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Studies on Asetivation of *Aspongopus viduatus* (Fabricius) (Dinidurdae, Hetroptera) in Rain Fed Areas, North Kordofan, Sudan

Mahgoub Ishag Abdalla[1*], Elsyed El Bashir[2], Ahmed Ismail Ahmed Safi[3] and Awad Elkarim Suliman Osman Khalifa[4]

[1]Departmenr of Crops Science, Faculty of Natural Resources and Environmental Studies, University of Piece, Elfulla, Sudan.
[2]Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Shambat, Sudan.
[3]Institute of Gum Arabic Research and Desertification Studies, University of Kordofan, Elobeid, Sudan.
[4]Department of Desertification Studies and Environment, Institute of Gum Arabic Research and Desertification, University of Kordofan, Elobied, Sudan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aspongopus viduatus is the most destructive pest threatening *Citrulus lanatus* (Thuab.), the pest is a troublesome in watermelon in Western States of Sudan, causes severe losses to the crop leading to a total failure. The insect disperses from watermelon crop in summer to find sheltering sites for aestivation to synchronize activities and allowing insects to continue unfavorable conditions while being ready to take advantage of good conditions as rapidly as possible. In general, very little knowledge in the aestivation of *A. viduatus* had been known therefore, this study was initiated to investigate some aestivation relations, namely, population density of aestivating insects, sex ratio, and loss in body weight as well as fat reserves depletion during aestivation. Field experiments were conducted at En-nahod North Kordofan State, Sudan. Plants (grasses, herbs, shrubs and trees) were sampled randomly, aestivated *A. viduitus* were handpicked, placed in polyethylene bags (50 x 40cm) and taken to the laboratory. Samples were counted to determine population density/ tree, male and female ratio /tree, male and female weighed, insect body fat extract was also determined. The results revealed that, there was a decrease in population density of both sexes with the progress of the aestivation period and that there were more females than males and that, the body weight and fat reserves declined as the aestivation period progressed, females were heavier and had more fat deposited than the males.

**Corresponding author: Email: mahgoub1967@gmail.com;*

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1. INTRODUCTION

1.1 *Citrulus lanatus*, Thuab. (Watermelon)

C. lanatus,Thuab. is very important crop. Its fruit contains saturated fats, a good source of vitamin A, and vitamin C, very low sodium and cholesterol content, the crop products compare favorably with the known protein- rich foods such as soybean, cowpea, pigeon peas and pumpkin [1] it also contains phenols, flavonoids, carotenoids, and lycopene contents [2], however the high concentrations of lycopene, can reduces the risk of cancer and other diseases, whereas the seeds and pulps contain antioxidants, essential amino acids such as arginine, isoleucine, leucine, phenylalanine as well as glutamic acid, aspartic acid, and also contain minerals such as Na, K, Ca, P, Cu, Mg, Mn, Zn, and Fe [1,3]. Natural products with antioxidant properties have been extensively utilized in pharmaceutical and food industry and have also been very popular as health-promoting herbal products [4], when the juice of watermelon used as supplementation to human for two weeks, it increased baseline plasma (nitrite), improved muscle oxygenation, during moderate- intensity exercise, increased resting blood pressure but, didn't improve time- to- exhaustion during severe-intensity exercise [5]. Further the natural content of antioxidants found in the phytochemicals of watermelon reduced the risk of chronic diseases [6].

C. lanatus is one of the important rain-fed and a multi-purposes crop in Sudan. It grown in Darfur and Kordofan regions of Sudan for cash, food, feed and water source in summer for both human and animals [7,8,9], however the crop is important source of hard currency in the country [10,11,12,8]. The crop fits very well in the cropping pattern of western Sudan [7]. *C. lanatus* is subjected to multiple insect attacks during the growing season. The most important pest is *Aspongopus viduatus* (melon bug) [7,9].

1.2 The Most Important Pest of Watermelon

1.2.1 Melon bug

Aspongopus viduatus (melon bug) is widely distributed in Middle East, Asia, Africa, with a higher incidence in Egypt, Israel, Iran and Turkey [13,14]. In Sudan the pest occurs mainly in Kordofan, Darfur, Kassala, Northern, Gadarif, Khartoum, and Blue Nile Provinces [15,16,17], [18]. It is commonly found in irrigated areas during winter and appears in rainfed areas during the rainy season [16]. The insect attacks other several crops, such as *Cucumis melo, Cucumis sativus*, *Lagenaria siceraria, Ecballium elaterium*, *Cucumis melo, Gossypium spp*, *Marah spp* and, *Citrullus colocyn* [15,19,20]. *A. viduatus* is a real threat and it is the most destructive pest threatening *C. lanatus*, (Thuab) crop [9,21]. Both nymphs and adults feed on leaves, stems and developing fruits causing wilting and shedding of the plants [15,7,22,9,19], as well as facilitating secondary infections of diseases [15,9]. Severe infestation may destroy the plant completely, and may lead to total crop failure [15,23,24,9,25].

