, Italy.

Ivie, M.A., Pollock, D.A., Gustafson, D.L., Rasolomandimby, J., Ivie, L.L. and Swearingen, W.D. 2002. Field-based evaluation of biopesticide impacts on native biodiversity: Malagasy Coleoptera and anti-locust entomopathogenic fungi. J. Econ. Entomol. 95(4):651−660.

Konstantopoulou, M.A. and Mazomenos, B.E. 2005. Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. Biocontrol 50(2):293−305.

Michaud, J.P. and McKenzie, C.L. 2004. Safety of a novel insecticide, sucrose octanoate, to beneficial insects in Florida citrus. Fla. Entomol. 87(1):6−9.

Ortu, S., Cocco, A. and Dau, R. 2009. Evaluation of the entomopathogenic fungus Beauveria bassiana strain ATCC 74040 for the management of Ceratitis capitata. Bull. Insectol. 62(2):245−252.

Pasqualini, E., Civolani, S. and Corelli Grappadelli, L. 2002. Particle Film Technology: approach for a biorational control of *Cacopsylla pyri* (*Rhynchota Psyllidae*) in Northern Italy. Bull. Insectol. 55(1−2):39−42.

Petacchi, R. and Minnocci, A. 1994. Impact of different *Bactrocera oleae* (Gmel.) control strategies on olive grove entomofauna. Acta Hort. 356:399−402.

Prokopy, R.J. and Haniotakis, G.E. 1975. Responses of wild and laboratory-cultured *Dacus oleae* to host plant color. Ann. Entomol. Soc. Am. 68:73−77.

Puterka, G.J., Glenn, D.M., Sekutowski, G., Unruh, T.R. and Jones, S.K. 2000. Progress toward liquid formulations of particle film for insect and disease control in pear. Environ. Entomol. 29:329−339.

Sabbahi, R., Merzouki, A. and Guertin, C. 2008. Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the tarnished plant bug, *Lygus lineolaris* L., in strawberries. J. Appl. Entomol. 132(2):124−134.

Saour, G. and Makee, H. 2004. A kaolin-based particle film for suppression of the olive fruit fly *Bactrocera oleae* Gmelin (*Dip*., *Tephritidae*) in olive groves. J. Appl. Entomol. 128:28−31.

Speranza, S., Bellocchi, G. and Pucci, C. 2004. IPM trials on attract-and-kill mixtures against the olive fly *Bactrocera oleae* (*Diptera Tephritidae*). Bull. Insectol. 57(2):111−115.

Tables

Table 1. Abundance of sampled Arthropods as density of activity (DAa) and dominance (Do).

	Kaolin		Naturalis		Control	
	DAa	Do	DAa	Do	DAa	Do
Araneae	0.33	4.3	0.37	5.0	0.7	9.9
Opiliones	0	0	0	0.0	0.03	0.5
Ichneumonidae	1.33	17.2	1.3	17.6	0.97	13.7
Other *Hymenoptera*	2.47	31.8	1.77	23.9	2.5	35.4
Coccinellidae	0.57	7.3	0.7	9.5	0.6	8.5
Lepidoptera	1.7	21.9	2.33	31.5	1.83	25.9
Neuroptera	0.07	0.9	0.2	2.7	0.07	0.9
Mecoptera	0.03	0.4	0.13	1.8	0.03	0.5
Syrphidae	1.27	16.3	0.6	8.1	0.33	4.7

Table 2. Percentage of reduction of selected taxa caused by treatments measured by Abbott's formula. Arthropod groups not reported in this table did not show negative effects caused by treatments.

	Kaolin	Naturalis
Araneae	57.6	72.7
Other *Hymenoptera*	39.0	35.5
Lepidoptera	7.2	-

- : no reduction observed.

Table 3. Results of the evaluation of Coenotic Balance computed using all sampled arthropods ($CB_{I/A}$) and only the order *Hymenoptera* ($CB_{hym/ichn}$).

	Kaolin	Naturalis	Control
$CB_{I/A}$	1.57	1.48	1.62
$CB_{hym/ichn}$	2.24	1.36	2.56

