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ŠTEFANA MICHIELIJA

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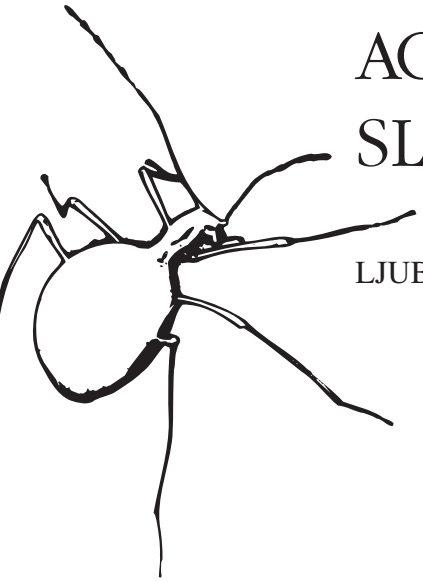

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Vsebina / Contents

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ACTA ENTOMOLOGICA SLOVENICA

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NEW RECORDS AND DISTRIBUTION OF THREATENED *CARABUS* (*VARIOLOSUS*) *NODULOSUS* CREUTZER, 1799 IN BOSNIA AND HERZEGOVINA (COLEOPTERA: CARABIDAE)

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Abstract – *Carabus* (*variolosus*) *nodulosus* Creutzer, 1799 is a species of European conservation concern listed in the Annexes II and IV of the Habitats Directive. In Bosnia and Herzegovina (BiH) it is a rare species, up till now known mainly from historic records. In this paper the authors summarize for Bosnia and Herzegovina all the available data of *C.* (*v.*) *nodulosus* from literature, museum and private collections. In total, records from 19 localities are presented, including records of five new populations from central, eastern and northern part of the country. New populations were found in wet habitats along the banks of streams in deciduous and mixed forests at altitudes between 390 and 1.190 m a.s.l. An old record from Trebević Mt. at 1.600 m a.s.l. represents the highest reported altitude for the species. Considering the large number of potentially suitable habitats, especially in the northern and eastern part of BiH, it is expected to confirm a wider distribution of the species in the future. The habitat characteristics of the new finding sites are in accordance with the ecological needs of the species described in detail in literature. The distribution of the species in the West Balkan area (former Yugoslavia countries) is outlined in the paper and a short discussion on the conservation status of the species in Bosnia and Herzegovina is appended.

KEY WORDS: Coleoptera, beetles, Natura 2000, Habitats Directive, Western Balkans

Izvleček – NOVI PODATKI IN RAZŠIRJENOST OGROŽENEGA MOČVIRSKEGA KREŠIČA, *CARABUS* (*VARIOLOSUS*) *NODULOSUS*, V BOSNI IN HERCEGOVINI (COLEOPTERA: CARABIDAE)

Močvirski krešič *Carabus* (*variolosus*) *nodulosus* Creutzer, 1799 je evropsko zavarovana vrsta, uvrščena na Prilogi II in IV Direktive o habitatih. Močvirski krešič je v Bosni in Hercegovini (BiH) redka vrsta, do sedaj večinoma znana le iz zgodovinskih podatkov. V prispevku podajamo vse podatke vrste za BiH, iz literature, muzejskih in osebnih zbirk. Predstavljeni so podatki za 19 lokalitet, vključno s podatki za pet novih populacij v osrednjem, vzhodnem in severnem delu države. Nove populacije smo našli v vlažnih habitatih na bregovih potokov v listnatem ali mešanem gozdu na višini med 390 in 1.190 m n.m.v. Zgodovinski podatek iz Trebevića s 1600 m n.m.v. predstavlja najvišjo znano lokaliteto za vrsto. V prihodnje pričakujemo povečanje znanega območja razširjenosti vrste v državi, zlasti v severnem in vzhodnem delu, kjer je veliko število potencialno primernih habitatov. Lastnosti habitata novih najdb sovpadajo z ekološko potrebo vrste, podrobno opisano v literaturi. V prispevku komentiramo tudi razširjenost vrste na zahodnem Balkanu (v bivših Jugoslovanskih republikah) in njeno varstvo v BiH.

Ključne besede: Coleoptera, hrošči, Natura 2000, Direktiva o habitatih, Zahodni Balkan

Introduction

Carabus variolosus (sensu lato) is an endemic European ground beetle belonging to the subgenus *Hygrocarabus* Thomson, 1845. The subgenus includes two (sub)species: *variolosus* Fabricius, 1787 and *nodulosus* Creutzer, 1799. The status of two closely related species or subspecies of one species isn't yet clear (Müller-Kroehling 2006). Although some authors are inclined towards the status of "sister species" because the differences in the male genital morphology and the non-overlapping ranges (e.g. Casale et al. 1982, Turin et al. 2003), the majority of authors favoured the subspecific rank of *C. nodulosus* (e.g. Březina 1994, 1999, Müller-Kroehling 2006). The European Carabologist's Meeting issued a statement in 2007 calling for inclusion of subspecies *nodulosus* in the interpretation of annex species *C. variolosus*. *Carabus variolosus* (sensu lato) is species of European conservation concern and one of 38 beetle species listed in the Annexes II and IV of the Habitats Directive (HD) (Council Directive 92/43/EC 1992, Council Directive 2013/17/EU 2013). At the time when *C. variolosus* was added to the Annexes II and IV of the HD in 2004 both taxa, *variolosus* and *nodulosus*, were regarded as subspecies to *C. variolosus* (Müller-Kroehling 2006, 2014). ETC-BD (2011) accepts that in context of the HD *Carabus variolosus* encompasses the species in the broader sense and includes *Carabus* (*variolosus*) *nodulosus*. The status of taxon *nodulosus* as Natura 2000 species is a subject of debate and additional studies are needed to clarify this issue (e.g. Müller-Kroehling 2014, Turin et al. 2003).

Since the taxonomic status of the species is not yet clear and this work has faunistic purposes we decided to treat this taxon as *Carabus (variolosus) nodulosus*. The specimens were inspected personally by I. Rapuzzi and ID keys by Turin et al. 2003 were used for the identification.

The ranges of these two taxa are separated. *C.* (*v.*) *nodulosus* has mostly central European distribution, compared to the eastern European distribution of *C.* (*v.*) *variolosus*. The distribution of both taxa is presented in Fig. 1. The species distribution in the western Balkans (Former Yugoslavia) is still insufficiently known. In this region the populations of *C.* (*v.*) *nodulosus* are mostly found in the Dinaric mountain region (Breuning 1926, Turin et al. 2003, Vigna Taglianti 2013). Slovenia lies in the core range of distribution of the species and most probably presents the global population stronghold with currently known at last of 200 localities (Vrezec et al. 2012, 2015). In Croatia *C.* (*v.*) *nodulosus* is mainly distributed in the north and east part of the country in mountain and hill areas, rare and localized in the western part (Gorski Kotar; Istria: Učka Mountain) (e.g. Breuning 1926, Rukavina et al. 2010, Turin et al. 2003). For Serbia it is known only from the east: Užice; Sokolska Planina (collection I. R.), while in Vojvodina (Fruška gora), central and eastern Serbia, *C.* (*v.*) *variolosus* is found (Müller-Kroehling 2014, Turin et al. 2003). The southernmost known locality of *C.* (*v.*) *nodulosus* is from Ljuboten on the Šar planina Mt., a mountain peak on the border between Kosovo and Macedonia (Turin et al. 2003).

In regard to morphology and life history, both species are very similar. In contrast to *C.* (*v.*) *variolosus*, *C.* (*v.*) *nodulosus* is slightly larger having relatively broader

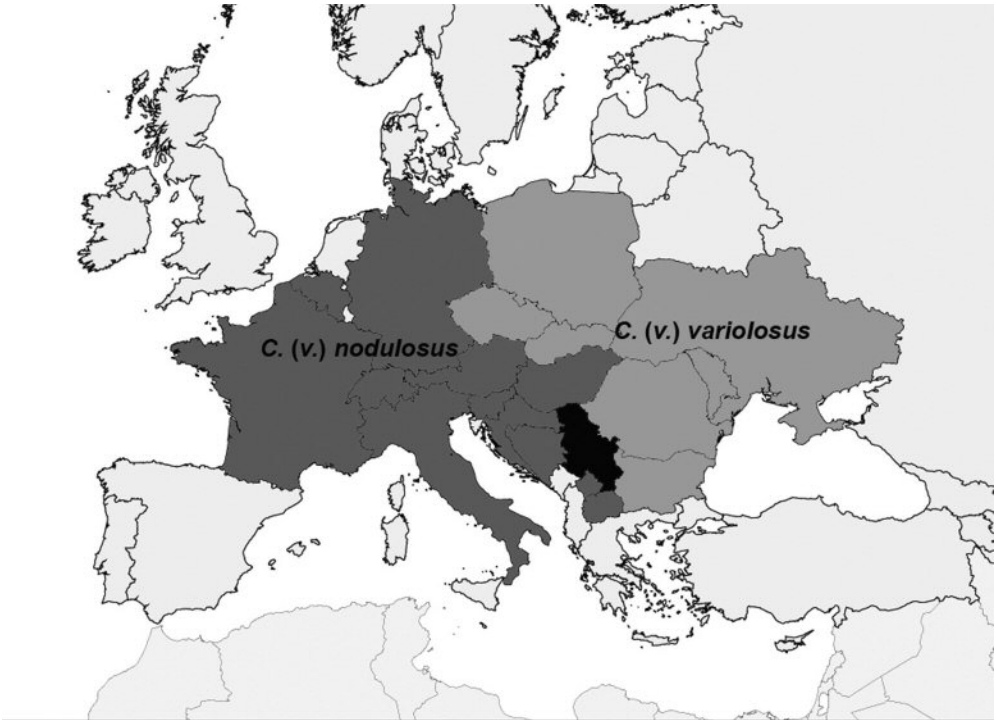

Fig. 1: Distribution of *Carabus* (*variolosus*) *variolosus* and *C.* (*variolosus*) *nodulosus* in Europe (light grey – *C.* (*v.*) *variolosus*; dark grey – *C.* (*v.*) *nodulosus*; black – both taxa; (Breuning 1926, Turin et al. 2003, Vigna Taglianti 2013).

body with smoother elytral sculpture and different male genital morphology (for details see Casale & Kryzhanovskij 2003).

The first written report of *C.* (*v.*) *nodulosus* for BiH was given by Möllendorff (1873), but he did not include any specific locality. The species was also mentioned for Bosnia by Reitter (1885), who collected various beetles around Sarajevo and Nemila in 1884. Reitter (1896) established var. *hydrophilus* for the *C.* (*v.*) *nodulosus* specimens from Bosnia (furthermore without indicating a more detailed locality), that was later synonymized by Breuning (1926).

Apfelbeck (1890) reported one specimen from Trebević Mt. and few years later in addition to Trebević he also said that more specimens are known from south and central Bosnia (Apfelbeck 1894). In his book „Käferfauna der Balkanhalbinsel" he mentions Travnik, Kiseljak, Sarajevo and Višegrad localities (Apfelbeck 1904). The same localities were listed by Gligić (1942) in his paper on the genus Carabus in BiH. Breuning (1926) reports the species from eight localities in BiH: Travnik, Kiseljak, Sarajevo, Višegrad, Preslica plan., Ivan plan., Žepče and Jablanica. In Drešković et al. (2011) the results of the country's first Natura 2000 project were summarised and five species records were listed: Travnik, Kiseljak, Sarajevo, Igman Mt. and Višegrad.

The aim of this paper is to present current knowledge of the distribution of *Carabus* (*variolosus*) *nodulosus* in BiH in order to support the establishment of Natura 2000 network and stimulate new research in the country.

Material and methods

Historical data were gathered from available literature: Apfelbeck 1890, 1894, 1904, Breuning 1926, Gligić 1942, Möllendorff 1873, Reitter 1885 and entomological collections: Biological Museum, Lund University (MZLU), Slovenian Museum of Natural History (PMSL), Zologische Staatssammlung Munich (ZSM) and private collection of A. Casale (Torino, Italia). The most important source of data were the collections of the National Museum of Bosnia and Herzegovina (NMBiH), particularly the entomological collection of Balkan Peninsula, the most significant insect collections for the country with app. 500.000 specimens gathered by Victor Apfelbeck at the end of XIX and the beginning of XX century (Hlaváč & Vít 2008, Kulijer & Marinov 2010, Mihajlova et al. 2008).

This paper also discusses results from two Natura 2000 projects that were implemented in the country (Anonymous 2014, Drešković et al. 2011).

In addition to historic data the paper presents five new records collected by authors from 2015 to 2017 (Fig. 2). New records were gathered during carabidological research in the Balkan Peninsula (I. Rapuzzi) or they represent random findings (A. Vesnić, D. Kulijer). Specimens from Majevica Mt., Motajica Mt. and Srebrenica are deposited in I. Rapuzzi's collection (Prepotto, Italy) specimens from Konjuh Mt. are deposited in the entomological collections of NMBiH while specimen from Vareš is deposited at the Faculty of Science in Sarajevo.

Old records obtained from literature and collections were often not accurate about the location, giving only the name of mountain or neighbouring town. In these cases the centre of town or the highest peak of the mountain is shown on the map.

Study area

Bosnia and Herzegovina is located in the western part of the Balkan Peninsula. It is predominantly mountainous country which's central and the largest part is occupied by Dinaric Alps, mountain chain that spreads from Slovenia to Albania, forming the largest continuous karst landscape in Europe. To the north of the mountain region lies the lowland region of Posavina, with the lower reaches of several large rivers and the Sava river that forms the natural border with Croatia. To the south the karstic mountains descend gradually towards the Adriatic Sea (Redžić et al. 2008).