1.2.2 Fat of melon bug

Crude oil recovered by hot water from *A. viduatus* was analyzed for oil content, fatty acid composition and tocopherol. The total lipid content was 45%. Oxidative stability was found to be 38 1h. Triacylglycerol was the major nonpolar lipid class and contribute nearly 70% of the total amount. Fourteen fatty acids were identified in the insect's fat, among which oleic acid contribute 46.5% of the total lipid followed by palmitic acid 44.2%, linoleic acid 3.4% and, traces of linolenic acid. The tocopherol content of the oil amount was 0.3 mg/100g oil. The total content of sterol was 17 mg/100g oil, whereas β-sitosterol was determined as the main compound with about 60% of the total sterol. The total amount of phenolic compounds in the oil extract was 20.7 mg/100goil, [26].

Fat of *A. viduatus* is used as food in western parts of Sudan and has traditional medicinal uses against human and animal skin diseases. It is stable, against rancidity and can be used for meat cooking and meat products preservation processes [27]. Melon bugs are edible in Namibia where the last soft stage which is called nakapunda is cooked and eaten. There is no poisonous material was found in *A. viduatus* fat. This bug fat is similar to most other animal fats [28]. Melon bug fats showed antibacterial activity against four food related bacteria; however, the crude of phenolic compound free oil showed the highest antibacterial activities [29].

1.3 Diapause in Insects

Conditions vary almost everywhere that insects live; insects have to adapt these changing of conditions. Physical and biological conditions suitable for growth, development and reproduction of the insect, generally prevail only during particular seasons. Therefore, to synchronize activities to favorable period and enhance survival during unfavorable periods, many insect species undergo a state of dormancy diapause) [30]. Dormancy is a mechanism used as a means to survive predictable, unfavorable environmental conditions such as low and high temperature, drought or reduction in food availability etc. Both intrinsic and extrinsic factors affect the behavior of insects or insects' stages. Dormancy can occur in embryonic, larval, pupal and/or adult stages of the insect life cycle [31]. In the adult stages reproductive diapause arrests the development of oogenesis, vitellogenesis, accessory glands activity and mating behavior. Male *Drosophila melanogaster* enter diapause with arrested spermatogenesis and development of testes and male accessory glands, increase in stored carbohydrates and an initial increase and then a decrease in lipids, an up-regulated expression of genes involved in metabolism, stress responses [32].

Reproductive diapause has been well studied in Lepidoptera (as monarch butterflies), several Orthoptera (as grasshoppers), and Diptera (as Drosophila), some of these studies suggested neuroendocrine control of reproductive diapause [33].

1.3.1 Phases of diapause in insects

1.3.1.1 Induction phase of diapause in insects

The induction phase of diapause occurs at embryonic stage of life and occurs obviously in advance of environmental stress [34]. This sensitive stage may occur within the lifetime of diapausing insect, or in its preceding generations [35]. During induction insects' response to external stimuli, that triggers the switch from direct development pathways to diapause pathways. However token stimuli can consist of changes in photoperiod, thermo period, or alletochemicals [36].

1.3.1.2 Preparation phase of diapause in insects

Preparation phase is the phase follows induction phase, though insects may go directly from induction to initiation phase without preparation. Before the insects enter diapause, physiological conditions of the internal organs of insects changed and protein, carbohydrates and fat reserves accumulated to maintain the insects during diapause, and provide fuel for development following diapause termination that depend mainly on the intensity of the insect feeding activity [37,38,34]. Over-wintering of *Biprorulus bibax* (Pentatomidae, Hemiptera) in South Australia is based on physiological and behavioral strategies. One of these strategies is the storage of lipids that are important for insect survival during winter [39].

When males' and females' caterpillars of *Spoddoptera litura* (Fabricious) provided with two choices of complementary foods (protein and carbohydrate) in the laboratory, females select significantly more protein than males with no difference in carbohydrate intake between males and females. When females and males confined to single diets with varying mixture of protein (P) and carbohydrate (C), females will consume more than males across on all protein and carbohydrate diets, late in the feeding stage of the final stadium. Males and females were differing in post- ingestive utilization of the ingested nutrients. Females utilized protein for body growth with greater efficiency compared to male. Adult females need more proteins to develop eggs, whereas males were more efficient in depositing lipids from carbohydrates in- take than females [40].