Figures

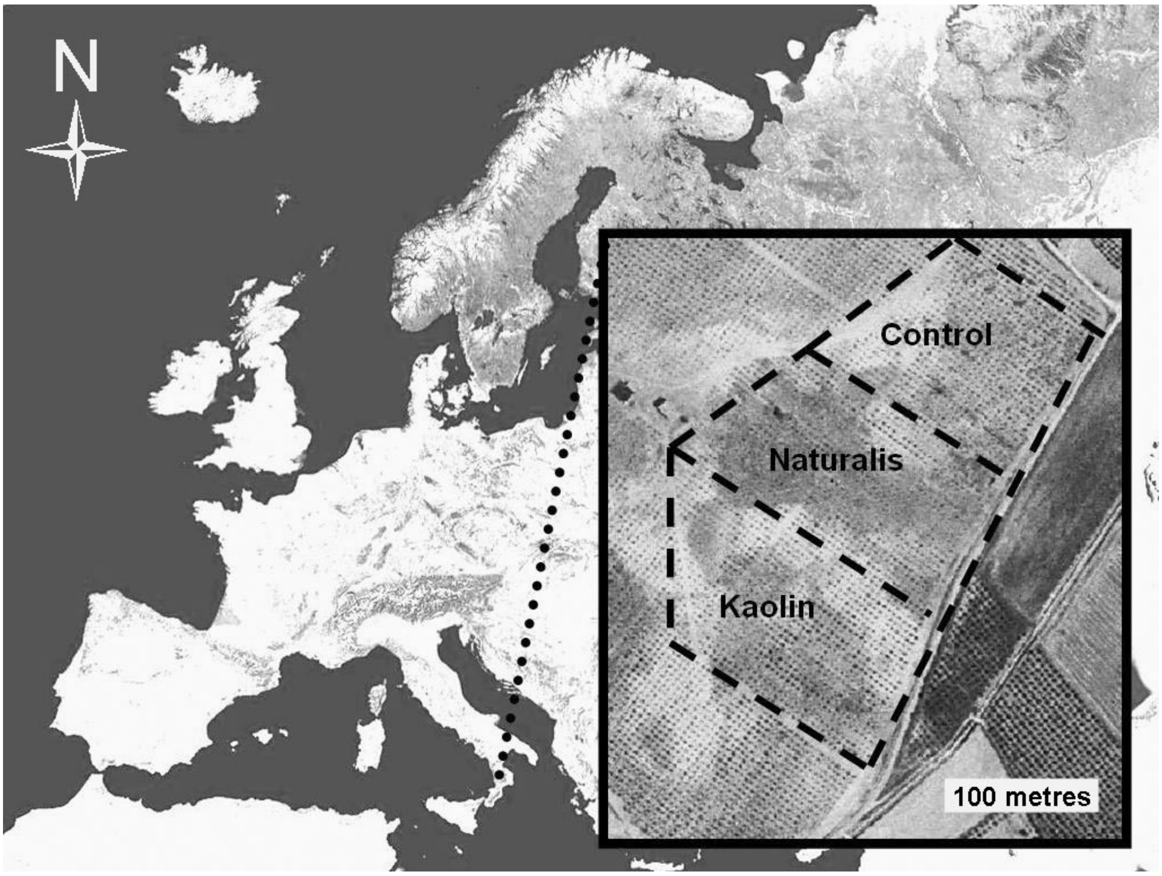

Fig. 1. Location of study area and experimental plots.

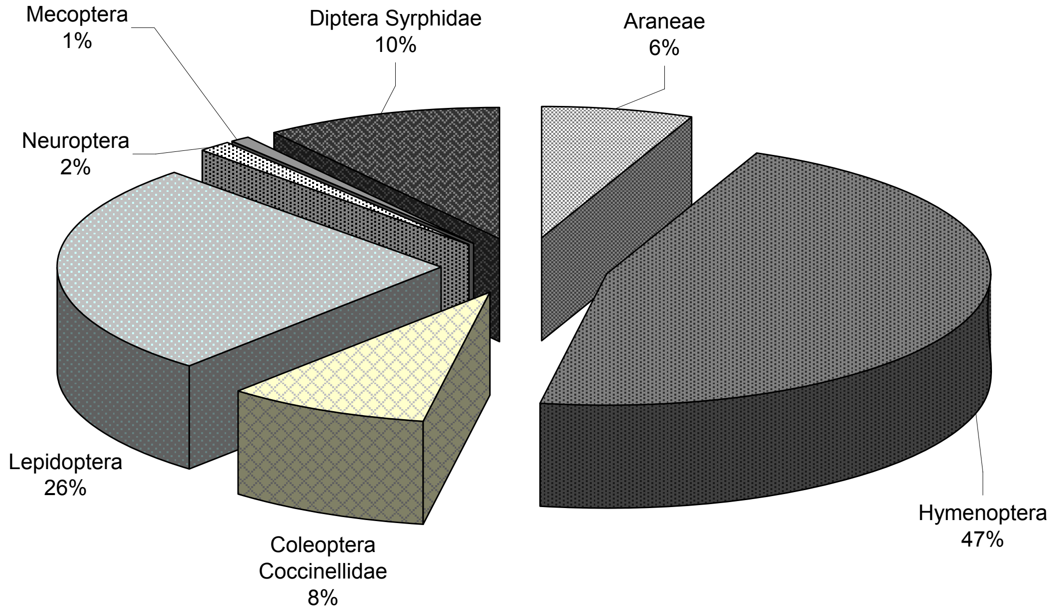

Fig. 2. Percentage of individuals collected, belonging to the different taxonomic groups. *Opiliones* were not reported because of their small populations.