Climate of the country is highly variable, with Mediterranean climate dominating in the south, alpine climate in the areas of high mountains and continental in the northern, lowland part of the country (Redžić et al. 2008). The country territory lies within the three biogeographical regions: Continental, Alpine and Mediterranean (EEA 2016).

Results

Material examined:

New data: Loc. 1. Vareš, Okolište, N 44.216323° E 18.266306°, 31/IV/2015, 1.190 m a.s.l., 1 adult, leg. & det. A. Vesnić; **Loc. 2.** Konjuh Mt., Mala Zlača River valley, N 44.336944° E 18.556389°, 06-07/V/2017, 390 - 540 m a.s.l., 4 adults, leg. & det. D. Kulijer & A. Vesnić (Fig. 3a); **Loc. 3.** Majevica Mt., Veselinovac env., N 44.581944° E 18.805000°, VII/2017, 450 m a.s.l., 2 adults, leg. & det. I. Rapuzzi & F. Kleinfeld (Fig. 3b); **Loc. 4.** Srebrenica, Karačići env., VII/2017, 700 m a.s.l., 1 adult, leg. & det. I. Rapuzzi; **Loc. 5.** Motajica Mt., Srbac env., VII/2016, 500 m a.s.l., 1 adult, leg. & det. I. Rapuzzi.

Collection and museum data: Loc. 6. Bjelašnica Mt., Kradenik, 01/IX/1974 (2 adults); 18/IX/1974 (1 adult); 11/IX/1976 (1 adult); 10/X/1976 (1 adult), 850 m a.s.l., coll. Mihljević, NMBiH; **Loc. 7.** Busovača, Tisovac, 08/V/1966, 1 adult, coll. Mihljević, NMBiH; **Loc. 8.** Jablanica, 1901, 1 adult, coll. MZLU; 1902, 11 adults, leg. Wgth., coll. Apfelbeck, NMBiH; 03/XI/1970, 1 adult, coll. Casale, A. (Torino, Italia); 1 adult, 1903 ZSM; **Loc. 9.** Ivan Mt., 1 adult, leg. Apfelbeck, coll. Apfelbeck, NMBiH; **Loc. 10.** Travnik env., 1 adult, leg. Brandis, coll. Apfelbeck, NMBiH; 1 adult, leg. Geschwind, coll. Apfelbeck, NMBiH; 04/XI/1970, 6 adults, leg. & det. Casale, A., coll. Casale, A. (Torino, Italia); **Loc. 11.** Sarajevo, Rečica, 1 adult, coll. Apfelbeck, NMBiH; **Loc. 12.** Trebević Mt., 3 adults, leg. Apfelbeck, coll. Apfelbeck, Apfelbeck, NMBiH; **Loc. 13.** Čemerno (pass between Foča and Gacko), 07/XI/1971, 2 adults, leg. & det. Casale, A., coll. Casale, A. (Torino, Italia); **Loc. 14.** Višegrad, Semeć, 1 adult, coll. Apfelbeck, NMBiH; **Loc. 15.** Nemila, 1 adult, leg. & det. Scheibel, PMSL.

Literature data only: Loc. 16. Žepče (Breuning 1926); **Loc. 17.** Kiseljak (Apfelbeck 1904, Drešković et al. 2011, Gligić 1942); **Loc. 18.** Igman (Drešković et al. 2011); **Loc. 19.** Preslica Mt. (Breuning 1926).

Discussion

Based on the records from museum collections and literature data *Carabus* (*v.*) *nodulosus* is historically known from 14 localities in BiH. With our five new records the total number of the localities for the country is 19, located in two biogeographical regions of the country, Alpine and Continental (Fig. 2). Out of 33 specimens in the

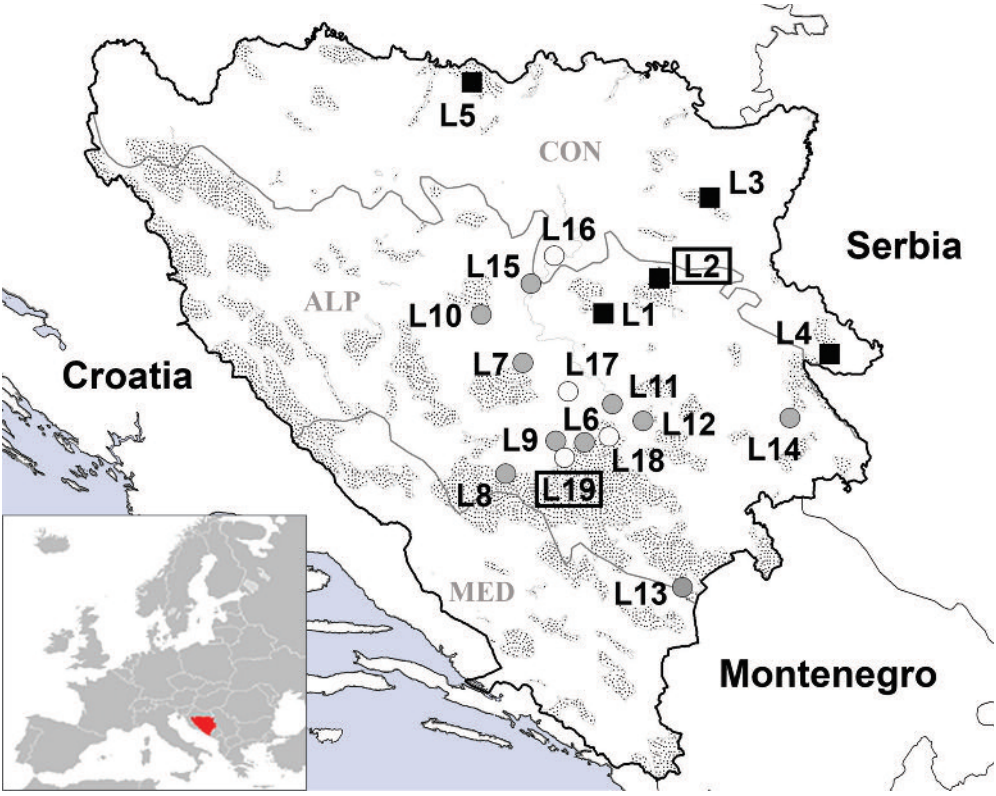

Fig. 2: The distribution of *Carabus* (*variolosus*) *nodulosus* Creutzer, 1799 in Bosnia and Herzegovina. The records from the entomological collections are marked with gray circles, records found in literature only with white circles and new data with black squares. Light gray lines on the map indicate the border between biogeographical regions: Continental (CON), Alpine (ALP) and Mediterranean (MED) (EEA 2016). The numbers of localities situated within the borders of proposed Natura 2000 Network areas (shaded) are shown in black frames. Geographical position of Bosnia and Herzegovina in Europe is shown on smaller map.

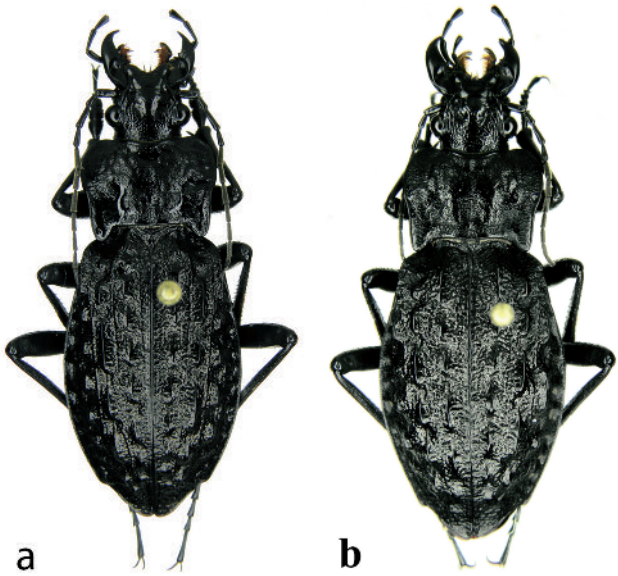

Fig. 3: Male of *Carabus* (*variolosus*) *nodulosus* from Konjuh Mt. (a) and female from Motajica Mt. (b) (photo: I. Rapuzzi)

collection of Viktor Apfelbeck in NMBiH, 20 originate from BiH. For the specimen with label "Damjanović", the name probably represents the name of the collector, not a locality name, while one specimen only has label „Bosnien (Reitter. Leder.)". In addition to the Apfelbecks collection, six specimens of *C.* (*v.*) *nodulosus* from two localities in Bosnia are found in the insect collection of Boro Mihljević that it is also stored in the NMBiH. These specimens were collected in 1966 (Busovača) and 1974/76 (Bjelašnica Mt.). A specimen from Jablanica collected in 1901 is stored in the collection of the Biological Museum, Lund University (MZLU) and one individual from Nemila is stored in the collections of the Slovenian Museum of Natural History in Ljubljana (PMSL). One specimen labelled "Jablancici" collected in 1901 and stored in the collections of the Zologische Staatssammlung Munich (ZSM) probably originates from Jablanica (north Herzegovina), as location with the name Jablancici could not be found in BiH and one specimen from Jablanica collected in 1901 is also present in the ZSM, as well as in MZLU collections. Additional specimens from three localities are stored in the collection of A. Casale (Torino, Italia).

Carabus (*variolosus*) *nodulosus* is stenotopic, silvicol and strongly hygrophilous species that inhabits fringes of water bodies and swampy areas in broadleaved and mixed forests (e.g. Casale et al. 1982, Sturani 1962, 1964, Turin et al. 2003). According to Pavičević et al. (1997) it inhabits various deciduous and conifer forest communities in hills and mountains. In Italy the species was reported from streams within a mixed secondary and tertiary deciduous beech forest (Rapuzzi, 2016), while in Croatia it was found at a brook in the beech forest (*Seslerio autumnalis-Fagetum* (Ht.) M. Wraber ex Borhidi 1963) (Rukavina et al. 2010). In north–west Germany the species is found in ancient woodland complexes with many near-natural springs and running waters in several types of deciduous forest (Matern et al. 2007), while in Bavaria it

lives at intact brook margins, seepages, spring swamps and spring marshland in forests or in natural marsh reedlands and occasionally in acidic bogs (Müller-Kroehling 2014).

Our observations of the species habitat in Bosnia and Herzegovina correspond to the descriptions available in literature. In the northern part of Konjuh Mt. the species was found along Mala Zlača River. The slopes on both sides of the river are covered with mixed beech, fir and spruce forest with the domination of broadleaved tree species at the finding sites and many small streams flowing down the hilly slopes (Fig. 5). The first individual was observed on a wet forest road in the evening, while additional three specimens were found during the day. Two were found under the logs in a small canalised stream along the road (Fig. 4) while the third specimen was found dead, trapped in a small dry concrete pool near the hotel, at the edge of forest. Observations distributed along the 5 km stretch of the river and the fact that four specimens were easily observed without target research, suggests the presence of good habitat for the species and possibly presence of larger population at this location.

At Vareš (Okolište) locality one specimen was found in mixed broadleaved and conifer forest (mixed forest of fir, spruce and beech) with trees over 20 meters high. The individual was found moving upstream through shallow water (2 – 5 cm deep) in small (50 cm wide) forest stream. The stream was on a slope with 5 – 10° inclination and south-west exposition. Many tree trunks that were left behind after last year forest logging were observed around the stream.

Fig. 4: *Carabus* (*variolosus*) *nodulosus* at Konjuh Mt. (photo: D. Kulijer)

Fig. 5: Habitat of *Carabus* (*variolosus*) *nodulosus* at Konjuh Mt. (May 7th 2017) (photo: D. Kulijer).

According to Turin et al. (2003) *C.* (*v.*) *variolosus* is distributed up to 1.000 m a.s.l. In Poland the highest known occurrence of *C.* (*v.*) *variolosus* is at 1.250 m a.s.l., but most localities are distributed from 300 to 700 m a.s.l. (Bobrek & Górska 2017). Similar altitudes were also reported for *C.* (*v.*) *nodulosus* (Matern et al. 2007, Rapuzzi 2016, Rukavina et al. 2010). New Italian population was found at altitudes between 430 and 500 m a.s.l. (Rapuzzi 2016).

In BiH new records are distributed between 390 (Konjuh Mt.) and 1.190 (Vareš) m a.s.l. For museum records, except records from Bjelašnica (850 m a.s.l.), the altitudinal data are not given and the localities are insufficiently precise for the extraction of even approximate altitudinal data. Interesting record is from Trebević Mt. where

Apfelbeck found one individual at 1.600 m a.s.l. (Apfelbeck 1890). This represents the highest locality in BiH and the highest observation of this species for the whole areal. Although the survey of the carabid fauna of this mountain and the surrounding area was conducted in 1980s, the species was not recorded (Tabaković-Tošić 1992).

Conservation reference

Carabus (*v.*) *nodulosus* is rare and threatened species across the whole distribution area that has lost significant part of its range, and the distribution within it (e.g. Turin et al. 2003). In the western part of its range main threats are drainage, brook regulation, forest fragmentation and habitat destruction (Müller-Kroehling 2014).

In Bosnia and Herzegovina *C.* (*v.*) *nodulosus* is not protected, neither it is included in the two existing red lists, The Red List of Protected Species of Flora and Fauna of Republika Srpska („Crvena lista zaštićenih vrsta flore i faune Republike Srpske") (Službeni glasnik Republike Srpske 2012) and Red List of wild species and subspecies of plants, animals and fungi („Crvena lista divljih vrsta i podvrsta biljaka, životinja i gljiva") in Federation of Bosnia and Herzegovina (Službeni list Federacije Bosne i Hercegovine 2014).