Differences in development of internal reproductive organs, feeding amount and nutrient storage between pre-diapause and pre-reproductive of *Harmonia axyridis* adults, were compared, the results indicated that, there were cleared morphological differences in internal reproductive organs of diapause and reproductive insects. The development of internal reproductive organs was suppressed at early adult stage in pre-diapause beetles compared to pre- reproductive beetles. Feeding amount of both pre- diapause and pre- reproductive beetles sharply decreased from the 15th days after eclosion in females and 14th day after eclosion in males, which implied the initiation of diapause. During the pre- diapause stage, carbohydrates and lipids were accumulated by females, whereas males mainly accumulated carbohydrates [41].

1.3.1.3 Initiation phase of diapause in insects

As mentioned earlier some insects may pass directly, from induction to initiation phase without preparation. Photoperiod is the most important stimulus initiating diapause [31]. However, initiation begins when morphological development stopped and diapause pathways started. In some cases, this change may involve moulting and/or change in color. Enzymatic changes may also take place. Fire bug *Pyrrhocoris apterus* has the enzymatic complement that allows them to accumulate polyhydric alcohols, molecules that help to lower their freezing points to avoid freezing [34,42].

Many external environmental factors such as light intensity, moisture, temperature, wind velocity and various biotic factors affect insect behavior. However, behavior is influenced by circumstances that vary from species to another and in ways that have survival value [43]. They may undergo behavioral changes and begin to aggregate, migrate or search for suitable over-wintering or summer sites for dormancy. However, dormancy is most often, observed in arthropods specifically among insects [42]. In most cases, it is the result of variations of atmospheric temperature and may be distinguished as hibernation or aestivation [44].

1.3.1.4 Maintenance phase of diapause in insects

During this phase insects learn how to lower metabolism and developmental arrest is maintained [34]. Also, their sensitivity to certain stimuli that prevent termination of diapause such as photoperiod and temperature increased as time progress.

Diapaused insects combine several types of adaptation to maintain water balance such as habitat choice, reduction of body water content, decreased cuticle permeability, absorption of water vapor and tolerance of low water levels, however many such features required energy and hence that metabolism albeit much reduced during diapause [45]. In any case the microclimatic conditions at the aestivating site may help decrease the metabolic rate and water loss, thus enhancing survival of the species. Composition of the cuticle may also change the hydrocarbon composition by adding lipids to reduce water loss [37,38].

1.3.1.5 Termination phase of diapause in insects

Termination of diapause in insects may vary according to the type of diapause. In obligated type of diapause, termination may occur without stimuli while, in facultative type termination must occur by the token stimuli that, depends on the environmental condition [32]. Stimuli are very important for preventing the insects from terminating diapause too soon, and as the diapause progressing, the depth of diapause slowly decreases and development can resume if the condition is favorable [46]. The termination of diapause accompanied by a rapid decline in upregulated genes and conversely an elevation in down-regulated genes during diapause [47].

The termination of the aestivation period and the movement of insects back to the breeding areas are due mainly to the onset of the rainy season and the search for food. When food resources are limited intra-specific competition may take place resulting in death or further migration to new sites, however migration from aestivation sites to the breeding sites may be in response to the onset of favorable conditions which lead to the start of the breeding period [48].

1.3.1.6 Post- diapause in insects

Diapause ends prior to the end of unfavorable conditions and follows by a state of quiescence from which the insects begin development, should condition change to become more favorable [34]. The first showers initiate vegetation growth, provide food and stimulate development of insects [49]. In Sudan *A. vidiuatus* migrated from aestivation sites to breeding sites, that depends mainly on the onset of the rainy season in July, where copulation took place [7,9].

1.3.2 Regulation of diapause in insect

Diapause in insects is regulated by environmental stimuli integrated with genetic programming to affect neuronal signaling, endocrine pass ways, and metabolic and enzymatic changes [50,47,36,35,51,52].

