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FULL LENGTH ARTICLE

First record and bionomics of the mycophagous ladybird *Psyllobora bisoctonotata* (Mulsant) (Coleoptera: Coccinellidae) in Sudan

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Abstract Powdery mildews caused by several fungi, particularly of the genera *Erysiphe*, *Sphaerotheca* and *Leveillula*, are destructive diseases of various cultivated and wild plants during winter season (December–March) in Sudan. Application of synthetic fungicides is the only control measure practiced. Fortuitously, during a field survey, congregations of coccinellid adults and grubs were found associated with powdery mildews on a wild plant, *Xanthium brasilicum* Vell., locally known as "Ramtouk", at Shambat area/Khartoum North. Therefore, the insect was subjected to some bionomical studies conducted during winter season 2011/12. Emphasis was devoted to morphometric investigations, supported with life cycle durations and seasonal trend on the foregoing host. Accordingly, the species was recognized as *Psyllobora bisoctonotata* (Muls.), a well known powdery mildew feeder in several countries. This is the first record of a mycophagous insect in Sudan. Hence, the important morphological features of the different stages were presented. The rearing of the insect on powdery mildew infected Ramtouk leaves in the laboratory (27.50 ± 3.75 °C and 15.83 ± 3.82% R.H.) revealed that the mean total lifecycle from egg to adult was 25.28 ± 1.57 days. The insect appeared on *X. brasilicum* in a very small number in December, peaked in February, and then gradually declined thereafter coinciding with an increase in temperature and a decrease in powdery mildew infection. So far, the real distribution of this mycophagous species and its host range are waiting for more investigations. Meticulous bio-ecological studies are important to ascertain the proper habitat for such insect throughout the year, and to evaluate its potential role as a biocontrol agent for the powdery mildews.

1. Introduction

The obligate biotrophic fungi of the family Erysiphaceae (Ascomycota: Erysiphales), commonly known as powdery mildews, are one of the most destructive pathogens infecting thousands of plant species worldwide. Economic yield losses due to

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powdery mildew infection were reported in various crops within several families including for instances, Asteraceae, Malvaceae, Cucurbitaceae, Verbenaceae, Solanaceae and Leguminosae, besides many cereals and fruit trees (Hasan, 1974; Amano, 1986; English-Loeb et al., 2007; Khodaparast and Abbasi, 2009). In Sudan, powdery mildew species, particularly of the genera *Erysiphe*, *Sphaerotheca*, *Leveillula* and others, are known to cause significant damage to several economic crops and natural flora, exclusively during winter season between December and March (Giha, 1987; Mohamed et al., 1995; Ahmed et al., 2001). Application of synthetic fungicides is the only means of control practiced, though studies in this field have proved that most of the powdery mildew diseases on important crops had already conferred resistance to the majority of these chemicals (Omer, 1972; McGrath, 2001). Moreover, a high cost of chemical control reported from elsewhere (Raja, 2010), besides the various drawbacks of chemicals on the environment and beneficial organisms (Sutherland et al., 2010), may put several questions on the significance of this approach.

However, numerous mycophagous insects and mites, and mycotoxicant fungi seem to offer promising potential to combat powdery mildews as biocontrol agents (Sutherland and Parrella, 2006; English-Loeb et al., 2007; Sutherland and Parrella, 2009a,b; Raja, 2010). In this respect, Sutherland and Parrella (2009a) referred to the mycophagy as an important and unique ecological niche for their role in natural control of plant pests and diseases. These authors stressed the members of the tribe Psylloborini (Halyziini), under the family Coccinellidae, as to possess several attributes conducive to biological control of powdery mildews; including host specificity and strong aggregative response to host density. Among the genera of this tribe, the genus *Psyllobora* Chevrolat, constitutes several species that are obligate feeders on all life stages (conidia, conidiophores, hyphae, cleistothecia, etc.) of Erysiphales powdery mildews. Although, they preferred powdery mildews, such bioagents were recently detected in rare cases to feed on other pathogenic Basidiomycota such as the rust fungus, *Phakopsora euvitis* (Culik et al., 2011), and many saprophytic fungi like *Alternaria* spp., *Cladosporium* spp. and *Curvularia* spp. (Patankar et al., 2009). The cosmopolitan distribution of *Psyllobora*, particularly in temperate and subtropical regions including several Arabian and African countries, besides their wide host range on different plants (Khan et al., 2007; Joshi and Sharma, 2008; Ahmad et al., 2003, 2009; Sutherland and Parrella, 2009b), may suggest their importance in ecological balance and natural control of the powdery mildews.