Two projects aiming to identify the presence and the distribution of habitats and species listed in Annexes I and II of the HD and prepare the proposition of the future Natura 2000 network in BiH, were conducted until now. The details are summarized by Milanović et al. (2015). *Carabus* (*v.*) *nodulosus* was included only in the first proposition of the country's Natura 2000 network when four sites were identified (Drešković et al. 2011). However, the species was excluded from the final reference list for BiH (Milanović et al. 2015). As shown in Fig. 2 only two localities of *C.* (*v.*) *nodulosus* fall within the borders of the currently proposed Natura 2000 network sites in BiH. Nevertheless, for most historical data the exact location could be only roughly determined and several localities are very close to the borders of proposed sites. The exact locations of these populations could possibly be within the borders of these sites, but this should be investigated, as well the possible current presence of the species in the area in general.

Although known data on the species distribution in BiH are mostly historical (most data were collected before 1930s), this cannot be regarded as a proof of disappearance of the species, but more likely illustrates the lack of recorders and investigations in the country. Recent discoveries of several new localities confirm this.

Considering that no research or monitoring exists in the country and that available data on the species are scarce and mostly very old, it is impossible to give reliable estimate of its threat status and populations trends in BiH. As in other parts of its range, the main threat for the species is the destruction of its habitats, while climate change, particularly in the south presents additional threat. However, Bosnia and Herzegovina lies in the central part of Dinaric Alps, large mountain chain that covers most of the country. This area is characterised with abundance of forest habitats and numerous streams and small rivers, particularly in altitudes between 200 and 1.000 m a.s.l. This zone occupies app. 62 % of country's territory (Lepirica 2009). These habitats that

are important for *C.* (*v.*) *nodulosus* are still well preserved so it can be expected that in BiH significant populations of this threatened European species are present and that its distribution in the country is much wider than currently known. It is probably present in all regions of the country apart from the driest part of the south Herzegovina region. Targeted research of potential habitats and the historical localities of the species should be one of the priorities of Natura 2000 process in the country.

We also expect that *C.* (*v.*) *nodulosus* is more common and has wider distribution in the western Balkan region and to find the species in Montenegro because the presence of suitable habitat and the geographical position just in between Bosnia and the population in Ljuboten. In BiH it was found at Čemerno (L13) that is app. 6 km from the Montenegro border. In Macedonia both (sub)species should be present in the country.

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FIRST RECORDS OF THE OAK LACE BUG *CORYTHUCHA ARCUATA* (SAY, 1832) (HEMIPTERA: TINGIDAE) IN NORTH MACEDONIA

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Abstract – The oak lace bug *Corythucha arcuata*, a Nearctic species, was first reported in Europe in 2000 in northern Italy. Since then it has spread to many European countries and regions, and there have been records of several recent outbreaks. We report the first find of *C. arcuata* in North Macedonia, one adult specimen, several nymphs and an egg cluster on a symptomatic leaf of *Quercus petraea* from the vicinity of Skopje, in July 2019. After inspecting additional 17 locations throughout North Macedonia we registered 3 more finds of the insect. We registered an egg cluster and nymphs on *Q. robur* var. *fastigiata*, and an egg cluster on *Q. cerris*, both in urban settings of Skopje. The only record further from Skopje was of an adult and nymphs registered on *Q. petraea*, some 25 km southeast, along a major road route. Although scarce and in low density, these few finds of *C. arcuata*, as well as recent records of its rapid expansion in Turkey, Bulgaria, Romania and outbreaks in Hungary and Russia, suggest that this insect pest will be found more consistently on a wider area in North Macedonia, and it potentially poses a health risk for oak species in the country.

Key words: Invasive species, alien species, *Quercus* spp., North Macedonia

Izvleček – PRVE NAJDBE HRASTOVE ČIPKARKE, *CORYTHUCHA ARCUATA* (SAY, 1832) (HEMIPTERA: TINGIDAE) V SEVERNI MAKEDONIJI

Hrastova čipkarka (*Corythucha arcuata*), nearktična vrsta, je bila v Evropi prvič opažena leta 2000 v severni Italiji. Od tedaj se je razširila v mnoge evropske države in območja, poročali so o več nedavnih izbruhih. Poročamo o prvi najdbi vrste *C. arcuata* v Severni Makedoniji, enem odraslem primerku, več ličinkah in skupini jajčec na značilno poškodovanem listu vrste *Quercus petraea* iz bližine Skopja, julija 2019. Ob pregledu dodatnih 17 lokacij po Severni Makedoniji smo zabeležili še tri najdbe.

Našli smo skupino jajčec in ličinke na vrsti *Q. robur* var. *fastigiata* in skupino jajčec na vrsti *Q. cerris*, oboje v urbanem okolju Skopja. Edina najdba izven Skopja so bili odrasel primerek in ličinke na vrsti *Q. petraea*, kakšnih 25 km jugovzhodno, ob glavni cesti. Čeprav redke in majhne gostote, nove najdbe vrste *C. arcuata*, ob nedavnih poročilih o hitrem širjenju v Turčiji, Bolgariji, Romuniji in izbruhih na Madžarskem in v Rusiji, kažejo na bodoče pogostejše pojavljanje tega žuželčjega škodljivca v večjem območju v Severni Makedoniji in postane lahko nevaren za zdravje hrastovih vrst v državi.

KLJUČNE BESEDE: Invazivne vrste, tujerodne vrste, *Quercus* spp., Severna Makedonija

Introduction

A Nearctic species, the oak lace bug *Corythucha arcuata* (Say, 1832) (Heteroptera: Tingidae), is one of 68 species of the genus *Corythucha* registered in the Western hemisphere and one of 49 registered in the USA (Neal and Douglass 1990). In its natural range in the USA there have been numerous early records of its presence in eastern (Gibson 1918) and central US, and west to Iowa (Osborn and Drake 1917) and southern Canada (Still *et al.* 1974; Emond and Still 1974).

Although it was first recorded in Europe in 2000, it was immediately obvious from the large areas of spread in the regions of Lombardy and Piedmont in Italy, that the insect was already present several years prior to its discovery (Bernardinelli and Zandigiacomo 2000). In 2002 it was reported in Switzerland (Forster *et al.* 2005) and already as far away as eastern parts of Turkey (Mutun 2003). In 2005 a find was reported in Iran (province of West Azarbayjan) (Samin and Linnavuori 2011); in 2012 it was recorded in Bulgaria (Dobreva *et al.* 2013); in 2013 in Croatia (Hrašovec *et al.* 2013), Hungary (Csóka *et al.* 2013) and Serbia (Pap *et al.* 2015); in 2015 in Russia (Karpun *et al.* 2018) and Romania (Don *et al.* 2016), and in 2016 in Slovenia (Jurc and Jurc 2017). For Bosnia and Herzegovina there are nearly simultaneous two first records in 2017, in June (Glavendekic and Vukovic Bojanovic 2017) and August (Dautbašić *et al.* 2018), while in Slovakia the oak lace bug was first recorded in 2018 (Zúbrik *et al.* 2018).

Reports of rapid expansion and larger areas of invaded territories in Italy (Bernardinelli 2007), Turkey (Mutun *et al.* 2009; Küçükbasmacı 2014), Bulgaria (Simov *et al.* 2018), Romania (Chireceanu *et al.* 2017), and outbreaks in Hungary (Csepelényi *et al.* 2017) and Russia (Neimorovets *et al.* 2017; Karpun *et al*. 2018) further underline the invasiveness and importance of this species and its potential to become a serious threat to oak forests in Europe and further.

Regarding plant hosts, *Quercus* spp., in particular species of the white oak group, are considered as the main hosts of *C. arcuata*. In the USA it has been registered on *Q. macrocarpa* (Drake and Ruhoff 1965) and as early as the beginning of the 20th century was considered as a serious pest on this host, causing discoloration and nearly total destruction of the leaves (Osborn and Drake 1917). This same tree species has also been reported as host in Canada (Still, Tidsbury, and Melvin 1974; Drake and

Ruhoff 1965). Other species reported as hosts in USA are *Q. muehlenbergii*, *Q. prinoides* and *Castanea americana* (*dentata*), and in Canada *Q. alba*, *Q. acuminata*, *Q. prinus* and *Q. rubra* (Drake and Ruhoff 1965).

In Europe, the oak lace bug has been registered on *Q. robur*, *Q. pubescens*, *Q. petraea*, presumed hybrids of *Q. robur* and *Q. petraea* (Bernardinelli and Zandigiacomo 2000), *Q. macranthera* and *Q. alba* (Csóka, Hirka, and Somlyai 2013), *Malus sylvestris* and *Ulmus minor* (Hrašovec *et al.* 2013).

In the Krasnodar region of Russia adults, nymphs and fifth-instar cases have been documented from the following species: *Q. hartwissiana, Q. pedunculiflora, Q. petraea, Q. pubescens, Q. robur, Prunus avium, Acer laetum* and *Robinia pseudoacacia* (Neimorovets *et al.* 2017).

Furthermore, Bernardinelli (2006) performed trials under controlled conditions in order to investigate plant host preference of the insect pest, as well as suitability of several plant hosts. In the trials, first instar nymphs were reared into adults on leaves of various plant species: *Q. pubescens*, *Q. petraea*, *Q. robur*, *Q. cerris*, *Rubus ulmifolius* and *Rubus idaeus* had the highest survival rate into adulthood (51–72%), while less than 25% reached adulthood on *Castanea sativa*, *Rosa canina* and *Rubus caesius*. In these same trials *C. arcuata* did not complete its life cycle when reared on leaves of *Q. suber*, *Q. ilex*, *Acer campestre*, *A. pseudoplatanus*, *A. platanoides*, *A. negundo* and *Malus domestica* and surprisingly on *Q. rubra*.

Also through trials in controlled conditions it has been presented that adults of *C. arcuata* avoid foliage grown under water deficit, when presented with a choice (Connor 1988). This finding could explain the environmental conditions for occurrence of outbreaks and might be used for prognosis and preventive control purposes.

Pure oak forests in the Republic of North Macedonia cover 289.973 ha (29,3%) of the 988.835 ha total area of forests (Statistical review – Forestry, 2013), and additionally oaks are in large percentage in 297.207 ha of mixed forests. The most prominent are *Q. petraea*, *Q. frainetto*, *Q. pubescens* and *Q. coccifera,* at least by volume of standing stock (Trajkov *et al.* 2016), with lesser presence of *Q. robur*, *Q. cerris*, *Q. ilex* and *Q. macedonica*. Since most of these species have been registered as hosts of C. *arcuata* and because of the fast spread in neighboring countries, our aim was to establish weather this alien insect species is present in Macedonia, and if so, to which extent, both in area coverage and in population density.

Materials and methods

Since the first record in July 2019 of a single adult specimen of *C. arcuata* near Skopje, until Sept 20th we inspected various sites of oak stands throughout North Macedonia. In total, we inspected 18 locations with occurrence of species of *Quercus*. We visually inspected oak leaves for general pathological symptoms and for symptoms specific for lace bug damage on the upper side, as well as for symptoms on the underside of leaves, specific for presence of egg clusters (Figure 1), adult specimens (Figure 2), nymphs and excrements (Figure 3) of oak lace bugs. For each visited location we recorded geographical coordinates. Finds with presence of specimens, or suspected

Fig. 1: Egg cluster of *C. arcuata* (photo Velian Jagev)

Fig. 2: Adult specimen of *C. arcuata* (photo Velian Jagev)

presence (eggs) were carefully collected and sealed in plastic bags and brought to the laboratory for detailed examination. Morphological identification was performed using various stereomicroscope magnifications (Zeiss, Stemi 305). Documentation photos of adults, nymphs and egg clusters were taken with Zeiss Axiocam 105 color camera.

Identification was based on the key of Forster *et al.* (2005) as well as on helpful notes in other references (Dobreva *et al.* 2013; Jurc and Jurc 2017; Hrašovec *et al.* 2013).

Specimens are stored in the entomological laboratory of prof. Nacheski at the Faculty of Forestry in Skopje.

Results and discussion

During an inspection of a mixed forest stand near Skopje with large presence of oak species, a single adult specimen, several nymphs and an egg cluster of *C. arcuata* were registered and collected from a leaf of *Quercus petraea* (41.928179°N, 21.521489°E; leg. Srebrova, 15.07.2019).

Additionally, in the timeframe of our research (15th July – 20th September 2019) we report of 3 other finds. We collected one living and one dead adult specimen on *Q. petraea* located on the road A1 E75 between Skopje and Veles (41.816788°N,

Fig. 3: Tar-like fecal deposit droplets, nymph and nymph molt of *C. arcuata* (photo Velian Jagev)

21.674875°E). We found 1 egg cluster of *C. arcuata* on a tree of *Q. robur* var. *fastigiata* within the arboretum of the Faculty of Forestry in Skopje (42.002590°N, 21.459605°E) and a single egg cluster on *Q. cerris* in urban setting in Skopje (41.991317°N, 21.444813°E).

All three oak species on which we have recorded presence of this alien insect pest are also mentioned by other authors as ones frequently infested, or pointed as main hosts (Bernardinelli 2006; Bernardinelli and Zandigiacomo 2000; Mutun, Ceyhan, and Sözen 2009; Dobreva *et al.* 2013; Hrašovec *et al*. 2013; Dautbašić *et al*. 2018).

All other inspections did not result in finds of this alien invasive insect pest, despite frequent observation of symptoms similar to ones of *C. arcuata*, which is discoloration and pale or chlorotic leaves.