1.3.2.1 Environmental regulation

Light and temperature commonly determine whether an insect is active or inactive. Since every insect has an optimum temperature ranges for its activities [48]. However, temperature and photoperiod are the most reliable seasonal changes that regulate diapause [35]. Diapause could be regulated environmentally. In general, it displays a seasonal characteristic pattern; photoperiodism is the most reliable seasonal changes: either short or long day can act as token stimuli. Temperature also acts as regulating factor by inducing diapause or commonly by modifying the response of insects to photoperiod [35,52]. Reproductive diapause of overwintering grasshopper, *Stenocatantops splendens* in natural condition was mainly regulated by photoperiod in the fall- long photoperiods promoted reproductive development and short photoperiods maintained reproductive diapause, and the sensitivity of the overwintering adults to photoperiod was over before the end of the winter [53].

Temperature has generally been known to influence the photoperiodic control of diapause (aestivation or hibernation); high temperature acts with long photoperiod (aestivation) and low temperature with short photoperiod (hibernation). The integration of vary photoperiod and temperature regimes and different cross- mating combination cleared the inheritance of diapause in insects. Both temperature and photoperiod combine are critical for induction and termination of diapause in spotted stem borer *Chlio partelius* [54,55]. When, a butterfly *Polygonla c-aureum* reared in the laboratory throughout its entire life cycle under a short photoperiodic at 21^c, all the adults have a strong tendency to enter diapause [56]. Moreover, high temperature favors the induction of aestivation (in summer) and the reverse is true to hibernation [57]. *Nazara viridula* (Hemiptera) has an adult aestivation control by a long-day photoperiods response and day length plays a leading role in aestivation induction [58].

1.3.2.2 Neroendocrine regulation of Diapause

Diapause also regulated by hormones, which enable survival during unfavorable conditions, Diapause may sometimes prolonged to second or late year as an adaptation to resources that are limited to a specific season but are erratic in availability or abundance from year to year [51]. Many hormones are involved in diapause regulation: such as Juvenile Hormone (JH), Diapause Hormone (DH) and Prothoracicotropic Hormone (PTTH) [36,47]. However, Prothoracic hormone stimulates prothoracic gland to produce ecdosteroids necessary to promote development. Larval and pupal diapause are often regulated by interrupting this connection, either by preventing release of Prothoracic hormone or by failure of Prothoracic glands to respond to Prothoracic hormone [47].

Corpora allata is responsible for juvenile hormone (JH) production. In *Riptortus pedestris* (bean bug) parslateralis neurons on the protocerebrum inhibit juvenile hormone production and maintain diapause [59]. However adult diapause is always associated with absence of juvenile hormone, while, larval diapause associated with presents of JH. Absence of JH in adults causes degeneration of flight muscle atrophy or cessation of development of reproductive tissue and halts mating behavior [60]. The activities of the neurosecretory cells and corpora allata are involved in reproductive aestivation [61]. Bean bug *Riptorus pedestris* maintains reproductive diapause by inhibiting JH production by the Corpora allata [59]. In the corn borer, *Diatraea gradiosella* JH required for the accumulation by the fat body of a storage protein that is associated with diapause [62]. Diapause hormone (DH) regulates embryonic diapause in the egg of the silkworm moth, *Bombyx mori* [63].

Food availability and quality may also help in regulating diapause; however in desert locust *Schistocerca gregeria* a plant hormone gibberellin stimulates reproductive development during the dry season, when their food plants lacking gibberellin the insect remain immature [50].

1.3.2.3 Genetic regulation of Diapause

Diapause in insects is also regulated genetically. Diapause- upregulated genes can be distinguished based on their expression patterns: genes upregulated throughout diapause, others are expressed only in early diapause, late diapause, or intermittently throughout diapause [47] However, when genetics of diapause of *Chlio partelius* examined, estimating the degree of dominance, the results revealed that, diapause, developmental and morphometric are governed by over dominance gene effects and mainly depend on parental diapause history [64].

Tow populations of multicolored Asian ladybird *Harmonia axyridis*, the high diapause population (HD) and low diapause population (LD), originated from two different places. Reciprocal first-generation hybrids and reciprocal backcrosses were investigated in laboratory, under strong diapause inducing photoperiod (12 h). The results indicated that photoperiod induction of diapause in the test insect mostly determine by several genes although, one of these genes is evidently plays a leading role with diapause being dominant over non-diapause, and that male and female genotypes are equally important in the determination of female reproductive diapause [65].