Therefore, studies are in progress worldwide to evaluate *Psyllobora* spp. for utilization in biological control against powdery mildews. Nevertheless, no research data are available in Sudan regarding mycophagous insects. Fortunately during a field survey before one year, an unfamiliar insect species was accidentally observed feeding on powdery mildews infecting *Xanthium brasilicum* Vell., locally known as “Ramtouk” (Family: Compositae), at Shambat area, Khartoum State. Accordingly, this research work was put forward to identify the insect species based on certain bionomical studies. The main parameter included morphometric investigation, supported with life cycle durations and population trend during winter season.

2. Materials and methods

2.1. Morphological studies

Adults of the mycophagous insect were collected from wild fields of *X. brasilicum* at Shambat area, Khartoum North, during February 2012. Plant leaves occupied with adults were cautiously picked, placed in Petri dishes and transported to the laboratory. The collected adults were reared in Petri dishes to get different developmental stages for various studies. They were provided with powdery mildew infected leaves of *X. brasilicum* for feeding. Samples from all stages of the insect were preserved in 70% alcohol to be used in morphological studies. Following metamorphosis of the insect, the prominent morphological characteristics regarding external shapes and colours of the different stages were closely observed and described under a stereo microscope. However, a binocular microscope provided with a camera (Labomed) and an eyepiece micrometer was used to take photos and measurements (length X width) for the various stages and important morphological parts. The body measurements of each larval stage were accompanied with head capsule measurements. The antennae of male and female adults and body hairs of the 1st larval stage were also measured. Additional photos were taken whenever possible, using Olympus digital camera X-785, 7.1 megapixels. A scale bar applicable to the whole plates was added using ImageJ program. The recorded data helped to identify the species in the light of available literature.

2.2. Life cycle studies

The life cycle durations of the mycophagous insect were followed under laboratory conditions at the Environment and Natural Resources Research Institute, Khartoum. The experiment was conducted during late winter season (mid February–mid April, 2012). The daily maximum and minimum room temperatures and relative humidity were recorded regularly; hence the overall means were worked out for the study period. Sexed pairs of the adults collected from the field were enclosed in Petri dishes and provided with leaves of *X. brasilicum* infected with powdery mildews for feeding and egg laying. The egg batches laid were transferred separately into new dishes to follow the incubation period. Upon hatching, ten newly emerged larvae were transferred separately in Petri dishes to track the durations of the different stages until the death of all adults. Such larvae were also provided with infected leaves of *X. brasilicum*, renewed daily, to secure feeding. The rearing dishes were always lined with semi wetted filter paper to delay drying of leaves. The Petri dish contents were inspected twice a day (early morning and evening) to check for moulting skins. Accordingly, the incubation, larval instars, pre-pupa, pupa and adult means durations were designated. Moreover, some data pertained to the egg stage (No. eggs/batch and hatchability) were also added.