Assaying physiological responses and biochemical parameters of *Q. robur,* Nikolić *et al.* (2019) infer that the rate of photosynthesis, transpiration and stomatal conductance were lowered in plants infested by *C. arcuata* by 59, 22 and 36%, respectively, when compared to non-infested plants, and that concentrations of photosynthetic pigments were also affected. This potential for damage to plant hosts, as well as its fast spread, tendency for outbreaks, the wide range of host and ecological preferences, designates *C. arcuata* as a very serious threat for oak species in Europe and Asia, with wide ranging environmental and economic implications. Furthermore, increase of frequency of stress events and adverse environmental factors due to global climate change, could be predisposing factors for easier spread and establishment of *C. arcuata* in populations of various oak species. On the other side, stress induced by oak lace bugs, especially in outbreak incidences, could be an additional factor for decline of oak populations and is a field for research in itself.

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CONTRIBUTION TO THE KNOWLEDGE OF NEUROPTERA FROM GEORGIA (SAKARTVELO)

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Abstract – Lacewing (Neuroptera) specimens collected during a short field trip in June 2018 in the Caucasian Republic of Georgia were examined. Fifteen species belonging to four families were found: Chrysopidae, Hemerobiidae, Coniopterygidae and Ascalaphidae. *Wesmaelius concinnus* and *Aleuropteryx loewii* are new species for the fauna of Georgia.

KEY WORDS: Neuroptera, faunistics, Georgia, Caucasus region

Izvleček – PRISPEVEK K POZNAVANJU MREŽEKRILCEV (NEUROPTERA) IZ GRUZIJE (SAKARTVELO)

Pregledali smo primerke mrežekrilcev (Neuroptera), ki so jih zbrali na kratkem terenskem delu v juniju 2018 v kavkaški republiki Gruziji. Ugotovljenih je bilo 15 vrst iz družin Chrysopidae, Hemerobiidae, Coniopterygidae in Ascalaphidae, med katerimi sta dve – *Wesmaelius concinnus* in *Aleuropteryx loewii* novi za favno Gruzije.

KLJUČNE BESEDE: Neuroptera, favnistika, Gruzija, Kavkaz

Introduction

In recent period, a number of papers on Neuropterid insects (Neuropterida: Megaloptera, Raphidioptera, Neuroptera) in the larger Caucasus region have been published (for review, see Dobosz et al. 2017, 2018). Neuropterids were in Georgia (native:

Sakartvelo) relatively well investigated, when compared to other countries of the larger Caucasus region. So far, 79 Neuroptera species were known from the country (reviews: Shengelia 1947, Aspöck et al. 2001, Duelli et al. 2015, Dobosz et al. 2017, 2018).

In June 2018, Slovenian zoologists Boris Kryštufek, Alenka Kryštufek, Tina Klenovšek and Franc Janžekovič conducted a field trip to Georgia with the goal to study endemic mole vole *Prometheomys schaposchnikowi*. Two of them (TK, FJ) occasionally collected neuropteran insects there. The aim of the paper is to present a list of lacewing species collected in the trip.

Material and methods

Lacewings were collected during the day with insect net and at night visiting the lights of buildings. Specimens are deposited in the first author's collection.

Results and discussion

During a four-days sampling period 68 individuals in 15 species were collected.

A list of species

Chrysopidae

***Chrysopa perla* (Linnaeus, 1758)**
Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 1 ♀.

***Chrysopa dorsalis* Burmeister, 1839**
Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ♀.

***Chrysopa fuscostigma* Esben-Petersen, 1933**
Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 2 ♀♀ (Fig. 1).
This species with characteristic head markings (Fig. 1a) was described by Esben-Petersen (1933) from Georgia. Later, the species was reported for the larger Caucasus region (Dorokhova 1979, Zakharenko 1984, 1986, Makarkin & Shchurov 2015) and Anatolia (Turkey) (Aspöck et al. 2001, Canbulat 2007, Arı 2014). Its recent occurrence in the country was confirmed by Dobosz et al. (2018).

***Pseudomallada prasinus* (Burmeister, 1839) – *'marianus'* sensu Duelli & Obrist 2019**
Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ♂.

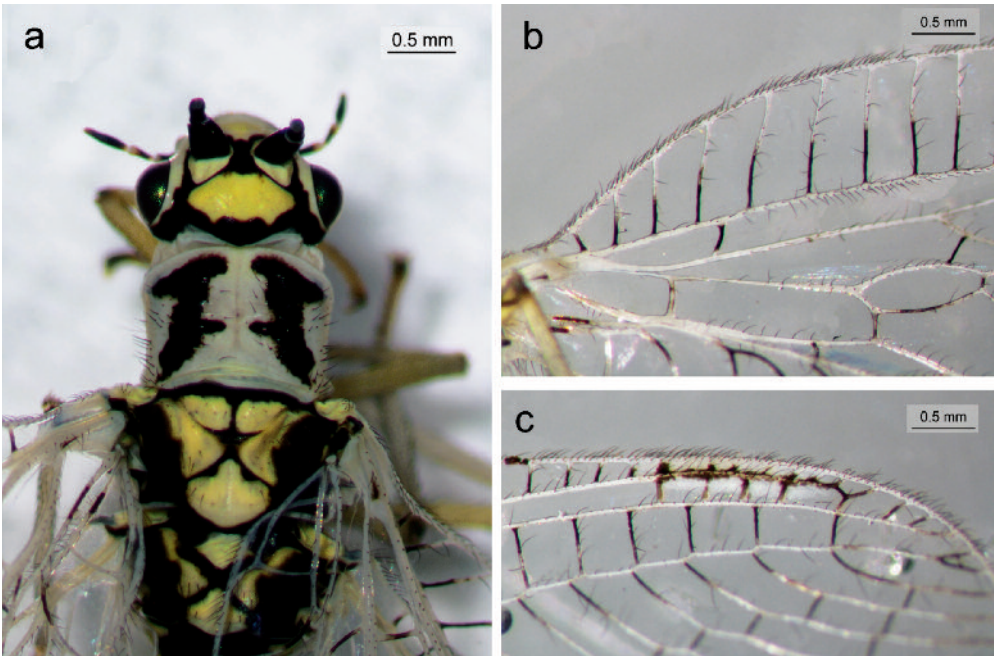

Fig. 1. Green lacewing *Chrysopa fuscostigma*: a, head and thorax, dorsal view: b, base of the forewing; c, pterostigma in the forewing. Photo D. Devetak.

Lesser Caucasus Mountains. Skhvilisi; mixed forest; 1080 m; 41°38'57.5"N 42°56'53.9"E; 23.VI.2018; 2 ♀♀.

Saparlo; meadow-bushes; 938 m; 41°17'34.3"N 44°19'07.3"E; 23.VI.2018; 1 ♂.

Bolnisi; pasture; 504 m; 41°26'56.9"N 44°36'47.2"E; 24.VI.2018; 1 ♀.

Just recently, Duelli & Obrist (2019) clarified taxonomic status of the *prasinus* group in the genus *Pseudomallada*. Freshly preserved individuals showed typical head morphology, without any colouration of the subantennal suture above the black genal stripe (Duelli & Obrist 2019).

***Chrysoperla* cf. *carnea* (Stephens, 1836) s.str.**

Greater Caucasus Mountains. Gudauri; meadow-pasture; 2258 m; 42°29'28.1"N 44°27'58.0"E; 20.-21.VI.2018; 1 ♂ 1 ♀.

Greater Caucasus Mountains. Gudauri; village, meadow-pasture; 2190 m; 42°28'41.4"N 44°28'41.2"E; 21.VI.2018; 4 ♂♂ 20 ♀♀.

Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 2 ♀♀.

Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ♂ 5 ♀♀.

Lesser Caucasus Mountains. Skhvilisi; mixed forest; 1080 m; 41°38'57.5"N 42°56'53.9"E; 23.VI.2018; 1 ♀.

Lesser Caucasus Mountains. Atskuri; mixed forest; 886 m; 41°44'40.1"N 43°11'57.5"E; 23.VI.2018; 1 ♂ 1 ♀.

This is a common green lacewing, reaching high altitudes. In the Gudauri area (Fig. 2), the species was collected even at 2258 m a.s.l.

***Chrysoperla* cf. *agilis* Henry, Brooks, Duelli & Johnson, 2003**

Greater Caucasus Mountains. Gudauri; village, meadow-pasture; 2190 m; 42°28'41.4"N 44°28'41.2"E; 21.VI.2018; 2 ♀♀.

Saparlo; meadow-bushes; 938 m; 41°17'34.3"N 44°19'07.3"E; 23.VI.2018; 1 ♀.

***Chrysoperla lucasina* (Lacroix, 1912)**

Greater Caucasus Mountains. Gudauri; village, meadow-pasture; 2190 m; 42°28'41.4"N 44°28'41.2"E; 21.VI.2018; 1 ♂ 3 ♀♀.

Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 1 ♂.

***Chrysoperla pallida* Henry, Brooks, Duelli & Johnson, 2002**

Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ♀.

Fig. 2. Mountains of the Greater Caucasus near Gudauri. Photo T. Klenovšek.

Saparlo; meadow-bushes; 938 m; 41°17'34.3"N 44°19'07.3"E; 23.VI.2018; 1 ♀.

***Cunctochrysa albolineata* (Killington, 1935)**

Greater Caucasus Mountains. Gudauri; village, meadow-pasture; 2190 m; 42°28'41.4"N 44°28'41.2"E; 21.VI.2018; 1 ♀.

Hemerobiidae

***Hemerobius handschini* Tjeder, 1957**

Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 1 ♀.

***Wesmaelius concinnus* (Stephens, 1836)**

Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ♀ (Fig. 3).

Distribution: Widely distributed in Europe (Aspöck et al 2001). Species known also from Turkey (Northeast Turkey, Isparta, Arhadan, Kars) (Arı 2014) and from north of the European part of Russia and Siberia (Makarkin 1995). From the Caucasus region for the first time recorded from Checheno-Ingushetia by Ábrahám (2000).

Remarks: New for Georgia.

***Megalomus tortricoides* Rambur, 1842**

Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 1 ♀.

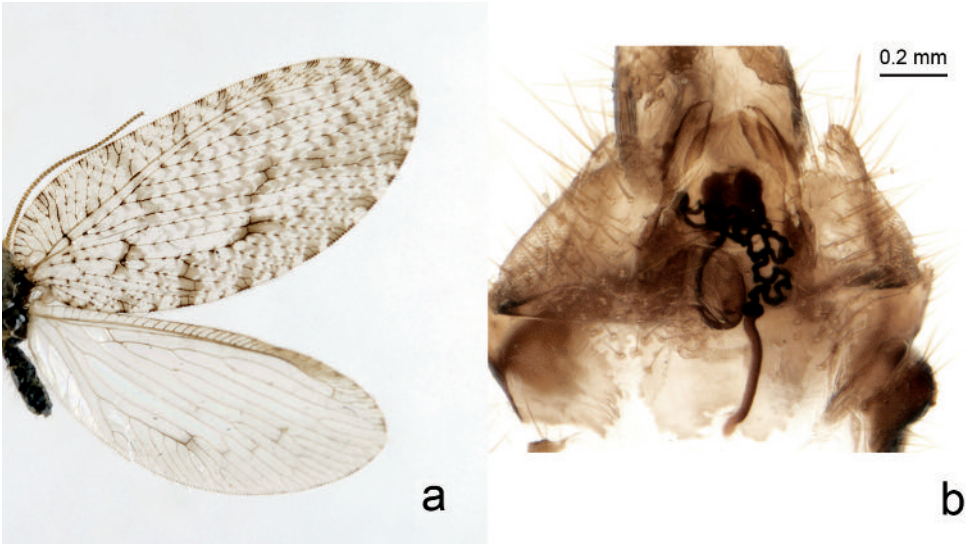

Fig. 3. Brown lacewing *Wesmaelius concinnus*, female: a, wings; b, female genitalia. Photo A. Larysz.

Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ind.

Distribution: Europe (with the exception of the Northern Europe); Asia: Middle and North Anatolia, Antalya, Burdur, Denizli, and Isparta (Arı 2014). From the Caucasus region recorded from Georgia, Armenia and generally from Caucasus (Aspöck et al. 2001).

Coniopterygidae

***Aleuropteryx loewii* Klapálek, 1894**

Lesser Caucasus Mountains. Abastumani; mixed forest; 1628 m; 41°47'08.2"N 42°50'34.4"E; 23.VI.2018; 1 ♂ (Fig. 4).

Distribution: Widely distributed in Europe, recorded generally from Anatolia (Aspöck et al. 2001) and from province Burdur (Canbulat & Kıyak, 2005).

Remarks: New for Georgia.

***Coniopteryx (C.) pygmaea* Enderlein, 1906**

Lesser Caucasus Mountains. Zekari pass, Abastumani; meadow-pasture; 2138 m; 41°51'08.6"N 42°48'28.2"E; 23.VI.2018; 1 ♂.

Lesser Caucasus Mountains. Abastumani; coniferous forest; 1937 m; 41°49'09.9"N 42°51'09.5"E; 23.VI.2018; 1 ♂ 3 ♀♀.

Ascalaphidae

***Libelloides hispanicus ustulatus* (Eversmann, 1850)**

Lesser Caucasus Mountains. Abastumani; coniferous forest, meadow; 1893 m; 41°47'33.7"N 42°50'25.5"E; 23.VI.2018; 1 ♀ (Fig. 5).