1.3.3 Tropical diapause (aestivation)

Tropical diapause (Aestivation) is a survival strategy used by many vertebrates and invertebrates to sustain harsh environmental condition. It includes strong metabolic rate suppression strategies to retain body water content, energy conservation and fuel reserves, altered nitrogen metabolism and mechanism to preserve and stabilize organs, cells and macro-molecules over many weeks and months of dormancy [66]. Tropical diapause often initiated in response to biotic rather than abiotic factors, because food in the form of vertebrate carcasses may be abundant following dry seasons or oviposition sites (fallen trees) may be more available following rainy seasons. Also, diapause may serve to synchronize mating season or reduce competition rather than to avoid unfavorable condition. However, diapause in tropic (aestivation) has several challenges to insects that are not faced in temperate zones. Insects must reduce their metabolism without the aid of cold temperature and may be faced with increased water loss due to high temperatures [67].

Aggregations are common among diapausing insects in tropics, specifically in Lepidoptera, Coleopteran and Hemiptera [67]. *Stenotarsus rotundus* (Fungus beetle) aggregates up to 70.000 individuals (measured about eight beetles deep), where relative humidity increases and water loss decreases [68]. Predators and parasites may be abundant among insects during the diapausing period [15,67,69]. Aggregations may also be used as protection against predators, since Aggregating insects are frequently toxic and predators quickly learn to avoid them [67].

Effective control measures of *A. viduatus*, in the growing season depend mainly on fundamental studies of the pest ecology, physiology, biology, behavior, etc. during favorable and un-favorable periods. Nevertheless, aestivation is a seasonal arrest of insects' development during unfavorable condition (summer), hence insects need to synchronize activities to favorable time. However, there is a very little data about *A. viduatus* aestivation had been reported. Based on the reported knowledge, this study was initiated to evaluate some of this important knowledge namely: population density of the aestivating insects, sex ratio, and the rate of loss in body weight as well as fat reserves depletion during the aestivation period.

2. MATERIALS AND METHODS

2.1 Study Area

Field experiments were conducted at En-nahod (latitudes 12^{c}. 4′ 13c.25′ N and longitudes 28^{o}. 0′ 29^{c}.0′ E) locality North Kordofan State, Sudan, about 800 and 200 km west of Khartoum and El Obied respectively. The study areas were heavily colonized by the aestivating insect *A. viduatus*.

2.1.1 Climate

The localities of the study area lie within the semi-arid desert climate with limited seasonal rain. The rainy season extends from July to October with maximum rain fall in August [70], average annual rainfall is 382.3-400 mm and temperature is 24-32^{d}C [71].

2.1.2 Soil and vegetation

The soil of the study area is generally sandy (Goz). Vegetation is a mixture of grasses, herbs, shrubs and trees. The trees include *Caleotropis procera* (Oshar), *Acacia senegal* (Hashab), *Balanites aegyptica* (Hejleej), *Guiera senegalensis* (khobeish), *Boscia senegelensis* (kursan), *Albizzia amara* (Arad), *Cobretum cordofanum* (Habiel), *Acacia nubica* (Laot), *A. nilotica* (Sunt) *Adanosonia digitata* (Tabaldi), *Ziziphus spina christi* (Sedir), *Scterocya birrea* (Himeid), *Ceratonia siliqua* (kharoob) as well as some grasses and herbs such as *Eragrostis megastachya* (Banu), *Geirgeria alatum* (Gadgad), *Aristida adscensionis* (Gau), *Cenchrus sp.* (Haskaneed), *Zornia glochidiata* (Shilini) and *Andropygon gayanus* (Abo-Rakhies) [70,71].

2.2 Sampling Methods

2.2.1 Population density, sex ratio, and loss in body weight

Different areas around En-nahod locality in two successive years were surveyed for the occurrence of *A. viduatus.* In the 1st year the test insect was found aestivating in the south and south-west En-nahod (6-10 km), North Kordofan State, Sudan. However, in the 2nd year the melon bug was found aestivating in other areas, 20 km west En-nahod, North Kordofan State, Selected areas were divided into four blocks of 1600 m^2 each and each block divided into 4 sub- plots of 400 m^2, arranged in Completely Randomized Block Design (CRBD) with four replicates to have homogenous experimental units (topography differences and presence of valleys). Five trees from each plot were randomly chosen and all aestivating *A. viduitus* at each tree were handpicked, placed in polyethylene bags (50 x 40cm) and taken to the laboratory in the Faculty of Education, University of West Kordofan, and En-nahod, Sudan.

Samples were counted to determine the population density per tree, sexed to determine male to female ratio/tree and weighed using sensitive digital balance to determine male and female weight. The same procedures and design were repeated during a period of three months (April, May and June) in both locations for the two successive years. At the end the data including: population density, sex ratio, as well as loss in body weight were determined and subjected to analysis using SAS software.