2.3. Field investigations

Sporadic field investigations were carried out on the wild plant, *X. brasilicum*, at Shambat area/Khartoum North during

2011/12. However, due to the fact that both the mycophagous insect and powdery mildews were disappeared from the plant between May and November (i.e., summer and autumn seasons), the insect counts were restricted merely to winter season (December–April). Meanwhile, some field observations on the insect habits and distribution on the plant leaves were recorded. For instance, the distribution percentages of egg batches between upper and lower leaf surfaces were compared. Simultaneously, the population numbers were counted on five randomly selected plants. All encountered stages (egg batches, larvae, pupae and adults) of the insect were counted, and then the insect mean numbers per plant were indicated. Accordingly, the monthly population means of this natural enemy were depicted for the winter period.

3. Results and discussion

3.1. Morphological studies

The results of the external measurements (mm) of all life cycle stages of the insect were presented in Table 1, in addition to some coloured plates in Fig. 1. The egg was the smallest stage (0.75 ± 0.03 × 0.36 ± 0.01) measured in the life cycle. It was oval in shape, nearly tapered and white yellowish or creamy in colour when freshly laid. As a result of embryonic development, the egg colour changed gradually to dark yellowish or dark creamy and lastly dark greyish before hatching, where the two eyes appeared as black spots on the upper portion (Fig. 1c). Upon hatching, the larval body showed gradual increases in sizes from the 1st instar (1.01 ± 0.08 × 0.40 ± 0.02) up to the 4th one (4.60 ± 0.21 × 1.50 ± 0.14), corresponding to a similar trend in sizes of head capsules. The larval body size seemed to be affected by the amount of food consumed, as some variations were detected among replicates. The first instar was almost homogeneously greyish or light brown without coloured markings and covered with long hairs. The second instar was also greyish, but manifested longitudinal rows of numerous black dots from head to caudal end, and two faint yellowish spots located dorsally on the anterior body segment. Such black dots were mostly associated with the bases of hairs. The third and fourth instars were yellowish grey and yellowish or greyish creamy, respectively. They were similar to the previous instar with respect to the hairs and black dots, but the two dorsal faint

Table 1 The mean measurements (mm) of the different stages of *Psyllobora bisoctonotata*.

Measurements (mm)/Life stage	Body (Mean ± S.D)		Head capsule (Mean ± S.D)	
	Length	Width	Length	Width
Eggs*	0.75 ± 0.03	0.36 ± 0.01		
1st instar	1.01 ± 0.08	0.40 ± 0.02	0.20 ± 0.01	0.26 ± 0.02
2nd instar	1.91 ± 1.10	0.69 ± 0.31	0.25 ± 0.00	0.33 ± 0.03
3rd instar	3.64 ± 0.53	1.13 ± 0.32	0.38 ± 0.01	0.60 ± 0.03
4th instar	4.60 ± 0.21	1.50 ± 0.14	0.54 ± 0.05	0.62 ± 0.01
Pupa	2.61 ± 0.41	1.62 ± 0.18		
Adult ♂	3.08 ± 0.25	2.19 ± 0.13		
Adult ♀	3.43 ± 0.13	2.40 ± 0.21		
Antenna (11 segments)♂	0.83 ± 0.00			
Antenna (11 segments)♀	0.88 ± 0.00			
1st instar hairs	0.15 ± 0.02			

* More information on egg stage is given in Table 2.