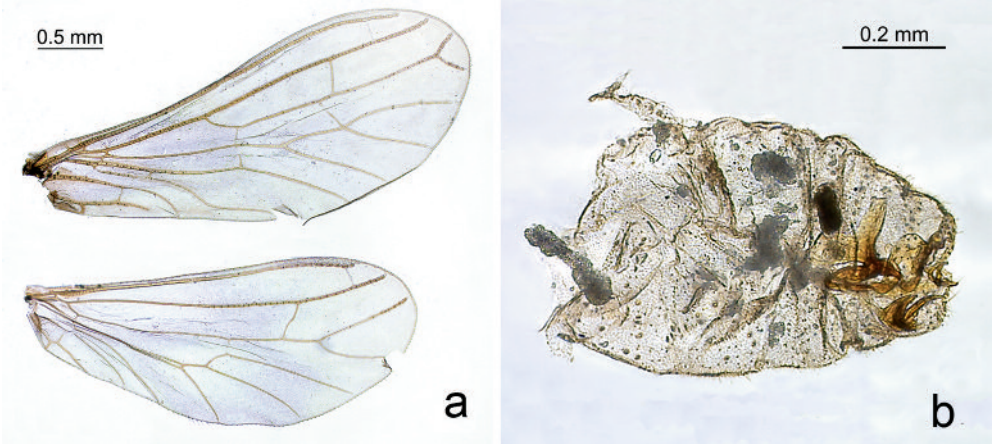

Fig. 4. Dustywing *Aleuropteryx loewii*, male: a, wings; b, male genitalia. Photo A. Taszakowski.

Fig. 5. Owlfly *Libelloides hispanicus ustulatus*, female. Photo D. Devetak.

The female was resting on an Apiaceae plant, in a meadow inside a coniferous forest (Fig. 6).

Distribution: Georgia, Armenia, Azerbaijan, Russia (Caucasus region), Turkey (Anatolia), north Iran (Kozhanchikov 1958, Aistleitner 1980, Aspöck & Aspöck 1994, Sziráki 1998, Aspöck et al. 2001, Dobosz & Ábrahám 2007, Krivokhatsky 2007, Makarkin & Shchurov 2010, Dobosz et al. 2017).

L. hispanicus hispanicus (Rambur, 1842) and *L. hispanicus ustulatus* are similar to each other, although their ranges are disjunctive. Hence, there are various hypotheses about their origin (Aspöck & Aspöck 1994, Aspöck et al. 2001). Some authors assume that these are taxa in the rank of subspecies (Krivokhatsky 2007; Makarkin & Shchurov 2010), and some recognize them as separate species (Aspöck et al. 2001, Sziráki 1998, Canbulat 2007).

Conclusion

During a short visit of the Caucasus in 2018, a survey of collected Neuroptera containing 15 species revealed two new species for Georgia – *Wesmaelius concinnus* and *Aleuropteryx loewii*.

Fig. 6. Meadow inside forest at Lesser Caucasus, where the owlfly *Libelloides hispanicus ustulatus* was found. Photo F. Janžekovič

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FOOD PLANT STERILIZATION AND ITS IMPACTS ON HEMOCYTE CELLS AND ACTIVITIES OF CARBOHYDRASES IN THE MIDGUT OF *ZONOCERUS VARIEGATUS* (L.)

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Abstract – Through feeding, phytophagous insects acquire their gut microbiota which assists in enzymes secretion and production of secondary metabolites. This study seeks to validate the role of microbes found on food plants by sterilizing cassava leaves eaten by *Zonocerus variegatus* and its impacts on hemocyte cells and the midgut carbohydrases (amylase, cellulase and α-glucosidase) activities. The insects were divided into two groups: insects fed with sterilized cassava leaves and others fed with unsterilized cassava leaves. T-test was used to analyze the results. Five (5) hemocytes cells (prohemocyte, plasmatocytes, granulocytes, sperulocyte and adipohemocyte) were detected in the insects' haemolymph with the granulocytes recording the highest frequencies. *Zonocerus variegatus* fed with unsterilized cassava leaves had significantly higher hemocyte cells than those that consumed sterilized leaves. Similarly, *Z. variegatus* that consumed unsterilized leaves had significantly higher carbohydrases activities than those whose feeds were sterilized. Activities of α-glucosidase were higher than other enzymes in the midgut of the experimental *Z. variegatus*. It can be concluded that removal of microorganisms on the food plants eaten by *Zonocerus* through sterilization affected its immune response and digestive physiology.

KEY WORDS: Cassava leaves, sterilization, hemocytes, midgut, carbohydrases, *Zonocerus*

Izvleček – STERILIZACIJA HRANILNIH RASTLIN IN NJEN VPLIV NA CELICE HEMOCITE IN AKTIVNOST KARBOHIDRAZ V SREDNJEM ČREVESU KOBILICE *ZONOCERUS VARIEGATUS* (L.)

S hranjenjem rastlinojede žuželke pridobijo svoje črevesne mikrobe, ki pomagajo pri izločanju encimov in produkciji sekundarnih presnovkov. V študiji smo skušali potrditi vlogo mikrobov, ki jih najdemo na hranilnih rastlinah, tako da smo sterilizirali liste kasave, ki jo jedo kobilice vrste *Zonocerus variegatus* in raziskati njihov vpliv na celice hemocite in aktivnost karbohidraz srednjega črevesa (amilaza, celulaza in α-glukozidaza). Žuželke smo razdelili v dve skupini: v eni so jedle sterilizirane liste kasave, v drugi nesterilizirane. Za analizo rezultatov smo uporabili t-test. 5 vrst hemocitnih celic (prohemociti, plazmatociti, granulociti, sperulociti in adipohemociti) smo našli v žuželčji hemolimfi in granulociti so dosegli največje gostote. Kobilice *Zonocerus variegatus,* hranjene z nesterileziranimi listi kasave, so imele občutno več hemocitnih celic kot tiste, ki so zaužile sterilizirane liste. Prav tako so imele tiste, ki so zaužile nesterilizirane liste, občutno višjo aktivnost karbohidraz od onih, katerih hrana je bila sterilizirana. Aktivnost α-glukozidaze je bila višja od drugih encimov srednjega črevesa poskusnih živali. Lahko zaključimo, da je odstranitev mikroorganizmov s hranilnih rastlin, ki jih jedo kobilice rodu *Zonocerus* s sterilizacijo vplivalo na njihov imunski odziv in prebavno fiziologijo.

KEY WORDS: listi kasave, sterilizacija, hemociti, srednje črevo, karbohidraze, *Zonocerus*

Introduction

The variegated grasshopper, *Zonocerus variegatus* (L.) was accorded pest status in Nigeria in 1974 by Centre for Overseas Pest Regulation, due to its polyphagus nature, consuming both food and cash crops (Toye, 1982; COPR, 1974). Chiffaud and Mestre (1970) reported that *Z. variegatus* consumed more than 250 plants among which are: citrus, cocoa, banana, vegetables and coffee. Also in the neighboring country, Cameroon, *Z. variegatus* was ranked 3rd most economically detrimental insect pest of agriculture (Kekeunou, 2006).

Methods adopted for its control include – physical, cultural, chemical and integrated pest management (Toye, 1982). However, due to its numerous side effects chemical method has been discouraged and other methods are now combined for effective control. One method yet to be fully harnessed is the biological control which involves the use of natural enemies as pathogens to control pest (Toye, 1982).

The roles played by hemocytes in the insects include phagocytosis of microbes, encapsulation of foreign materials, coagulation of haemolymph and distribution of nutritive materials (Gupta, 1985). Idowu and Sonde (2004) observed that food types consumed by insects influenced the types and number of hemocyte cells.

The gut of polyphagus insects like *Zonocerus* harbors numerous microorganisms. Namely: bacteria and fungi (Chapman, 1990; Campbell, 1990). The colony forming unit (cfu) of these microbes increased as the size and age of the insect increased

(Idowu and Edema, 2004). Among other things, the microbes synthesize digestive enzymes especially cellulase which cannot be synthesized by animals (Idowu *et al*, 2009). Alcohols, vitamins and minerals are other by-products of fermentation process anchored by these microbes (Chapman, 1990). These microorganisms are not vertically transmitted from mothers to their offspring in *Zonocerus* but through the food plant consumed (Ademolu and Idowu, 2011).

The roles played by microbes in the physiology of insects are numerous, however, their significant roles at micro level (enzymes and immune response) has not received full attention in literature. The study aims to investigate the role of microbes in digestion process and effects on immune response. The focus of this study is to evaluate the effect of food (leaves) sterilization on gut enzymes and hemocytes number in *Z. variegatus.*

Materials and methods

INSECT COLLECTION AND MAINTENANCE

Variegated grasshoppers (*Z. variegatus*) of various stages of development were collected from uncultivated farmland near the Health Centre of Federal University of Agriculture, Abeokuta, (FUNAAB) Nigeria early in the morning (6:30 am – 7:30 am). The insects were sorted by sex and later instars (5^{th} and 6^{th} instars) were maintained on fresh cassava (*Manihot esculenta*) leaves until they reached the adult stage. Male adults of 3 days old were recruited for this study.

EXPERIMENTAL PROCEDURE

Forty (40) male adults *Z. variegatus* were separated into two groups (A and B) of twenty individuals each. Group A were fed with sterilized cassava leaves while insects in group B were fed with unsterilized cassava leaves. The two groups of insect were fed *ad-libitum* for 6 weeks.

The sterilization of the cassava leaves was done following procedures described by Rutala *et al.* (2008) and Ademolu *et al.* (2015).

HEMOCYTE ESTIMATION

Hemocytes estimation was done by making a thin smear of the insect haemolymph, air dried and hemocyte cells were detected with drops of Leishman's stain (Gupta, 1979). Freshly prepared slides were used for identifying cell types under microscope (x 400). Estimates of cell counts were made by counting number of different cells in three different fields of view (Jibir,1981). Quantification was performed in triplicate for each animal.

GUT PREPARATION

Three individual insects per group were frozen in a deep freezer for 30 minutes after which their guts were dissected out and the midguts were carefully removed and homogenized in 0.05M KCl and centrifuged at 500 rpm for 30 minutes at 5 ^{0}C. The enzymes extract was decanted into bottles and kept for enzyme analysis.

GUT ENZYMES ANALYSIS

The activities of carbohydrases (α-glucosidase, amylase and cellulase) in the midgut of *Z. variegatus* were found out following methods described by Adedire and Balogun (1995). The enzymes activities were determined quantitatively by using Dinitrosalicyclic Acid Reagent (DNSA). The amount of reducing sugar produced at the end of incubation period was estimated spectrophotometrically at 550nm. The experiment was replicated three times.

STATISTICAL ANALYSIS

Data collected from the experiments were analyzed using t-test (t = 0.05).

Results

The number and types of hemocytes in the *Zonocerus* fed sterilized cassava leaves are presented in Table 1. Five (5) different types of hemocyte cells were detected in the insect haemolymph with Granulocytes representing the highest number of cells. Also, insects fed unsterilized cassava leaves had significantly higher number of hemocyte cells than those fed sterilized cassava leaves.

Table 1: Estimate of hemocyte cells in *Zonocerus variegatus* (L) fed cassava leaves ($x10^5$ per ml of haemolymph)

HEMOCYTE CELLS	INSECTS FED STERILIZED LEAVES	INSECTS FED UNSTERILIZED LEAVES
Prohemocyte	8	10
Plasmatocyte	7^b	12^a
Granulocyte	10^b	15^a
Oenocytoid	0	0
Sperulocyte	3^b	10^a
Adipohemocyte	7^b	11^a
Total	35	58

*Means values in the same row having different superscript are significantly different at ($p<0.05$)

The activities of carbohydrases in the midgut of *Zonocerus* fed sterilized cassava leaves are shown in Table 2. Insects fed unsterilized leaves had significantly higher cellulase and α-glucosidase activities than those fed sterilized leaves. The highest enzymatic activity in the midgut was detected for α-glucosidase, while amylase activity was the lowest.

Table 2: Carbohydrases activities (Abs/min) in the midgut of *Zonocerus variegatus* fed cassava leaves

S/N	SAMPLE	CELLULASE	AMYLASE	α - GLUCOSIDASE
1	**Grasshopper fed sterilized leaves**	0.678^{b}	0.215	0.861^{b}
2	**Grasshopper fed unsterilized leaves**	0.712^{a}	0.221	0.868^{a}

*Means values in the same column having different superscript are significantly different at ($p<0.05$).

Discussion

The number and types of hemocyte cells present in the haemolymph of *Z. variegatus* and in other insects generally varies with the physiological state of the insects (Mullin, 1985 and Idowu and Sonde, 2004). *Zonocerus* fed with unsterilized leaves had more hemocytes than their counterparts fed with sterilized leaves. Ademolu *et al* (2010) observed that the number of hemocytes increased as *Z. variegatus* increased in age (during embryonic development) and this is attributed to the increase in food consumption as the insect ages. The food plants consumed by *Zonocerus* harbor microorganisms which elicit or activate host defense system of the insect (Eslin and Provost, 1998). Hence, the lower hemocytes in the insect fed with sterilized leaves might be due to reduced/low microbial load on the leaves which elicit less of the insect defense system.

Five hemocyte types were detected in the haemolymph of the experimental insects namely prohemocyte, plasmatocyte, granulocyte, sperulocyte and adipohemocyte. Oenocytoids were not detected in the experimental insects and this runs contrary to the six (6) hemocyte cells earlier recorded in *Z. variegatus* by Idowu and Sonde (2004). Gouli *et al.* (2000) earlier observed that Oenocyloids were extremely rare and few numbers were also seen in *Adelges tsugae*, likely due to their insignificant role in fighting foreign agents. Similarly, Jone (1962) pointed out that Oenocytoids were either absent or questionably present in same orthopterans as they rapidly disintegrate outside the host. On the other hand, granulocytes were the most abundant, followed by plasmatocytes and prohematocytes. This might be connected to their

functions. Both granulocytes and plasmatocytes are involved in phagocytosis of the foreign agents and also important in wound healing (Gupta, 1985).