2.2.2 Insect body fat extraction

Fat of aestivating *A. viduatus* was extracted using the method described by Balla [72] with some modifications. Five grams of sexed (male / female) grounded sexed insects were put in a sochxlet apparatus *with* 200 ml of hexane in a round bottom flask (250 ml). The sochxlet was operated for three hours. Temperature was adjusted at 68°C, and the obtained fat was weighed and the amount of fat was determined for each of the three months (April, May and June) for the two successive years. The experiment and units were arranged in a randomized complete design CRD with three replicates. At the end Data were subjected to analysis using SAS software.

3. RESULTS

3.1 Body fat Loss of *A. viduatus* during Aestivation

The depletion of energy reserves of aestivating males and females of *A. viduatus* during the two successive years is displayed in Table 1. Table 1 is presenting the means body fat at different times following the onset of the aestivation period. The results indicated that there was loss in the fat reserves with time the aestivation progressed. Results also revealed that, the fat content of the females was significantly greater than that of the males (Table 1).

3.2 Loss in Body Weight of *A. viduatus* during Aestivation

Loss of body weight of the aestivating A. *viduatus* at different times following onset of aestivation was achieved. Both males and females during April, May, and June in the two successive years were displayed in Table 2. The results indicated that there was a significant difference in body weight loss of both males and females. Weight loss of insects decreased with the time after the onset of the aestivation period. It is also evident that the weight of females was always significantly greater than that of males (Table 2).

Table 1. Mean body fat (gram) of *A. viduatus* at different times following the onset of the aestivation period during April, May and June in two successive years

Year / Month	Mean males and females body fat (g) in the 1st year		Mean males and females body fat (g) in the 2nd year		Mean total of males and females body fat (g)	
	Males (5g)	Females (5g)	Males (5g)	Females (5g)	Males (5g)	Females (5g)
April	1.51 a	1.86 a	1.54 a	1.85 a	1.525	1.855
May	1.41 b	1.65 b	1.35 b	1.70 b	1.380	1.675
June	1.30 c	1.45 c	1.17 c	1.65 b	1.235	1.550
Mean total	1.41	1.65	1.35	1.73	1.380	1.690
LSD	0.05	0.06	0.06	0.09		
SE±	±0.06	±0.06	±0.07	±0.10		

Means with the same letters within each column are not significantly different at (P<0.05) according to LSD

Table 2. Loss in body weight at different times following the onset of aestivation of *A. viduatus* during April, May and June in two successive years

Month \ Year	Loss in male and female body weight (g) in the 1st year		Loss in male and female body weight (g) in the 2nd year		Mean total of male and female weight loss (g)	
	Male	Female	Male	Female	Male	Female
April	0.26 [a]	0.31 [a]	0.26 [a]	0.30 [a]	0.26	0.30.5
May	0.25 [a]	0.29 [b]	0.25 [b]	0.29 [b]	0.250	0.290
June	0.23 [b]	0.27 [c]	0.23 [c]	0.28 [c]	0.230	0.275
Total mean	0.24	0.29	0.24	0.29	0.240	0.290
LSD	0.01	0.01	0.01	0.003		
SE±	±0.01	±0.01	±0.01	±0.003		

Means with the same letters within each column are not significantly different at (P<0.05) according to LSD

3.3 Changes in Population Density and Sex Ratio of *A. viduatus* during Aestivation

The population density of *A. viduatus* during aestivation period was changed at different times following the onset of the aestivation period and, it was presented in Table 3. There was significant difference in the mean number of both females and males/tree in the two successive years. Insect densities decreased with time after the onset of aestivation period. It is also evident that the number of females was always significantly greater than that of the males. Further, the number of both males and females decreased as the aestivation period progressed (Table 1, Plate 1).

Plate 1. Female and male of *A.viduatus* during aestivation period (on the right =female/ on the left = male)

Table 3. Changes in population density and sex ratio of *A. viduatus* at different times during aestivation period (April, May and July) in two successive years

Month \ Year	Mean No. of Males and Females/ tree in the 1st year		Mean No. of Males and Females/ tree in the 2nd year		Mean total No. of Males and Females / tree	
	Males	Females	Males	Females	Males	Females
April	20.62 [a]	26.44 [a]	14.06 [a]	20.34 [a]	17.34	23.39
May	16.63 [b]	23.65 [a]	11.99 [b]	16.61 [b]	14.31	20.13
June	11.86 [c]	13.99 [b]	10.01 [c]	14.50 [b]	10.94	14.25
Total mean	16.20	21.36	12.02	17.15	14.19	19.26
LSD	2.69	4.22	1.64	2.91		
SE±	±3.02	±3.76	±1.46	±2.59		

Means with the same letters within each column are not significantly different at (P<0.05) according to LSD

4. DISCUSSION

4.1 Body fat Loss of *A. viduatus* during Aestivation

The results indicated that there was significant loss in the fat reserves with time following aestivation at the rates of approximately 7-13% and 7-12% each month, for males and females respectively. Results also concluded that, the fat content of the females was significantly greater than that of the males in most cases (Table 1).