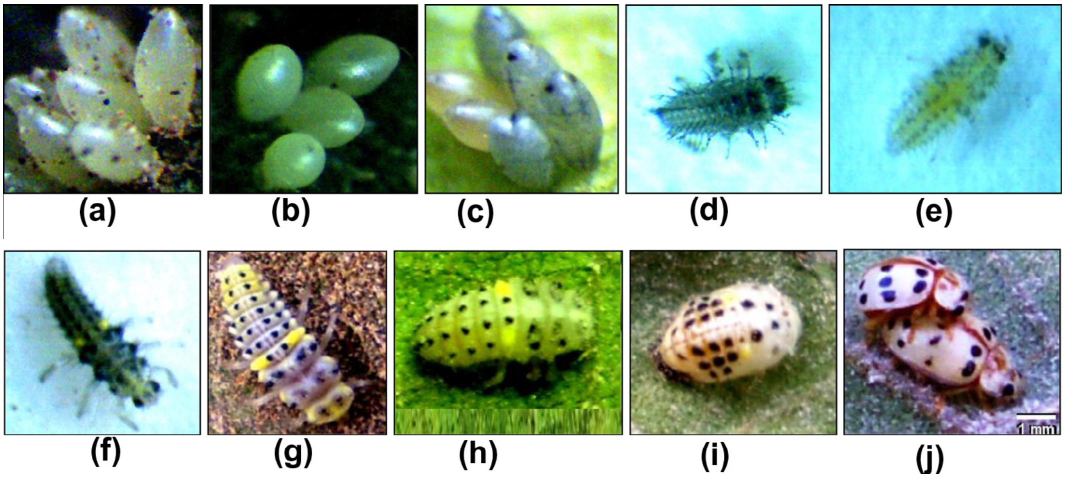

Figure 1 Stages of *Psyllobora bisoctonotata*: (a–c) Egg batches at different ages, (d–g) First, second, third and fourth larval instars, respectively, (h) Pre-pupa, (i) Pupa and (j) Mating male and female adults.

spots became more prominent and bright yellowish in these instars. However, all larval instars were elongated in shape, slightly tapered posteriorly and carried numerous hairs on the whole body. But, these hairs looked as if they were longer and denser in the first instar (0.15 ± 0.02) due to the smallbody size. The 4th instar represented the largest stage measured in the life cycle lengthwise, as the insect size was greatly diminished at the pupal stage (2.61 ± 0.41 × 1.62 ± 0.18). The pre-pupa, almost analogous to the fourth instar larva, moulted in an exarate pupa type. The pupa was yellowish creamy to greyish in colour, decorated dorsally with numerous distinctive black spots besides the two yellowish spots, as stated for the larvae. Adults were relatively larger than pupae, with the male being smaller than the female. They were nearly oval in shape with convex dorsum, yellowish brown abdomen and white creamy elytra and head shield. Each elytron was found to be marked with eight black spots arranged in four rows (2:3:2:1; front to back), to make a total of 16 spots per two elytra. These spots were more or less corresponding with those spots observed on larval stages as mentioned above, but with some reduction in number. The adult head was broader than long (like in larvae), carried two black compound eyes and clubbed antennae with 11 segments. Hence, the current morphological descriptions, which largely came in consistency with previous literature (Omkar and Pervez, 2004; Joshi and Sharma, 2008; Kumar et al., 2010), have proved the detection of the *Psyllobora bisoctonotata* (Mulsant) (Coleoptera: Coccinellidae) as the first record of a mycophagous insect in Sudan.

3.2. Life cycle studies

The data regarding the number of eggs per batch and hatchability levels are presented in Table 2. The female *P. bisoctonotata* was found to insert its eggs vertically on plant leaves. The eggs were mainly deposited attached together in groups, but in few cases laid singly. A mean of 6.00 ± 2.86 eggs/batch was obtained, while the most frequent numbers detected were 4, 7 and 10 eggs/batch. However, few un-hatched eggs (< 10%) were attributed largely to some infertile eggs in each batch, or sometimes to certain adverse environmental factors. Some authors stated that coccinellid beetles generally deposit extra infertile eggs with the fertile eggs (Banks, 1956; Perry and Roitberg, 2005).