One essential role of microbes in the gut of *Zonocerus* is to synthesize enzymes needed to break down food plants consumed by insects. Idowu *et al* (2009) reported that microbes inhabiting the gut of *Zonocerus* secrete cellulolytic enzymes used by the insects. The source of these microbes has been linked to the food plants (Ademolu and Idowu, 2011) and their removal by sterilization shown to influence the production of these enzymes. This explains the reduced carbohydrases activities in the midgut of *Zonocerus* fed sterilized leaves.

It is worthy to note that the two groups had the highest activities of α – glucosidase and lowest activities of amylase in the midgut. The main diet of the insect is cassava leaves which is a form of polysaccharide and requires high activity of amylase to break it down to disaccharides. However, digestion of polysaccharide begins at the foregut as the enzymes move backward from the site of secretion (midgut) to the fore gut (Hill and Orchard, 2015). Hence, higher α – glucosidase activities in the midgut is needed to hydrolyse the inflow of disacharides from the foregut. Detection of cellulase in the midgut of the insects suggests the presence of microbes as animals lack the ability to synthesize cellulase. Microorganisms live symbiotically in the gut of phytophagous insects and assist by synthesizing the enzymes required for the hydrolysis of cellulose portion of their diet.

Conclusion

This study suggests that food microbes present on the food plants of *Z. variegatus* aid digestion through enzymes secretion and elicit the insect's defense system.

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EVALUATION OF POWDER MIXTURES OF SELECTED PLANTS AS PROTECTANTS OF COWPEA (*VIGNA UNGUICULATA* [L.] WALP.) AGAINST *CALLOSOBRUCHUS MACULATUS* (F.)

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Abstract – *Callosobruchus maculatus* (F.) is an important storage pest of cowpea seeds in Nigeria. Although synthetic pesticides are effective at controlling the pest, environmental and health hazards of these chemicals are of increasing concern. Thus, efficacy of leaf powders of *Carica papaya* (CP), *Tithonia diversifolia* (TD), *Acalypha ciliata* (AC), *Azadirachta indica* (AI) and *Vernonia amygdalina* (VA) in control of *C. maculatus* on cowpea was investigated. Cowpea seeds (200 each) were infested separately with 20 newly emerged adults (10 males and females each) of *C. maculatus* respectively in 1 litre kilner jars. The jar contents were mixed separately with 5g and 10g of each of CP, TD, AI, AC or VA per 100g seeds to determine both the toxicity on the adult insects and production of F_1 progenies for the first 28 days and percentage grain weight loss were assessed after 28 days. In the storage material experiments, 5kg of each cowpea seeds were separately weighed into polyethylene bags and plastic containers. A 500g of the five botanicals and their different mixtures (CP+TD+AI+AC+VA; TA+AI+VA; CP+TD+AI+AC; CP+TD+AI+VA; CP+TD+AC; TD+AI+AC; CP+AI+AC; CP+VA+AC) were added separately into cowpea seeds in the storage materials. Untreated cowpea grains and Actellic ® dust treatments served as positive and standard controls respectively. The trials were laid in a completely randomized design (CRD) in three replicates for 5 months in the laboratory at ambient temperature (25±3ºC). Data were collected at one month intervals on seed germination, seed weight loss and insect infestations and analysed using ANOVA and significant means separated with DMRT at $\alpha_{0.05.}$ There were significant differences ($p<0.05$) in mortality rate of the *C. maculatus* among the leaf powders. *Acalypha ciliata* at both 5g and 10g/100g seeds compared to other botanicals resulted in the highest mortality at day 21, with 47.5% - 77.5% mortality recorded for *C. mac-*

ulatus. All the plant powders, irrespective of concentration reduced the F_1 progeny emergence of *C. maculatus* (14% - 78.2%) significantly (P<0.05) compared to the control. Efficacy of *Acalypha ciliata* and its mixtures were significantly different to synthetic insecticide from second month of storage. Leaf powders effectively improved seed germination and reduced weight loss and insects damaged more grains in the polyethylene bags than in plastic containers. Results show that Actellic dust completely inhibited progeny emergence while leaf powders only reduced the F1 progeny emergence. Therefore *A. ciliata* could be used as an alternative control option to synthetic pesticides.

KEY WORDS: *Callosobruchus maculatus,* cowpea, botanicals, Bruchinae, crop protection, pesticides, Actelic dust

Izvleček – VREDNOTENJE MEŠANIC PRAHU IZBRANIH RASTLIN ZA ZAŠČITO KITAJSKEGA FIŽOLA (*VIGNA UNGUICULATA* [L.] WALP.) PRED HROŠČEM *CALLOSOBRUCHUS MACULATUS* (F.)

Callosobruchus maculatus (F.) je pomemben skladiščni škodljivec semen kitajskega fižola v Nigeriji. Čeprav so sintetični pesticidi učinkoviti pri zatiranju škodljivca, povzročajo okoljska in zdravstvena tveganja teh kemikalij vedno večjo zaskrbljenost. Zato je bila preverjena učinkovitost prahu iz listov rastlin *Carica papaya* (CP), *Tithonia diversifolia* (TD), *Acalypha ciliata* (AC), *Azadirachta indica* (AI) in *Vernonia amygdalina* (VA) za zatiranje hrošča *C. maculatus* na kitajskem fižolu. Semena fižola (po 200) so bila ločeno okužena s po 20 sveže izleženimi odraslimi hrošči (po 10 samcev in samic) v litrskih kilnerjevih posodah. Vsebina posod je bila ločeno zmešana s 5g in 10g vsakega od praškov, CP, TD, AI, AC ali VA na 100g semen za ugotavljanje strupenosti za odrasle žuželke in proizvodnje potomcev generacije F_1 v prvih 28 dneh, po tem času je bilo ocenjeno zmanjšanje teže zrnja v odstotkih. V poskusih s skladiščnim materialom je bilo po 5 kg kitajskega fižola ločeno odtehtanega v polietilenske vreče in plastične vsebnike. 500g petih rastlinskih pripravkov in njihovih različnih mešanic (CP+TD+AI+AC+VA; TA+AI+VA; CP+TD+AI+AC; CP+TD+AI+VA; CP+TD+AC; TD+AI+AC; CP+AI+AC; CP+VA+AC) je bilo ločeno dodanih semenom fižola v skladiščnem materialu. Netretirana zrnja fižola in tretiranje s praškom Actellic ® sta služila kot pozitivna in standardna kontrola. Poskusi so potekali po povsem naključni zasnovi v treh 5-mesečnih ponovitvah v laboratoriju pri sobni temperaturi (25±3°C). Na en mesec smo beležili podatke o kaljivosti semen, izgubi teže semen in napadi žuželk, analizirali z uporabo ANOVA in z DMRT pri $\alpha_{0.05}$ ločili statistično pomembne srednje vrednosti. Bile so statistično pomembne razlike (p<0.05) v smrtnosti hroščev *C. maculatus* med različnimi listnimi praški. *Acalypha ciliata* je tako s 5g kot 10g/100g semen povzročala najvišjo smrtnost na 21. dan, 47.5% - 77.5% smrtnost pri *C. maculatus*. Vsi rastlinski praški, ne glede na koncentracijo, so zmanjšali izleganje potomcev generacije F_1 hroščev *C. maculatus* (14% - 78.2%), statistično pomembno (P<0.05) glede na kontrolo. Učinkovitost praška *Acalypha ciliata* in njegovih mešanic je bila pomembno drugačna od sintetičnega insekticida po drugem mesecu skladiščenja. Listni praški so učinkovito izboljšali

kaljivost semen in zmanjšali izgubo teže, žuželke pa so poškodovale več semen v polietilenskih vrečah kot v plastičnih vsebnikih. Rezultati kažejo, da je prah Actellic popolnoma zaustavil izleganje potomcev, medtem ko so listni praški le zmanjšali izleganje generacije F_1. Torej bi lahko prašek *A. ciliata* uporabljali kot zamenjavo za zatiranje s sintetičnimi pesticidi.

KLJUČNE BESEDE: *Callosobruchus maculatus,* kitajski fižol, rastlinski praški, Bruchinae, zaščita pridelkov, pesticidi, prah Actelic

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a staple food crop of significant economic and nutritional importance worldwide (Emeasor *et al*., 2007; Akunne *et al*., 2013). Cowpea seeds, pods and leaves are consumed in fresh form as green vegetables in some African countries (Ghaly and Alkoaik, 2010), while the rest of the cowpea plant after the pods have been harvested serves as a nutritious fodder for livestock (Abebe *et al*., 2005). Nigeria is the largest producer of cowpea grain with approximately 3.80 million hectares under cultivation and an annual production estimate of 2.95 million MT (FAO, 2015).

Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries with a pre- and post-harvest loss ranging from 10 to 100% (Talukder *et al.,* 2004). These substantial losses are caused by *Callosobruchus* on legumes. *Callosobruchus maculatus* is a major constraint to cowpea storage often leading to loss of economic value (Musa *et al*., 2010; Baidoo *et al*., 2010).

Callosobruchus maculatus is a field-to-store pest of cowpea as its infestation starts in the field when the mature pod dries. It multiplies very rapidly in storage where it causes losses from 10 to 90% on the grains (Caswell, 1981; Boateng and Kusi, 2008; IITA, 2015) and also reduces seed quality, market value and seed viability to 2% after 3 months of storage (Caswell, 1981; Ofuya and Credland, 1995; Boateng and Kusi, 2008).

Chemical method such as the use of phosphine, methyl bromide to control these storage pests has been the most efficient and effective means of protecting grains (Rajendran and Sriranjini, 2008; Mesele *et al*., 2013). However, with the increasing cost of synthetic pesticide, development of resistance such as the case of phosphine and their hazards to the environment such as ozone depletion by methyl bromide which is being phased out completely, alternative controls measure needs to be investigated (Price, 1984; Banwo and Adamu, 2003).

One viable alternative option is the use of botanical pesticides, despite having different active constituents, their modes of action are target-specific, relatively safe, af-

fordable and readily available (Chebet *et al*., 2013). Insecticidal activity of several plant essential oils, powders and other extracts has been evaluated against several insect pests of legumes (Chebet *et al*., 2013; Bohinc *et al*, 2013) and found to have contact toxicity (Asawalam *et al*., 2006; Ogendo *et al.,* 2008), repellence (Kéita *et. al*., 2001; Rosman *et al.,* 2007), fumigant toxicity (Lee *et al*., 2003; Rajendran and Muralidharan, 2005) and anti-feedant (Saxena *et al.,* 1992a, b) effects.

Researches have shown that botanicals have been extensively used on agricultural pests and to very limited extent on insect pests of stored products (Ijeh and Ejike, 2011; Akunne *et al.,* 2013). Moreover, *Carica papaya, Tithonia diversifolia*, *Acalypha ciliata*, *Azadirachta indica*and *Vernonia amygdalina* have been screened *in vivo* and *in vitro* and found to be effective as fungicide and insecticide though with different degree of success compared to the synthetic pesticides (Owolade *et al*., 2004; Moses and Dorathy 2011; Brisibe *et al.*, 2011; Onyeani *et al.,* 2012; Akunne *et al.,* 2013; Chebet *et al*., 2013). Although Akunne *et al.* (2013) reported no improvement in the efficacy of *V*. *amygdalina* and *A. indica* mixtures compared to single application of each of the botanicals against *C. maculatus*, he recommended further research using higher concentration of the botanicals and on a wide range of other common insect pest of stored products in order to increase the efficacy since the plant materials are cheap and readily available.

Also in Nigeria, information on the mixture of two or more botanicals against insect pest of stored products and the impact of different storage materials on the effectiveness of the botanicals is limited. Therefore, the objectives of this study were to

1. evaluate the effects of *Carica papaya, Tithonia diversifolia*, *Acalypha ciliata*, *Azadirachta indica* and *Vernonia amygdalina* at different concentrations in the control of *Callosobruchus maculatus;*
2. evaluate these plant extract combinations for control of F_1 progeny of *Callosobruchus maculatus* and;
3. investigate the influence of the storage materials on effectiveness of the plant extracts.

Materials and methods

Experimental sites

Experiment was carried out in the Entomology laboratory of the Nigeria Agricultural Quarantine Service (NAQS), Moor Plantation, Ibadan located on latitunde 7. 38N and longitude 3·83E. The studies were conducted between the months of August, 2013 through September, 2014.

Collection of plant materials and seeds

Fresh leaves of *Carica papaya* (Pawpaw), *Tithonia diversifolia* (America sunflower), *Acalypha ciliata* (Red Acalypha)*, Vernonia amygdalina* (Bitter leaf) and *Azadirachta indica* (Neem) were collected from Ibadan. Seeds of cowpea (Ife Brown)

were obtained from the seed store of Institute of Agricultural Research & Training (IAR&T), Ibadan while polyethylene bags and plastic containers were purchased from the market.

Preparation of plant powders

Fresh leaves of *Carica papaya* (Pawpaw), *Tithonia diversifolia* (America sunflower), *Acalypha ciliata* (Red Acalypha) *Azadirachta indica* and *Vernonia amygdalina* were washed with sterile distilled water and drained on the laboratory table to remove the water. The plant materials were then air-dried on the laboratory table at 25±3ºC. The dried botanicals were ground using the hammer mill and sieved to obtain fine powders. The plant powders were put in air tight containers separately to ensure that the active ingredients are not lost. The powders were stored in a cool dry place until needed.