During preparation and initiation phases of aestivation, dormant insect accumulates protein, carbohydrates and fat reserves to provide fuel for the aestivation, and development following aestivation termination that depend mainly on the intensity of the insect feeding activity [37,38,34]. In crickets *Teleogryllus commodus*, both male and female longevity were maximized their nutrient on a high- carbohydrates and low-protein diet in the field, but male and female lifetime reproductive performances were maximized in markedly different parts of the nutrient intake landscape [73]. There may be a sex-specific response to feeding [74]. A recent study using geometric framework for nutrition suggested that trade-offs are often regulated by the intake of specific nutrients, but a formal approach to identify and quantify the strength of such trade-offs is lacking [75].

Male and female caterpillars of *Spoddoptera litura* (Fabricious) provided with two choices of complementary foods in the laboratory (one was a protein-based diets and the other was a carbohydrate- based diets) the consumption preferred by the two differed, with females selecting significantly more protein than males with no difference in carbohydrate intake between the two sexes. When confined to single diets with varying mixture of protein and carbohydrate {protein P: carbohydrate C ratios i.e. Protein p42%: carbohydrate c0%(p42% :c0%), p35% :c7%, p28% :c14%, p21% :c21%, p14% :c28%, p7% :c35, females consumed more than males across on all P:C diets, late in the feeding stage of the final stadium. Sixes were differing in nutrients utilization. However, females utilized protein more efficiently than male, whereas males were more efficient in depositing lipids from carbohydrates in take than females [40]. Similar findings of carbohydrates and protein intake on the trade-off between reproduction and aspects of immune function were achieved in male and female decorated crickets *Gryllodes singllatus* [75]. During the pre-diapause stage, carbohydrates and lipids were accumulated by females, whereas males mainly accumulated carbohydrates [41]. Females of *Agonoscelis pubescens* sister order of the test insect live longer during adverse conditions than males, and that they accumulate more energy reserves [76,77].

Temperature during aestivation affects survival by influencing the rate of depletion of energy reserves [78], so the metabolic rate is maintained at an extremely low level [79]. Dormant insects combine so many types of adaptation to maintain water balance such as habitat choice, reduction of body water content, decreased cuticle permeability, absorption of water vapor and tolerance of low water levels, however many such features required energy so, metabolism albeit much reduced during aestivation [45]. Higher temperature increases the metabolic rate in insects and food reserves are rapidly consumed [80].

Dormancy in female *Drosophila melanogaster* and other female flies characterized by arrested development of ovaries, altered nutrient stores, lowered metabolism, increased stress and immune resistance and drastically extended lifespan. However male *D. melanogaster* enter a state of dormancy with arrested spermatogenesis and development of testes and male accessory glands, increase in stored carbohydrates and an initial increase and then a decrease in lipids, an upregulated expression of genes involved in metabolism, stress responses. Dormant male flies deplete stored nutrients faster than females [32].

For the same reasons reported earlier the accumulation of protein, carbohydrates and fat reserves to provide fuel for dormancy and the development following dormancy termination depends mainly on the intensity of insect feeding prior to dormancy, however, females shown to have better ability than males in feeding intensity during their normal life before aestivation, hence females' potentiality to store more fuel is higher. As males and females would subject to the same environmental factors during aestivation, the depletion of stored fuel of males was faster and bigger.

4.2 Loss in Body Weight of *A. viduatus* during Aestivation

The results revealed that the weight of both sexes significantly decreased with the time after the onset of the aestivation but the weight of females was significantly greater than that of males in most cases (Table 2). The findings agreed with Bilal and Mostufa [76,77], they found similar results in *Agonoscelis pubescens* sister order of the test insect.

As reported earlier dormant insects combine so many types of adaptation to maintain water balance such features required energy hence, metabolism albeit much reduced during aestivation [45]. Habitat at the aestivating site may also help in decreasing the metabolic rate and water loss, thus enhancing survival of the species. Composition of the dormant insect's cuticle may also change the hydrocarbon composition by adding lipids to reduce water loss [37,38]. Such feature may help dormant insect to adapt and maintain water loss and fuel depletion better but, metabolism and water content during aestivation consumed the stored nutrients and water content much higher resulting in body weight loss (Table 2).