Durations of different life cycle stages of *P. bisoctonotata* under laboratory conditions were indicated. The average room temperature and relative humidity during the study period were recorded to be 27.50 ± 3.75 °C and 15.83 ± 3.82%, respectively (Table 2). The results of different durations are presented in Table 3. Newly emerged adults stayed about 3.50 ± 0.58 days on an average before start of egg laying. In most instances, the eggs in each batch were not hatched together, though were simultaneously laid. According to this study an egg batch took between 1–3 days to complete hatching of all viable eggs. The egg stage was followed by four larval instars, and the last larval stage entered a short resting period (1.29 ± 0.25 days) as non feeding pre-pupa before moulting into pupa. It is clear that the incubation took longer time (6.00 ± 0.82) than each of the subsequent pre-adult stages. Also, there was a gradual increase in the life durations from the 1st larval instar (2.80 ± 0.27) up to the 4th (4.71 ± 0.51). However, the total egg to adult period was 25.28 ± 1.57 days, in an average, which is nearly similar to what has been reported by Ahmad et al. (2003). The average adult longevity of male was shorter than that of the female. In general, the detected trends in the life cycle durations were found to agree with some previous studies on *P. bisoctonotata* (Ahmad et al., 2003; Kumar et al., 2010), thus confirming the identity of the present species. Nevertheless, the current durations were mostly shorter than those obtained by Kumar et al. (2010) in India. The reasons could be related to differences in several factors such as climatic conditions and the species of powdery mildews fed upon. According to Ahmad et al. (2009), *Psyllobora vigintiduopunctata* (L.) showed different life cycle durations when reared on various species of powdery mildews.

As microscopically observed from the laboratory rearing, the larvae, except the first instar, seemed to be the most voracious stages in the life cycle of the insect. They used to wipe out large areas of powdery mildews in rearing dishes,

Table 2 Some aspects on egg stage of *Psyllobora bisoctonotata* and laboratory climatic conditions, during the study period (winter 2011/12).

Aspects on egg stage		Laboratory conditions	
Eggs/batch (mean)	6.00 ± 2.86	Temperature (mean)	27.50 ± 3.75 °C
Egg/batch (range)	1–11 eggs	Temperature (range)	21–34 °C
Egg batch (hatching period)	1–3 days	R.H. (mean)	15.83 ± 3.82%
Hatchability level	90.63%	R.H. (range)	10–24%

Table 3 The mean durations (day) of the different life cycle stages of *Psyllobora bisoctonotata*, during late winter period (mid February-mid April) 2012.

Life stage	Mean(±S.D)	Life stage	Mean(±S.D)
Pre-oviposition	3.50 ± 0.58	Pre-pupa	1.29 ± 0.25
Incubation	6.00 ± 0.82	Pupa	4.60 ± 0.38
1st instar	2.80 ± 0.27	Total egg-adult period	25.28 ± 1.57
2nd instar	2.88 ± 0.57	Adult longevity ♂	18.83 ± 0.68
3rd instar	3.00 ± 0.88	Adult longevity ♀	23.67 ± 0.73
4th instar	4.71 ± 0.51		

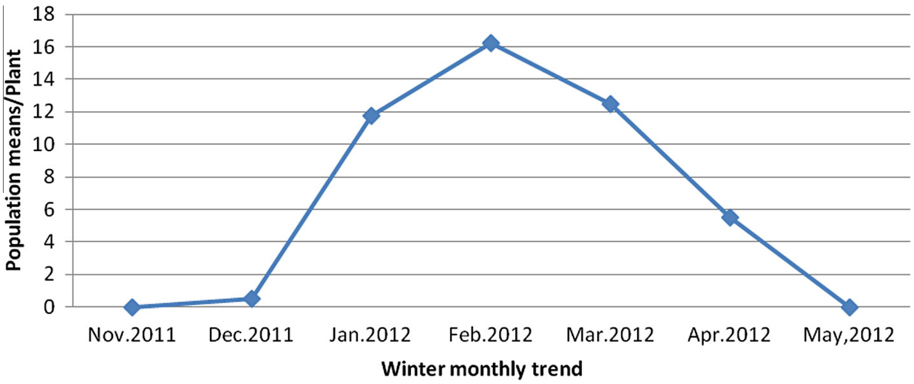

Figure 2 The population means trend of *Psyllobora bisoctonotata* recorded on *Xanthium brasilicum* at Shambat area (Khartoum North), during winter season 2011/12.