Culture of the experimental insects

Adult *Callosobruchus maculatus* used for the experiment were reared in plastic containers under ambient laboratory temperature of 30±3ºC and relative humidity of 75±3%. Weevil-infested cowpea were purchased from Bodija Market Ibadan, Oyo State and were put in culture vial (19cm in diameter) before incubating in the laboratory cupboard so that the old insects will mate and oviposit. This was left undisturbed for two months and the newly emerged adults were used for the experiment.

Cowpea seeds treatment and introduction of test insects

Cowpea seeds with 12-13% moisture content were separately cleaned using 1mm sieve-mesh screen before disinfested in a freezer at -4°C for two weeks. The cowpea seeds were counted (200 seeds) separately into 1L plastic jars, replicated thrice for each of the five plant powders at two different concentrations (5g/100g and 10g/100g of seeds). The seeds were then separately admixed with the plant powders and the contents of each jar were mixed thoroughly to allow even distribution of the powder in the whole grain mass. Actellic super® (0.175g /jar) and untreated cowpea grain treatments served as standard and control respectively.

Twenty, 24hr old *Callosobruchus maculatus* and *Sitophilus zeamais* (ten males and ten females each) were introduced into the plastic jars containing cowpea and maize respectively using camel hair brush. The jars were placed in the shelves at a temperature of 28±2°C and relative humidity of 70±5% for 28 days in a completely randomized design (CRD).

Mortality was assessed for the first 28 days of exposure using the technique of Ceruti and Lazzari (2005) and Nukenine *et al*. (2011). Insects were considered dead on failure to respond by moving to three probings with small paint brush. Percent mortality was determined as follows:

$$\text{Mortality} = \frac{\text{number of dead insects}}{\text{Total number of insects introduced}} \times 100$$

Determination of control efficacy

Toxicity efficacy on F_1 progeny emergence:

After 28 days of exposing the beetles to the treatments, 100 seeds of maize and cowpea were randomly removed from each jar containing the treatments and the number of eggs oviposited on them were counted. These were placed in another equal volume plastic jar separately until progeny emergence. Progeny emergence in each treatments and replicates were taken for F_1 after 28 days of observation, the newly emerged progenies were sieved out, counted and recorded.

Control efficacy on mortality of F_1 progeny:

For each of the five plant extracts powder and their different mixtures, observations were made and recorded for effect on mortality rates of F_1 progeny in all the jars at 24, 48, and 72 hours. The mortality rate was expressed as percentage progeny reduction.

Storage Bioassay with plant extracts and different mixtures:

Bioassay was conducted based on the method described by Talukder and Howse (1994) and modified by Owolade *et al*. (2003). One concentration of 100g/1kg of seeds (1g/10seeds) of each extracts from the leaves of *Carica papaya*, *Tithonia diversifolia*, *Azadirachta indica,Vernonia amygdalina* and *Acalypha ciliata* including the different extracts combination was mixed properly into 5kg of each of the cleaned maize and cowpea seeds separately inside polyethylene bags and plastic containers. Twenty 24hr old *Callosobruchus maculatus* (ten male and ten female each) were introduced into the storage materials containing cowpea using camel hair brush The storage materials containing the seeds were placed on the laboratory table at ambient temperature of 25±3ºC for 5 months.

The experiment was laid up in complete randomized design with three replicates.

Data collection and Statistical Analysis

Data were collected at monthly intervals for five months of storage. The data collected include the weight of seeds, percentage seed infestations, total insect counts and weight of damaged seeds. Data collected were analysed using the Statistical Analysis Software (SAS, 2010). Means were separated using the New Duncan's Multiple Range Test at $P \leq 0.05$.

Results

Effect of the botanical leaf powders on the mortality rate of *Callosobruchus maculatus* at 28 days is shown in Table 1. The efficacy of the plant extracts increased with increase in concentrations irrespective of the botanicals. *Acalypha ciliata* at 5 g and 10g/100 seeds was the most effective compared to other leaf powders at day 28 with 47.5- 77.5% mortality recorded for *C. maculatus* which was not significantly

different at 10g/100 seeds from the standard control using 2% Actellic dust. This was closely followed by powdered extracts from *Azadirachta indica* at 10g/100seeds which recorded 41.0% in *C. maculatus*. Powders of *C.a papaya* and *V. amygdalina* recorded the least number of dead adult insects. The highest mortality was recorded at 0.175 of Actelic dust.

Table 1: Effect of the botanical leaf powders on the mortality rate of *Callosobruchus maculatus* at 28 days.

Treatment	Concentration (g/100g of seed)	Number of dead adults
Control	0.0	1.2e
Actellic	0.175	20.0a
C. Papaya	5	1.5e
	10	2.5de
T. diversifolia	5	3.7d
	10	7.3c
A. indica	5	4.8d
	10	8.2c
V. amygdalina	5	1.2e
	10	2.5de
A. ciliata	5	9.5c
	10	15.5a

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

Mean number of adult *C. maculatus* emergence after 42-day incubation period is shown in Table 2. The F_1 progeny emergence differed significantly with plant extracts and concentrations ($p<0.05$). All the plants extract irrespective of concentration reduced the F_1 progeny emergence of *C. maculatus.* However, the higher the concentrations of the extracts, the higher the percentage reduction in adult emergence of both insects. The percentage reduction in F_1 progeny emergence was significantly higher with the 10g/100g of seeds of *A. ciliata* among the plant extracts relative to the control. The results also indicated that the powdered extract of *A. indica* also significantly reduced the number of adult emergence at a higher concentration when compared with other botanicals. Similar trend was observed for the extracts in the control of *C.maculatus* on cowpea seeds. However, Actellic dust completely inhibited progeny emergence in *C. maculatus*.

Table 2: Effect of botanical leaf powders on adult F_1 progeny emergence of *Callosobruchus maculatus*

Treatment	Concentration (g/100g of seed)	No of live Adults	% PRD
Control	0.0	25.7a	0.0
Actellic	0.175	0.0f	100.0
C. Papaya	5	22.1b	14.0
	10	10.3d	59.9
T. diversifolia,	5	15.2c	40.8
	10	10.5d	59.2
A. indica	5	12.0d	53.1
	10	9.5d	63.1
V.amygdalina	5	18.7bc	27.3
	10	15.8c	38.5
A.ciliata	5	6.5e	74.7
	10	5.6e	78.2

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

% PRD= Percentage progeny reduction relative to control

Table 3 shows that the seed weight loss increased with storage period irrespective of the treatments in the cowpea seeds stored in polyethylene bags. There was a significant difference ($p<0.05$) in weight loss of cowpea seeds among the different treatments from the first to fifth month of storage with the mixtures (except for CP+TD+AI+AC+VA; TD+AI+VA and CP+TD+AI+AC) performing better than their single application. All the mixtures involving *A ciliata* also significantly resulted in the least weight loss due to weevil damage followed by the single application of *A. ciliata* which was not significantly different from the effects of all its mixtures. The effects of other plants extracts in reducing weight loss during storage were not significantly different from the untreated control. However, the application of *A ciliata* was comparable to Actellic dust in the reduction of weight loss during the storage trial. The weight at 5 months of storage ranged between 2.3g (Single application of *Tithonia diversifolia* and 11.2g (Actellic Dust) using polyethylene bags as storage material.

Result in Table 4 revealed that the germination of cowpea seeds was significantly influenced by the type of protectant applied during storage. Using polyethylene bags as storage material for cowpea seeds the percentage of germination reduced to 69.3%

from 96% in the control after a month of storage, which was significantly different ($p<0.05$) from other treaments except *Carica papaya, Vernonia amygdalina* and combinations of *Carica papaya* + *Tithonia diversifolia*,+ *Azadirachta indica* + *Acalypha ciliata* + *Vernonia amygdalina*. Although there were significant differences among the treatments after 2 months of storage, the germination percentage was reduced to zero from the 4th month of storage irrespective of the plant extracts.

Table 3: Effects of plant powders on weight of 100 cowpea seeds stored in polyethylene bags during 5 months of storage.

Plant Extract	Cowpea 100-seed weight (g)					
	INW	WA1M	WA2M	WA3M	WA4M	WA5M
CL	22.3	15.6 b	11.2 b	8.1 b	4.7 b	4.6 b
CP	23	15.5 b	12.0 b	8.0 b	4.2 b	3.2 bc
TD	22.5	15.0 b	12.2 b	8.3 b	4.4 b	2.3 c
AI	23.5	14.8 b	11.5 b	7.4 b	4.1 b	2.4 c
VA	22	15.2 b	10.8 b	8.1 b	3.8 b	3.5 c
CP+TD+AI+AC+VA	23	15.2 b	10.8 b	7.7 b	3.8 b	3.3 c
TD+AI+VA	22	14.6 b	11.2 b	7.8 b	4.4 b	3.1 c
CP+TD+AI+AC	21.4	15.2 b	11.4 b	8.8 b	4.6 b	2.9 c
CP+TD+AI+VA	23.7	20.4 a	17.5 a	17.7 a	14.5 a	8.8 a
CP+TD+AC	22.6	21.2 a	17.9 a	16.0 a	13.2 a	7.9 a
TD+AI+AC	23	21.8 a	18.9 a	17.8 a	13.2 a	9.1 a
CP+AI+AC	22.8	20.3 a	19.3 a	17.5 a	14.7 a	9.2 a
CP+VA+AC	23	20.1 a	19.8 a	17.2 a	15.2 a	8.9 a
AC	22	19.5 a	17.2 a	16.5 a	14.5 a	9.2 a
AD	22	21.8 a	18.6 a	16.3 a	13.5 a	11.2 a

INW = Initial weight, WA1M-WA5M= Seed weight 1 -5 months after storage. Values followed different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control (untreated seed) ; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina*, AC= *Acalypha ciliata;* AD= *Actellic dust*

Table 4: Effect of the powdered plant extracts on the percentage of germination of cowpea seeds stored in polyethylene bags during 5 months of storage.

Plant Extract	Percentage of germination (%)					
	INW	PG1M	PG2M	PG3M	PG4M	PG5M
CL	96.0	69.3 e	42.7 f	19.6 d	0.0 a	0.0 a
CP	97.0	69.7 e	51.7 d	26.3 cd	0.0 a	0.0 a
TD	99.0	73.7 d	48.3 ed	22.3d	0.0 a	0.0 a
AI	98.6	73.3 d	56.0 d	34.3 bc	0.0 a	0.0 a
VA	99.0	70.0 e	50.3 ed	32.7 bc	0.0 a	0.0 a
CP+TD+AI+AC+VA	97.0	71.3 e	44.7 e	24.7 d	0.0 a	0.0 a
TD+AI+VA	99.0	72.7 e	48.0 ed	28.3 c	0.0 a	0.0 a
CP+TD+AI+AC	98.7	71.3 e	48.3 ed	31.7 c	0.0 a	0.0 a
CP+TD+AI+VA	99.7	76.3 ed	49.0 ed	25.0 c	0.0 a	0.0 a
CP+TD+AC	96.8	87.0 b	82.3 b	40.3 a	0.0 a	0.0 a
TD+AI+AC	99.0	97.0 a	95.3 a	43.3 a	0.0 a	0.0 a
CP+AI+AC	98.0	86.7 b	85.3 b	39.0 ab	0.0 a	0.0 a
CP+VA+AC	98.7	79.3 c	74.0 c	42.0 a	0.0 a	0.0 a
AC	99.0	87.0 b	86.0 b	39.3 ab	0.0 a	0.0 a
AD	98.5	84.0 bc	84.0 b	41.0 ab	0.0 a	0.0 a

ING = Initial germination PG1M-PG5M= seed percentage germination 1 -5 months after storage

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina*, AC= *Acalypha ciliata;* AD= *Actellic dust*

The synthetic insecticide (Actellic dust) effectively controlled the infestation of *C. maculatus* (Table 5). The mixtures of *Tithonia diversifolia* + *Azadirachta indica* + *Acalypha ciliata* (1:1:1) and that of *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* (1:1:1) as well as the single application of *Acalypha ciliata* significantly reduced infestation of cowpea seeds stored in polyethylene bags throughout the period of storage. There were however 100% infestations by the cowpea weevil in other treatments as well as the untreated control.

Infestation of *C. maculatus* in cowpea stored in polyethylene bags during 5 months of storage (Table 5) revealed that only single application of *A. ciliata* and its mixtures

significantly reduced (P≤0.05) the percentage of *C. maculatus* infestation throughout the duration of storage. However, effect of *A. ciliata* and its mixtures on *C. maculatus* infestation was not significantly different from actellic dust treatment in the first month of storage but was significantly different from the second month (9%) to 5th month (13%) of storage. The Actellic treated seeds were devoid of insect attack (0%) from the second month of storage onward in polyethylene bag. It was observed that cowpea seeds stored with the untreated control and other extracts were completely (100%) infested by *C. maculatus* from the 4th month of storage.

Table 5: Effect of the plant powders on the percentage of seed infestation by *Callosobruchus maculatus* stored in polyethylene bags during 5 months of storage.