Females consume significantly more protein than males, but they both consumed carbohydrate at the same rate, however, females consumed more than males across on all protein to carbohydrate C: P diets [40]. Similar findings were achieved in male and female of decorated crickets *Gryllodes singllatus*, which insured the same way of carbohydrates and protein intake [75]. Aestivated male insects depleted stored nutrients faster than females and that males take longer to recover reproductive capacity after reintroduction to favorable conditions [32].

For the same reasons mentioned earlier the accumulation of protein, carbohydrates and fat reserves to provide fuel for aestivated insects and later for the growth following aestivation termination depends fundamentally on the insect feeding before aestivation, however females shown better ability than males in nutrient intake during their development and then high potentiality for storing more food for unfavorable condition (aestivation). As males and females subjected to the same environmental factors during aestivation, they all depleted fuel and loss water, correspondingly, they lose weight. As, females accumulated more energy reserves than males, they were heavier, bigger in size than males (Table 2 and Plate 1), and thus, allow females to be more capable to live adverse condition better and continue to withstand harsh conditions better and effective.

4.3 Population Density and Sex Ratio

The population density of *A. viduatus* during the aestivation showed significant decreased as the aestivation period progressed. It is also evident that the number of females was significantly greater than that of the males (Table 3) in most cases. Increase the aestivation period (April-June) resulted in decreasing the population density, however males decreased greater. These results agreed with that of Bilal, and Mustafa, [76,77], who reported that females of *Agonoscelis pubescens* sister order of the test insect live longer during adverse conditions than males. The sex ratio in most insects in general is approximately 1:1 [48]. According to Romoser the test insect *A. viduatus* and Dora antad *Agonoscelis pubescens* may among his exception. They showed that female's number was significantly greater than that of the males that may because some strains of insects are species specific, also environmental condition changes during aestivation may affected the dormant insect sexes and their performance severely and differently.

Study on pupal sexes of the most common mosquitoes species in the course of biweekly censuses (with replacement) of the contents of 3-7 tree holes from 1980-2003 in Vero beach, a significant male bias was detected over the time, for the most species abundant, *Aedes triseriatus* specially in February- May, August, and December months. No significant deviation from a 1:1 sex ratio was detected among pupae of *Toxorhynchites nutilus* or *Ae. triseriatus*. Sex-specific responses to hatching stimuli are judged to be present but less pronounced in eggs of *Ae. Albopictus*. Male biases in container Aedes are likely associated with sexual selection, which may also explain seasonal changes in sex ratio. The overproduction of *Ae. Triseriatus* males may be counterbalanced by increased fitness of females which are known predominate in delayed hatches [74].

As females accumulated more energy reserves, more weight than males (Tables 1, 2), they were more capable to sustain well, live adverse condition better, and continue to withstand harsh conditions better therefore, their sex ratio were significant female bias (Table 3).

The fact that *A. viduatus* is one of the major pests of watermelon. Both adults and nymphs are very destructive [22,9], and may even cause total failure of the crop [25]. However, the crop is very important in Sudan, as food, feed, cash, water and hard currency sources. There is an additional merit for fundamental studies of the test insect, such as ecology, behavior, physiology, etc. since there is a very little knowledge had been attempted. Applicability of fundamental studies of *A. viduatus* in the field is highly needed. Therefore, this study initiated to evaluate some of these studies in the field namely, population density, sex ratio, the rate of loss in body weight, as well as fat reserves depletion of *A. viduatus* during aestivation period in the field. Validation of these results is to be added to other related studies as well as studying more environmental, physiological, behavioral and ecological parameters during aestivation period in different locations, since the reproductive ability of the test insect during the rainy season is very high [49], and the insect is of highly economical importance. Further investigations will be addressed to more biotic and abiotic factors, various behavioral and ecological parameters, as well as improving methods of sampling with encouraging results.

5. CONCLUSIONS

- Population density of *A. viduatus* decreased as aestivation period progressed and number of females were greater than that of males.
- The weight of both sexes of *A. viduatus* significantly decreased with the time after the onset of the aestivation period and the weight of females was significantly greater than that of males.
- *A. viduatus* lost weight and fat reserve continuously and gradually as the aestivation progressed and females usually had heavier weight and more fat content.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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