hence necessitated daily renewal of infected leaves to secure feedings. This attribute might support the potential role of *P. bisoctonotata* in biological control of powdery mildews. Yigit and Soylu (2002) reported that each *P. bisoctonotata* individual larva (from the first to the fourth instar) consumed an average of 12.32 cm^2 *Erysiphe cichoracearum* powdery mildew infected area on okra leaves, whereas an average adult consumption was measured as 1.54 cm^2. Moreover, investigations conducted abroad also revealed that *P. bisoctonotata* induced a significant decrease in powdery mildew infected leaf area which reached up to 92%, as compared with the control (Soylu et al., 2002; Sutherland and Parrella, 2006).

3.3. Field investigations

The wild plant, *X. brasilicum*, was observed to be the most susceptible host plant to powdery mildews under field situations. It acquired the disease early in the season, and showed symptoms for an extremely long period extending mainly to the onset of summer season, thus providing an attractive habitat for mycophagous insects. However, several species of powdery mildews (e.g., *E. cichoracearum*, *Sphaerotheca fuliginea* and *Podosphaera xanthii*) were reported to infect this plant and other members of Compositae worldwide (Kenneth and Palti, 1984; Kachooeian-Javadi et al., 2006; Mir et al., 2012). Studies proved that there are different olfactory cues (e.g., 1-octen-3-ol) emitted by powdery mildew infected plants that guide foraging by mycophagous insects (Tabata et al., 2011). Currently, the plant occupying a vast area at the River Nile bank of Shambat, where the present insect (*P. bisoctonotata*) was encountered. According to the field surveys all stages of *P. bisoctonotata* were detected on this plant, concentrating mainly on lower sides of the leaves. For instance, counts of eggs detected per five plants showed that 80% of batches were confined to the lower sides of leaves, where the subsequent emerged larvae equally pupated. This may be referred to the relatively higher densities of powdery mildews on lower sides of leaf blades. Such leaf sides also seemed to protect the different stages of the insect from sun heat particularly during the day time.

The results of the monthly mean population counts of *P. bisoctonotata* on *X. brasilicum* during winter 2011/12 are depicted in Fig. 2. The insect was scarcely found early in the season (November–December), but increased rapidly with the start of January. It showed its peak population level in February (16.25 ± 2.22 insects/plant), then gradually declined towards the end of the season and disappeared between May and November. This diminishing trend was found to be coincided with two factors, namely the increasing temperature and declining powdery mildew infection. Accordingly, the host was free of any powdery mildew infection during May–November; meanwhile the insect was expected to seek other habitats which may provide alternative foods. Therefore, more meticulous studies are needed to cover various bio-ecological aspects on this newly discovered mycophagous insect. These may include; surveys of other host plants in different locations so as to find out those habitats where the insect spends the summer and autumn seasons, besides monitoring of population dynamics on the major cultivated crop harbouring the insect. However, a part from the impact of climatic conditions on the activity of *P. bisoctonotata* appeared to be the effect on food availability. In Syria, this mycophagous species was reported to be abundant from April until November, a period of high powdery mildew prevalence on various plants (Ahmad et al., 2003), whereas in India, it showed the highest levels in December and January as the disease also appeared in an epidemic state (Kumar et al., 2010). Hence, the obtained results were nearly similar to what have been recorded in India, where cold weather and mildew infection seemed to start a little bit earlier with more severities and longer durations (Kumar et al., 2010).

4. Conclusion

The current findings revealed the presence of *P. bisoctonotata* as an important mycophagous insect among the beneficial fauna of the Sudan. The species is expected to be more abundant in cooler areas with extended winter season, where high incidences of powdery mildews occur for longer periods. Therefore, additional investigations are required to cover the various aspects of this insect, and to evaluate its potential role in combating the disease. Since powdery mildew control is completely dependent on chemical fungicides, despite its economic burden and numerous disadvantages, the results may enhance a new trend in disease management through biological control and other ecologically sound components.

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