Plant Extracts	Percentage of insect infestation (%)				
	INF1M	INF2M	INF3M	INF4M	INF5M
CL	49.7 a	58.7 a	94.3a	100.0 a	100.0 a
CP	25.3 b	41.7 c	80.3 b	100.0 a	100.0 a
TD	26.0 b	43.3 bc	79.7 b	100.0 a	100.0 a
AI	25.7 b	47.0 b	68.7 bc	100.0 a	100.0 a
VA	26.7 b	43.7 bc	75.0 b	100.0 a	100.0 a
CP+TD+AI+AC+VA	17.3b	37.0 c	60.7 c	100.0 a	100.0 a
TD+AI+VA	22.7b	44.3 bc	80.3 b	100.0 a	100.0 a
CP+TD+AI+AC	24.0 b	37.0 c	70.7 bc	100.0 a	100.0 a
CP+TD+AI+VA	23.3 b	42.0 bc	74.7 b	100.0 a	100.0 a
CP+TD+AC	6.7 c	12.7 d	12.0 d	16.7 b	19.7 b
TD+AI+AC	0.7 c	6.0 d	12.0 d	15.0 b	12.0 c
CP+AI+AC	6.0 c	10.3 d	13.0 d	12.0 c	20.0 b
CP+VA+AC	5.0 c	12.0 d	13.0 d	10.0 d	12.0 c
AC	5.0 c	9.0 d	12.0 d	15.7 b	13.0 c
AD	3.0 c	0.0 e	0.0 e	0.0 e	0.0 d

INF1M – INF5M = Percentage seed infestation 1 -5 months after storage

Values followed by different letter in each column are significantly different *P*≤0.05 according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina;* AC= *Acalypha ciliata;* AD= *Actellic dust*

The result in Table 6 shows that seed weight reduced with increase in storage period irrespective of the treatments in cowpea seeds stored in plastic containers. There were significant differences ($p<0.05$) in weight loss among the different treatments. All the mixtures involving *A ciliata* also significantly resulted in the least weight reduction due to weevil damage followed by the single application of *A. ciliata* which was not significantly different from the effects of all its mixtures. The results also revealed that the percentage decrease in 100-seed weight 5 months after storage was 2g for the untreated control while it was 15.8g for Actellic treated seeds. However, weight reduction for the seeds stored with mixtures of *Carica papaya + Vernonia amygdalina + Acalypha ciliata* and single application of *A. ciliata* was the least with 11g and 11.3g respectively. The weight reduction for other treatments ranged from 5.4g in mixture of *Carica papaya +Tithonia diversifolia +Azadirachta indica + Vernonia amygdalina* to 9.9g for *Carica papaya+ Azadirachta indica + Acalypha ciliata* in cowpea seeds stored in plastic containers after 5 months of storage.

Table 6: Effect of the plant powders on the weight of 100 cowpea seeds stored in plastic container during 5 months of storage.

Plant Extracts	Cowpea 100-seed weight (g)					
	INW	WA1M	WA2M	WA3M	WA4M	WA5M
CL	22.3	12.9 e	9.9 c	8.6 d	4.7 e	2.0 de
CP	23.0	15.4 bc	12.6 b	10.3 c	8.0 c	6.9 d
TD	22.5	15.7 b	12.4b	9.7 c	7.5 cd	7.1 d
AI	23.5	13.7 ed	12.55b	9.5 c	7.6 cd	6.5 d
VA	22.0	13.4 e	12.3 b	9.7 c	8.4 c	6.2 d
CP+TD+AI+AC+VA	23.0	14.8 cd	18.9 b	9.3 c	8.1 c	6.2 d
TD+AI+VA	22.0	14.0 cd	12.1 b	9.7 c	7.9 cd	6 d
CP+TD+AI+AC	21.4	15.6 bc	12.5 b	9.7 c	7.2cd	5.4 d
CP+TD+AI+VA	23.7	15.4 bc	11.2 b	9.2c	6.8 d	6.2 d
CP+TD+AC	22.6	17.3 a	15.2 a	12.5 b	10.9 b	9.2 c
TD+AI+AC	23.0	17.8 a	15.5 a	12.9 b	11.1 b	8.4 c
CP+AI+AC	22.8	16.7 b	15.1 a	12.8 b	11.4 b	9.9 bc
CP+VA+AC	23.0	18.0 a	16.3 a	15.2 a	12.9 b	11.0 b
AC	22.0	17.3 a	15.2 a	13.6 b	12.3 b	11.3 b
AD	22.0	18.2 a	17.5 a	16.2 a	16.0 a	15.8 a

INW = Initial weight, WA1M-WA5M= seed weight at 1 -5 months of storage.

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; *AI=Azadirachta indica*; VA= *Vernonia amygdalina*; AC= *Acalypha ciliata;* AD= *Actellic dust*

Table 7 shows the effect of the different plant powders on the percentage of germination of cowpea seeds stored in plastic container for 5 month. The percentage of germination of the cowpea seeds was significantly influenced by the type of plant extracts and mixtures. The result revealed that there was drastic reduction in seed germination at 3 months of storage with the untreated control recorded 0% germination while the Actellic treated control recorded 98%. *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* and single application of *A. ciliata* recorded significantly higher ($P \leq 0.05$) percent of germination of 78.7% and 77.7% respectively compared to treatments with other extracts after three months of storage.

All the plant extract treated seeds except *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* and single application of *A. ciliata* recorded between 0% and 25.4% of germination after four months of storage. The same trend was observed after five months of storage.

Table 7: Effect of the plant powders on the percentage of germination of cowpea seeds stored in plastic containers during 5 months of storage.

Plant Extract	Percentage of germination (%)					
	ING	PG1M	PG2M	PG3M	PG4M	PG5M
CL	98	85.0cd	57.0 e	0.0 e	0.0 d	0.0 d
CP	97.9	84.0cd	80.7 c	25.7cd	0.0 d	0.0 d
TD	99.2	87.7cd	81.0 c	33.0 c	0.0 d	0.0 d
AI	98.7	85.7cd	81.7 c	25.0cd	0.0 d	0.0 d
VA	97.5	85.0 d	81.0 c	22.6 d	0.0 d	0.0 d
CP+TD+AI+AC+VA	99	85.7cd	83.0 c	30.0 c	0.0 d	0.0 d
TD+AI+VA	98.5	84.5cd	79.0 c	31.0 c	0.0 d	0.0 d
CP+TD+AI+AC	97.5	80.0 d	73.0 d	30.0 c	0.0 d	0.0 d
CP+TD+AI+VA	96.8	82.0 d	76.7 d	28.6cd	0.0 d	0.0 d
CP+TD+AC	99	98.0 a	90.3 b	20.5 b	0.0 d	0.0 d
TD+AI+AC	98.3	95.2 b	92.7ab	31.0 b	20.5 c	6.2 c
CP+AI+AC	98.7	93.7 b	91.3 b	49.0 b	25.4 c	5.2 c
CP+VA+AC	99	95.3 b	90.3 b	78.7 b	62.7 b	40.5 b
AC	97.2	97.0ab	91.4 b	77.7 b	66.0 b	45.4 b
AD	100	99.2 a	98.0 a	98.0 a	90.0 a	85.2 a

ING= Initial germination

PG1M-PG5M= seed percentage of germination after 1 -5 months of storage

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina;* AC= *Acalypha ciliata;* AD= *Actellic dust*

Infestation by *C. maculatus* as affected by the efficacy of the plant extracts is shown in Table 8. The results revealed that only the extracts from *Carica papaya* + *Vernonia amygdalina* + *Acalypha ciliata* and single application of *A. ciliata* among the extract mixtures caused significant ($p<0.05$) reduction (100%) in the percentage of insect infestation in seeds stored in plastic containers for the period of five months. It was observed that cowpea seeds stored with the untreated control and other extracts/mixtures were completely infested by *C. maculatus.* The actellic treated control was devoid of insect attack throughout the period of storage.

Table 8: Effect of the plant powders on the percentage of cowpea seed infestation by *Callosobruchus maculatus* stored in plastic containers during 5 months of storage.

Plant Extract	Percentage of insect infestation				
	INF1M	INF2M	INF3M	INF4M	INF5M
CL	34.3 a	56.0 a	100.0 a	100.0 a	100.0 a
CP	33.0a	48.7 ab	100.0 a	100.0 a	100.0 a
TD	30.7 a	48.3 ab	100.0 a	100.0 a	100.0 a
AI	30.7 a	47.7 b	100.0 a	100.0 a	100.0 a
VA	31.7 a	48.0 ab	100.0 a	100.0 a	100.0 a
CP+TD+AI+AC+VA	27.3 b	49.7 ab	100.0 a	100.0 a	100.0 a
TD+AI+VA	30.7 a	45.7 b	100.0 a	100.0 a	100.0 a
CP+TD+AI+AC	27.3 b	48.7 ab	100.0 a	100.0 a	100.0 a
CP+TD+AI+VA	34.6 a	56.0 a	100.0 a	100.0 a	100.0 a
CP+TD+AC	4.2 c	12.3 d	32.0 b	100.0 a	100.0 a
TD+AI+AC	4.6 c	10.5 d	33.0 b	100.0 a	100.0 a
CP+AI+AC	5.2 c	9.7 d	24.0 c	100.0 a	100.0 a
CP+VA+AC	3.7 c	11.2 cd	22.7 c	48.7 b	56.7 ab
AC	2.3 c	9.6 d	11.2 d	25.3 c	54.7 c
AD	0.0 c	0.0 e	0.0 e	0.0 d	0.0 d

INF1M – INF5M = percentage of seed infestation after 1 -5 months of storage

Values followed by different letter in each column are significantly different $P \leq 0.05$ according to New Duncan's Multiple Range Test (DMRT).

CL= Control; CP= *Carica papaya*; TD=*Tithonia diversifolia*; AI=*Azadirachta indica*; VA= *Vernonia amygdalina;* AC= *Acalypha ciliata;* AD= *Actellic dust*

Discussion

Effects of five leaf powders of *Carica papaya*, *Tithonia diversifolia*, *Acalypha ciliata*, *Azadirachta indica* and *Vernonia amygdalina* in control of *C. maculatus* on Ife Brown cowpea was studied. Adult mortality of *C. maculatus* was found to increase with increase in concentration levels, although in some cases the mortality with 5g treatments was the same with 10g concentration, and time of exposure to treatments. This trend of results compared favourably with the reports given by Gupta and Tandon (2004) with the use of *Withania somnifera* (leaf, stem, fruit and root extracts) applied at 5% and 10% against *Callosobruchus chinensis* L. infesting green gram (*Vigna radiata*). High mortality of storage beetles have been recorded in treatments of lemon grass products and Eucalyptus products (Dike and Mbah, 1992; Oparaeke, 1997; Ojiako and Adesiyun, 2013). Higher concentration levels in the treatments of this study as well as duration of exposure of the pest to treatments caused higher mortality of *C. maculatus*.

Higher concentrations of the treatments recorded lower oviposition in all the plants under trials which was similar to the findings of Ofuya *et al., (*2010) who reported that 2.0 g plant powder added to 500 cowpea seeds reduced oviposition and egg hatch in *C. maculatus.* Oviposition rates between and within treatment were significantly lower than the control, which confirms Ivbijaro (1983a) work that neem seed powder drastically reduced egg laying in female *S. oryzae* from 154 in the untreated control to only 9 and 3 at neem powder doses of 0.5 and 1.0/20 g maize grains, respectively. Dike and Mbah (1992) reported similar conclusions on cowpea treated with lemon.

Results from the present study indicate that botanical leaf powders can be used to control *C. maculatus* in storage. The utilisation of different plant products as stored crop grain protectants has been reported by Swella and Mushobozy (2007); Araya and Emana (2009) and Mulungu *et al.* (2010).

It was also observed that the type of storage materials used to store cowpea seeds had significant effect on the germination capacity. Seeds stored in plastic containers had higher percentage of germination than polyethylene bags, which is in consonance with data obtained by Kamara *et al.* (2014).

In Nigeria, some of the plant materials used in this study have been found individually effective in storage pest's control (Oparaeke, 1997). Information on the use of plant extracts for field pests control is limited. However, Oparaeke (2004) and Olaifa *et al.* (1987) have shown that these plant extracts exhibited varying degrees of efficacy on pests of field cowpea. This is the first time a mixture of plant extracts are employed to effectively checkmate the nuisance of pests on cowpea and maize seeds.

The present study showed that the effectiveness of botanical pesticides decreases with time and they therefore need constant reapplications. The effectiveness of botanical pesticides was better with *A. ciliata* leaf powder and its mixtures (CP+VA+AC, CP+AI+AC; TD+AI+AC and CP+TD+AC) used to treat maize grain at 10g. There was a low mortality rate of maize weevils at application rates of 5 g and 10 g for all the botanicals though there was a significant mortality rate for *C. papaya* at 5 g after

3 months. The toxic and repellent action of the leaf powders of *A. ciliata*, *T. diversifolia, A. indica* and *C. papaya* on *C. maculatus* indicates that these botanical products can be utilised as good alternatives to synthetic pesticides in the control of the pest in stored maize grain. Where synthetic grain protectants like Actellic dust are unavailable or unaffordable, small holder farmers can use traditional techniques to confer some measure of protection of their maize grain against weevils.

Results obtained from this study also suggest that plastic containers are better packaging materials than polyethylene bags. The applications of *Tithonia diversifolia + Azadirachta indica + Acalypha ciliata, Carica papaya + Azadirachta indica + Acalypha ciliata, Carica papaya + Vernonia amygdalina + Acalypha ciliata*, as well as single application of *Acalypha ciliata* in airtight plastic containers reduced *C. maculatus* infestation of cowpea seeds significantly. The results of this investigation showed that botanical mixtures could form the basis for a successful formulation and commercialization of biopesticides in developing countries, where low input agriculture is in vogue. In Nigeria, these plants are readily available in the local markets all the year round for farmers' use to protect their crops. Since the materials are used in ethno-botany for the treatment of various ailments, they are safe, cheap, easily biodegradable, and technologically and environmentally friendly. They could provide valuable alternatives to synthetic chemicals which have been proven to be hazardous to man and environment.

Further work should be done to identify and isolate active compounds contained in these plant powders to determine the efficacy and methods of formulations. This may involve chemists, biochemists and environmental scientists. These botanical powders should be incorporated into grain protection practice of resource-poor farmer. In addition, there is the need to investigate the shelf life of the powders to find out if repeated application is needed after a given period and also the health implication to man, if any.

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